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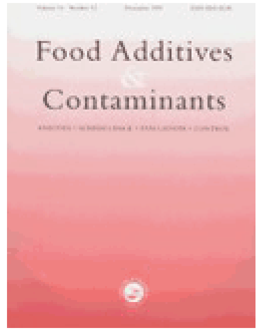

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Deoxynivalenol and other *Fusarium* mycotoxins in bread, cake and biscuits produced from UK-grown wheat under commercial and pilot scale conditions.

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Abstract

Bread, cakes and biscuits were manufactured from flour containing deoxynivalenol (DON) and low concentrations of zearalenone (ZON) and nivalenol (NIV). The results show that these mycotoxins remain mostly unaffected during manufacture. Although the results indicate that the mycotoxins are largely stable and survive processing, when concentrations were determined on an 'as is' basis as stipulated in legislation, levels in finished products were usually lower than in the starting flour due to the dilution effect of other ingredients such as fat, sugar and water. Thus mean concentrations of DON in bread were reduced by about 35% and 39% in white and wholemeal bread respectively which are in close agreement with the reduction required by the regulations although the changes that occur during milling white and wholemeal flour from whole wheat also need to be taken into account. The reduction of DON during cake manufacture is greater than for bread because flour makes up only about 25% of the starting ingredients. However, in biscuit production, particularly for crackers for which flour constitutes about 90% of the recipe ingredients, the reduction indicated by the regulations is not achieved. It is concluded that for some commercial processes, the whole-wheat or flour ingredients used will need to contain DON levels lower than those set by legislation to ensure that the final products will still meet statutory limits. Limited results with consignments containing low concentrations of ZON and NIV suggest that their stability and survival are similar to those for DON.

Keywords: Cereals, Fusarium, mycotoxin, bread, cake, biscuits, processing

Introduction

Cereals including wheat are often infected by Fusarium fungi in the field that can cause diseases such as Fusarium head blight (Edwards 2004) and the formation of mycotoxins that include deoxynivalenol, zearalenone, nivalenol, HT2-toxin (HT2) and T2-toxin (T2). Many mycotoxins are inherently chemically stable so may survive processing to remain in the products purchased by the consumer. It is thus important to know the extent to which this occurs to assist in assessing the risk that Fusarium mycotoxins might pose to the consumer. To achieve this it is necessary to study both the occurrence of the mycotoxins in primary agricultural crops such as wheat and the effect of processing and food manufacturing on their concentrations in the retail products (Hazel and Patel 2003).

The consumer can then be protected by the implementation and observation of appropriate legal limits such as have already been introduced in the EC for DON and ZON in wheat and in other cereals (EC 2006). The need of, and values for, further legislation to address HT2 and T2 are currently being considered. Different limits are set for mycotoxins at selected stages during their processing so that for wheat there are values at intake, in milled ingredients, in retail products and for baby and infant foods (Table 1). Legal limits refer to the product "as is" and the acceptance of a product must also take into consideration the measurement uncertainty.

INSERT TABLE 1

While considerable data are available (e.g. Gareis et al. 2003) to show the frequency and levels of Fusarium mycotoxins, particularly DON, occurring in wheat there is much less information on their transfer to wheat-based products such as bread, cakes, pastries and biscuits at the retail point. Samar et al. 2001 found that fermentation reduced naturally occurring deoxynivalenol in Argentinean bread processing technology within a pilot scale plant. French bread and Vienna bread were prepared from wheat flour naturally contaminated with deoxynivalenol at 150 µg kg^{-1} in which dough was fermented at 30-50º C according to standard procedures used in Argentina. The maximum reduction obtained in dough at 50º C was 46% for the Vienna bread and 41% for French bread. This agreed with a study by Neira et al. 1997 in which 8 types of product were prepared in a low technology bakery that showed a significant reduction of deoxynivalenol during the bread making process.

However, Gilbert et al. 1984 showed that 80% of deoxynivalenol survived in both spiked and naturally contaminated wheat although Abbas et al. 1985 obtained variable losses from 19 to 69%. Studies have further suggested that DON may sometimes be present in cereals in a bound form and that an acid solvolysis procedure may be required to release this bound mycotoxin (Liu et al. 2005). Boyacioglu et al. 1993 examined the effect of additives such as potassium bromate, L-ascorbic acid, sodium bisulphite, L-cysteine, ammonium phosphate at varying levels on deoxynivalenol during baking. The reduction was about 7% in flour without additives. Potassium bromate and L-ascorbic acid had no effect, but sodium bisulphite, L-cysteine and ammonium phosphate resulted in about 40% reduction.

ZON is also usually regarded as stable or only partly decomposed by heat. However, Matsuura and Yoshizawa 1981 found ZON was reduced in bread by approximately 40% while about 50% was lost during the production of noodles although it is unclear whether their results for bread were corrected for water content. However, baking at 170ºC did not degrade ZON or NIV (Tanake et al. 1986). During extrusion of maize, Scudamore et al. 2008a found that ZON was reduced particularly under low moisture conditions (13.5%) but remained virtually unchanged when forming pellets under high moisture extrusion conditions (28%).

In related studies (Scudamore and Patel 2008b) the occurrence and fate of DON during commercial preparation of whole-wheat breakfast cereals has shown that exhaustive cleaning regimes can remove up to about 50% of DON from wheat received directly from UK farms and that this could be further reduced in whole-wheat breakfast cereals. However, on investigation this was shown to be principally due to solution of DON in aqueous liquid run off from the cookers, and not to chemical breakdown.

The variety of products manufactured from flour involves many different production methods and uses a large range of ingredients based on a complex and specialised food technology. Flour is a combination of protein, starch, sugar, fat and mineral salts but the quality and quantity of the gluten - forming proteins is the most significant factor in determining its suitability for specific products. It is recognised that the behaviour of mycotoxins in laboratory or pilot scale studies may not accurately reflect what occurs in large commercial operations such as in mills, bakeries and other processing plants using naturally contaminated cereals. The work reported here forms part of a Project studying the occurrence and fate of Fusarium mycotoxins in UK cereals, and the changes in mycotoxin concentrations during the commercial and pilot plant manufacture of specimen samples of bread, cake and biscuit. The results are discussed in relation to the existing EC regulations.

Materials and Methods

Bread manufacture and sampling

The manufacture of bread from naturally contaminated flour was carried out in a pilot scale commercial bakery with 4 kg mixes containing 2.25 kg flour using the Chorleywood Process (Chamberlain et al. 1962, Axford et al. 1963). In summary this process involves mixing the ingredients of the dough typically for a few minutes to a set work input in order to achieve a dough temperature ex-mixer of 30°C, the dough was divided, moulded and proved for a maximum of 60 minutes at 40-45 °C followed by baking for approximately 21 minutes at 210°C. Dough ingredients were flour, yeast, salt, fat, ascorbic acid fungal alpha-amylase and water. The bread was then cooled to 27°C. Samples (500 g) made up of 5 incremental samples were taken post mixing and post proving. A composite final bread sample of four loaves constituted the final product sample. These were mixed together in a food processor and stored frozen at -20ºC until required for analysis. 25 g samples were taken for analysis

Cake production

There is a very large range of cake types and two different recipes were examined here; a plain Genoa (cake 1) in which the ingredients were baked for 15 minutes at 170°C and a stressed high ratio cake (cake 2) baked for longer (30 minutes) but at a lower temperature of 150°C. The ingredients in both cake recipes consisted of flour, sugar, egg, water, margarine and other ingredients High ratio refers to the sugar to flour ratio while stress is a property of the dough. The dry ingredients were mixed first and then the liquids added and mixed. The mixtures were deposited immediately, stood for a set period of time then baked in the oven. The cakes were allowed to cool, ground together in a food homogeniser and stored at –20C until required for analysis.

Biscuit manufacture

Semi-sweet biscuits were produced by using a recipe containing about 70% flour, the remaining ingredients being mainly fats and sugar. Cracker biscuits were manufactured by baking a lean cracker recipe containing about 90% flour and with significantly less fat and sugars. Both commercial flour and flour from experimental trials were used and blended to provide a range of concentrations in batches of between 5 and 10 kg of ingredients. The biscuits and crackers were baked in a 3 zone travelling oven for 5 minutes at a maximum temperature of 245ºC for semi- sweet biscuits and 3 minutes at a maximum temperature of 280ºC for crackers.

Analytical methods

DON (and other related trichothecene mycotoxins) was determined following the method of Patel *et al*. 1996 as used by Scudamore *et al*, 2007. Ground or chopped samples (20 g) were extracted with 100 ml acetonitrile/water (84:16). An aliquot of the extract was cleaned using a charcoal/alumina column. After taking to dryness and re-dissolving in acetonitrile DON was derivatised to form the trichothecene -trimethyl silyl (TMS) derivatives and determined by GC/MS operating in selected ion mode, using 4 ions for confirmation. ZON was determined by HPLC with fluorescence detection as used by Scudamore *et al*, 2007. Ground samples (25 g) were extracted with 125 ml of acetonitrile/water (75/25). The extract was cleaned-up using an immunoaffinity column and determined by HPLC with fluorescence detection..

The analytical methods were UKAS (ISO 17025) accredited. Analyses were conducted with a spiked sample, i.e. a known amount of toxin was added to each sample matrix each day prior to extraction, clean up and determination for each batch of 1-5 samples. The spike level was 200 µg kg^{-1} for DON and 50 µg kg^{-1} for ZON. These results were used to assess recovery and all reported results were corrected using the values obtained. Recoveries in the range 70-110% were considered acceptable. The limit of detection and limit of determination respectively for each mycotoxin were 5 µg kg^{-1} and 10 µg kg^{-1} for DON, NIV and other trichothecenes and 1.5 µg kg^{-1} and 3 µg kg^{-1} respectively for ZON. The limit of detection is defined as 3 times the electronic baseline noise and the limit of determination as 6 times baseline noise. Calibration curves for each mycotoxin were plotted with the lowest calibration points respectively being equivalent to 10 and 3 for DON and ZON. After analysis samples were retained and stored at -20°C.

In house reference material (wheat and maize) contaminated at 220 and 550 µg/kg of DON was spiked with 200 and 500 µg/kg respectively. Typical mean recovery was 89% with a coefficient of variability of 8.1% for 10 replicates. For fumonisins, maize in house reference material contaminated at 498 µg kg^{-1} was then spiked at 400 µg kg^{-1}. Typical mean recovery was 86% with a coefficient of variability of 6.5% for 10 replicates. For ZON, ground maize in house reference material contaminated at 50 µg kg^{-1} of ZON was spiked at 53 µg kg^{-1}. Typical mean recovery was 88% with a coefficient of variability of 7.2 % for 10 replicates.

Results and Discussion

Tables 2 and 3 show the levels of DON in white bread and wholemeal bread manufactured from commercial consignments of white and wholemeal flour respectively. Legislation applies to ingredients and products on an 'as is' basis, that is, the concentration determined without any correction for moisture content or the presence of other components. The analytical values obtained for flour and for the bread produced are converted to a dry weight values. Changes in concentrations are then presented 'as is' (relevant for legislation) and to show the true change in concentration (using a standard moisture content of 12.5% for flour and 40% for bread). The mean of 13 and 7 baking runs respectively for white bread and wholemeal bread show that the ‘as is’ levels detected in bread were 35% and 39% lower than the concentration in the flour. The EC limits for DON in flour and bread are 750 µg kg^{-1} and 500 µg kg^{-1} respectively, Table 1, indicating that a 33% fall in concentration should be expected. The results reported here are thus consistent with the current legislative limits which in themselves are required to take into account measurement uncertainty..

INSERT TABLE 2

The maximum concentration of DON found in commercial consignments of flour over the period of these studies was 284 µg kg^{-1}, which is approximately 40% of the maximum allowed limit. When the values for DON of each flour and the corresponding bread sample are plotted, Figure 1, a linear relationship is obtained with an R^2 value of 0.91. This implies that results can be extrapolated safely up to the EC statutory limit.

INSERT FIGURE 2

The results for bread in Tables 2 and 3 have also been corrected for moisture content. When this is done the mean concentrations show an actual loss of only 5% for DON in white bread and 11% for the wholemeal bread although the changes in concentration obtained between runs were quite variable. Any further correction to take into account the presence of other minor ingredients would only increase the calculated values in bread by a small amount so that it is clear that DON is virtually unaffected by the baking procedure. However, there is a large variability in the milling fractionation pattern between different consignments of wheat. On 6 occasions samples were taken at intermediate stages in the bread making process including the dough after mixing and after dividing, from the dough after its first poof and after its final proof. After correcting for the moisture content at each stage the results confirm that there is no significant change in DON during these intermediate stages. These results are not shown.

INSERT TABLE 3

Elsewhere Edwards et al. (in press) have shown that during milling flour from whole wheat a mean concentration of DON is reduced by about 30 % for white flour but this is virtually unchanged with wholemeal flour because the latter consists of all the components of the whole grain. This emphasises that millers and bakers cannot use wholemeal wheat containing DON at the legislative limit if they are to produce wholemeal bread within the legal limits and would need to lower the acceptable concentration in the unprocessed wheat.

INSERT TABLE 4

Table 4 gives the results for cake manufacture. On an 'as is basis DON in both types of cake was reduced on average by at least 55% so that the reduction expected from the statutory limits is easily met. The results are calculated on the basis that flour only constitutes 255 of the cake produces, the other ingredients being mostly fat, sugar and water. While the total number of bakes was small, the relative concentration of DON in the 2 Genoa type cakes compared with the flour was lower than those in the high stress type of cake. When the other ingredients are considered there is close to 100% survival of DON in the Genoa cake but an apparent increase in DON levels in the high stress cake bakes. However, the starting concentrations of DON in flour are low and the increases seen are only equivalent to about 20-30 $\mu g\ kg^{-1}$ of the mycotoxin. The reason for this is unclear but could be due to the release of 'bound' DON or the presence of DON in one or more of the other ingredients although these were checked for the presence of DON in selected samples. The apparent small increase in DON during manufacture of cereal products, including breakfast cereals, has been noted in other processes (Scudamore et al. 2008b)

INSERT TABLE 5

The changes in DON and ZON concentrations during the manufacture of biscuits are given in Table 5. Because concentrations of both mycotoxins were low in commercial biscuit flour, portions milled from wheat grown on experimental plots was used to carry out bakes to study DON at levels up to and above the regulatory limits and to also carry out a very limited study with NIV and ZON. Intermediate concentrations were obtained by blending commercial consignments of flour with experimental consignments of flour. The relative concentrations of DON and ZON in the flour and sweet biscuits were 25% and 30% lower respectively on an 'as is' basis. Because the sweet biscuits studied were produced from ingredients which contained about 70% flour this indicates that there was no loss of either DON or ZON during manufacture. Crackers are produced from a recipe containing about 90% flour so that the mean reduction in concentration of DON on an 'as is' basis indicates again that DON (and probably) ZON are not affected during baking. NIV occurred in low concentrations in some of the flour from the experimentally grown wheat and this allowed some limited studies. The results are shown in Table 6. This suggests that there was little loss in sweet biscuits with the reduction in concentration seen being due to the presence of other ingredients. The mean reduction in NIV concentration in crackers was >28% although the results obtained were quite variable ranging from 0 to 42%.

INSERT TABLE 6

The results obtained in this study have been used to calculate the levels expected in the products manufactured when starting with whole wheat containing DON extrapolated to the regulatory limit and are shown in Table 7. This shows that limits for DON in consumer products are only met in cakes because of the large dilution factor due to other ingredients. White bread and a cracker variety of biscuit would have levels of DON in excess of the statutory limits, while those in wholemeal bread will be higher still because the flour will contain all DON present in the whole wheat. The studies also show that there is quite a large variability in results from batch to batch so allowance for this also needs to be taken into account.

One outcome is that buyers will decide to purchase whole wheat or flour containing levels of DON below the maximum allowed to have confidence that legislation will be met in the susceptible finished products. In some circumstances this will increase costs and/or necessitate wheat being used for other purposes.

Conclusions

DON appears to be very stable in all processes examined. Where results obtained for DON were lower in the products, this was due to a dilution effect from other ingredients including water. This dilution enabled the regulatory limits to be met in the consumer product because legislation applies to results on an 'as is' basis. Within the limits of analytical variation the level of DON at each intermediate stage in the baking process was the constant when corrected for moisture content. The baking of white bread from naturally contaminated flour using the Chorleywood Baking Process resulted in little change in DON levels.

Concentrations of DON in both white and wholemeal bread were shown to be reduced by 35-40% during bread making compared to the flour ingredient when concentrations were determined on an 'as is' basis. If moisture content and the presence of other ingredients were taken into account the loss of DON was less than 5 or 11% respectively confirming that it is stable during the processes. In the absence of ZON in flour, the stability of this mycotoxin could not be determined.

In the same way, the concentration of DON in cakes and biscuits when compared to that in the flour was only reduced by an amount due to dilution with the other ingredients added. Because biscuits are manufactured from ingredients containing a high percentage of flour, the reduction demanded by the legislation would appear not always to be met. However, because allowance must also be made for the uncertainty of the measurement (typically about 44%), the need to reject these products in practice would be extremely unlikely.

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References

Axford DWE, Chamberlain N, Collins TH, Elton, GAH. 1963. The Chorleywood Process. Cereal Science Today 8:265-270.

Abbas HK, Mirocha CJ, Rosiles R, Carvajal M. 1988. Effect of Tortilla-preparation process on aflatoxin B_1 and B_2 in corn. Mycotoxin Research 4:33-36.

Boyacioglu D, Hettiarachchy NS, Dappolonia BL. 1993. Additives affect deoxynivalenol (vomitoxin) flour during baking. Journal of Food Science 58:416-418.

Chamberlain N, Collins TH, Elton GAH. 1962. The Chorleywood Bread Process. Bakers' Digest 36:52–53.

Edwards SG. 2004. Influence of agricultural practices on fusarium infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. Toxicology Letters 153:29-35.

European Commission 2006, Commission Regulation (EC) No 1881/2006, of 10 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364: 5-24.

Gareis M, Schothorst RC, Vidnes A, Bergsten C, Paulsen B, Brera C, Miraglia M. 2003. Collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states, European Commission, Final Report of SCOOP task 3.2.10, 605 pages.

Gilbert J, Shepherd MJ, Startin JR. 1984. The analysis and occurrence of *Fusarium* mycotoxins in the United Kingdom and their fate during food processing. In *Toxigenic fungi and Health Hazards*. Edited by H. Karata and Y. Ueno. Amsterdam: Elsevier, pp. 209-216.

Hazel CM, Patel S. 2004. Influence of processing on trichothecene levels. Toxicology Letters 153:51-59.

Liu Y, Walker F, Hoeglinger B, Buchenauer H. 2005. Solvolysis procedures for the determination of bound residues of the mycotoxin deoxynivalenol in fusarium species infected grain of two winter wheat cultivars pre-infected with barley yellow dwarf virus. Journal of Agricultural Food Chemistry 53:6864–6869.

Matsuura Y, Yoshizawa T. 1981. Effect of food additives and heating on the decomposition of zearalenone in wheat flour. Journal of the Food Hygiene Society of Japan 22:293–298.

Neira MS, Pacin AM, Martinez EJ, Molto G, Resnik SL. 1997. The effects of bakery processing on natural deoxynivalenol contamination. International Journal of Food Microbiology 37:21-25.

Patel S, Hazel CM, Winterton AGM, Mortby E. 1996. Survey of ethnic foods for mycotoxins. Food Additives and Contaminants 13:833-841.

Samar MM, Neira MS, Resnik SL, Pacin A. 2001. Effect of fermentation on naturally occurring deoxynivalenol (DON) in Argentinean bread processing technology. Food Additives and Contaminants 18:1004-1010.

Scudamore KA, Baillie H, Edwards SG, Patel S. 2007. The occurrence and fate of Fusarium mycotoxins during the commercial processing of oats in the UK . Food Additives and Contaminants, 24: 1374-1385.

Scudamore KA, Guy RG, Kelleher B, Macdonald SJ. 2008a. Fate of the fusarium mycotoxins fumonisins B_1, B_2 and B_3, deoxynivalenol and zearalenone in maize flour and maize grits during extrusion cooking. Food Additives and Contaminants 25:1374-1384.

Scudamore KA, Patel S. 2008b. The fate of deoxynivalenol and fumonisins in wheat and maize during commercial breakfast cereal production. World Mycotoxin Journal 1:315-323.

Tanaka T, Hasegawa A, Yamamoto S, Matsuki Y, Ueno Y. 1986. Residues of *Fusarium* mycotoxins, nivalenol, deoxynivalenol and zearalenone, in wheat and processed food after milling and baking. Journal of the Food Hygiene Society of Japan 27:653-655.

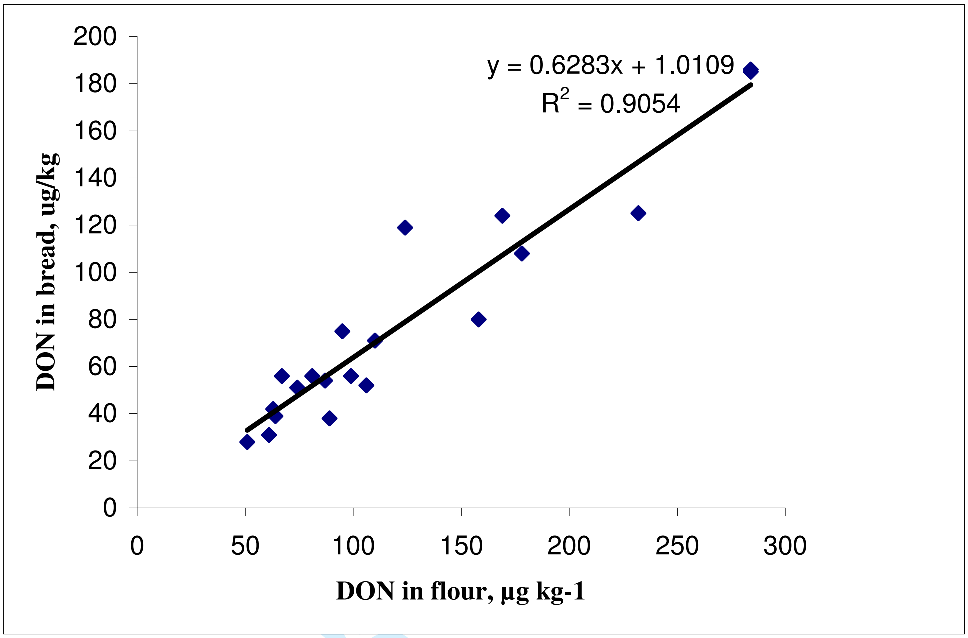

Figure 1: Correlation of the percentage of DON in flour with DON in white and wholemeal bread –all bread results included, results on an 'as is' basis

Table 1. EC, Maximum permissible limits for DON and ZON in wheat. (EC 2006)

Wheat component	Mycotoxin, µg kg^{-1} DON	ZON
Unprocessed wheat (other than durum wheat)	1250	100
Wheat intended for direct human consumption, wheat flour, bran and germ as end product marketed for direct human consumption	750	75
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500	50
Processed cereal-based and baby foods for infants and young children	200	20

Table 2. Change in DON concentration from flour to white bread on an 'as is' basis and on a true basis after correction

	µg kg^{-1}				change, %	
	flour		white bread			
bake no.	"as is"	corrected*	"as is"	corrected*	"as is"	true
1	87	99	54	90	-38	-10
2	89	101	38	63	-57	-38
3	63	72	42	70	-33	-3
4	284	323	185	307	-35	-5
5	284	323	186	309	-34	-4
6	67	76	56	93	-16	+22
7	158	180	80	133	-49	-26
8	61	69	31	51	-49	-26
9	106	121	52	86	-51	-28
10	64	73	39	65	-39	-11
11	169	192	124	206	-27	+7
12	124	141	119	198	-4	+40
13	95	108	75	125	-21	+16
mean					-35	5
range					-4 to -57	+40 to -38

* = dry weight basis

Table 3. Change in DON concentrations from wholemeal flour to wholemeal bread on an 'as is' basis and on a true basis after correction

	µg kg^{-1}				change, %	
	flour		bread			
Bake no.	"as is"	corrected*	"as is"	corrected*	"as is"	true
1	81	92	56	93	-31	+1
2	74	84	51	85	-31	+1
3	51	58	28	46	-45	-20
4	110	125	71	118	-35	-6
5	99	113	56	93	-43	-18
6	178	203	108	179	-39	-12
7	232	264	125	208	-46	-21
mean					-39	-11
range					-31 to -46	+1 to -21

* = dry weight basis

Table 4. Change in DON concentrations from flour to cake on an 'as is' basis and on a true basis after correction.

		μg kg^{-1}				change, %	
		flour		cake			
type	cake run	"as is"	corrected[#]	"as is"	corrected[##]	"as is"	true
1	a	115	131	27	136	-76	+4
1	b	24	27	5[*]	25	≥-79	-7
2	c	75	85	28	141	-63	+168
2	d	51	58	23	116	-55	+203
2	e	17	19	10	50	-41	+279
2	f	19	22	5[*]	25	≥-74	+118
2	g	19	22	5[*]	25	≥-74	+118
1	mean					≥ -77	-1.5
	range					-76 to ≥ -79	+4 to -7
2	mean					≥ -61	+ 177
	range					-41 to ≥ -74	118 to 279

[*] = value used was half the LoQ [#] =corrected on dry weight basis
[##] =correction based on a combined factor of moisture dry weight and on flour being 24% -25% of the ingredients

Table 6. Change in NIV concentrations occurring during the manufacture of biscuits, on an 'as is' basis

commercial (C) experimental (E) or flour blend (B)	biscuit type	NIV, µg kg^{-1} flour	NIV, µg kg^{-1} biscuit	change, %
bake no.				
E 2	semi sweet	31	20	-35
E 3	semi sweet	32	22	-31
Mean				-33
C 5	crackers	11	11	0
E 6	crackers	31	30	-3
C 7	crackers	14	<10	≥ -64
B 8	crackers	20	14	-30
B 9	crackers	40	23	-42
B 10	crackers	23	16	-30
Mean				≥ -28

Table 7. Concentrations of DON in wheat based bread, cakes and biscuits extrapolated to the statutory limit in whole wheat, µg kg^{-1}

	legislation	study results	
	white flour and wholemeal	ex white	ex wholemeal
whole wheat	1250	1250	
flour	750	875*	1250
bread	500	569	762
cake 1	500	236	
cake 2		394	
semi sweet biscuits	500	656	
crackers		779	

* = value obtained in related study (Edwards et al in press)

Table 5: Change in DON and ZON concentrations occurring during the manufacture of biscuits, on an 'as is' basis, μg kg^{-1}

commercial (C) experimental (E) or flour blend (B) bake no.		biscuit type	Mycotoxin, μg/kg DON flour	DON biscuit	DON change, %	ZON flour	ZON biscuit	ZON change, %
C	1	semi sweet	71	62	-13	<3	<3	-
E	2	semi sweet	3830	2390	-38	21.7	17.4	-20
E	3	semi sweet	3480	2630	-24	102	66	-35
Mean					-25			-27.5
C	4	crackers	71	82	+13	<3	<3	-
C	5	crackers	1040	846	-19	<3	<3	-
E	6	crackers	3830	3450	-10	<3	<3	-
C	7	crackers	429	411	-4	<3	<3	-
B	8	crackers	736	665	-10	11.4	11.6	+2
B	9	crackers	904	601	-33	<3	<3	-
B	10	crackers	1120	961	-14	28.8	27.9	-3
Mean					-11			-1