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# *Fusarium* mycotoxins in milling streams from the commercial milling of maize imported to the UK, and relevance to current legislation.

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***Abstract***

A study in three large commercial UK maize mills showed that *Fusarium* mycotoxins such as deoxynivalenol, zearalenone and fumonisins present at mill intake are distributed in milling streams approximately according to their occurrence in the maize seed structure. Fractions derived from the endosperm tended to contain the lowest levels of mycotoxins. Concentrations of mycotoxins within the endosperm are also related to the particle size. However, the products derived from the embryo or outer seed layers contained the highest mycotoxin levels being concentrated up to 5 times or more, although these components are normally used for animal feed or industrial use. The general pattern of mycotoxin distribution found when milling French and Argentinean maize was similar although very variable and it is concluded that this variability stems from different milling strategies used at each mill and from the nature and condition of each consignment of maize.. Mycotoxins in maize grits (particle sizes >500 µm) were usually reduced by the greatest amount when compared to the whole maize, while flour (≤500 µm) could be both reduced or increased

depending on the mill and consignment. Thus in most situations mycotoxin concentrations in whole maize that meet EC legislation on intake should give rise to levels in milled ingredients that should also do so. However this was not always true in some ingredients, especially for fumonisins in those fractions with particle size ≤500 µm.

**Keywords:** *maize, deoxynivalenol, zearalenone, fumonisins, nivalenol, milling, flour, grits, bran*

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## Introduction

Maize often contains a range of mycotoxins that sometimes occur in combination. These include aflatoxins (Andersen et al. 1975), ochratoxin A (Shotwell et al. 1969), a number of *Fusarium* mycotoxins, deoxynivalenol (DON) (Gilbert et al. 1983), nivalenol (NIV) and other trichothecenes (Vesonder et al. 1973), zearalenone (ZON) (Shotwell et al. 1971), fumonisins (Gelderblom et al. 1988), moniliformin (Sharman et al. 1991, Scudamore et al. 1997, 1998) and beauvaricin, fusaproliferin and fumaric acid (Ritieni et al. 1997). In the UK, occurrence of mycotoxins in imported maize has been reported previously during a 10-month period in 2000 (Scudamore and Patel 2000) and more recently from the 2004-2007 harvests (Scudamore and Patel, in press).

In order to protect the consumer, EC legislation relevant to mycotoxins in raw maize and derived products now applies to aflatoxin, ochratoxin A, DON, ZON and

the sum of fumonisins $B_1$ and $B_2$ ($FB_1$ + $FB_2$). Limits have been set within the EC for DON, ZON and fumonisins in maize (EC 2006, EC 2007) and are under consideration for T2-toxin and HT2-toxin.

Fumonisins, first reported in 1988, have been shown to occur widely and sometimes in high concentrations in maize (Shephard et al. 1996) but less commonly and in much lower amounts in other commodities. Legislation for *Fusarium* toxins applies to the unprocessed maize, which includes both in-take maize and whole maize after cleaning. Maize mills use comprehensive cleaning regimes to remove stones, metal objects and other such contaminants and also dust, straw, maize cobs and broken maize seeds. Because mycotoxins are often concentrated in the latter impurities, overall concentrations can be reduced during cleaning although the extent to which this occurs in cereals can be very variable (Brekke et al. 1975, Scott et al. 1984, Abbas et al. 1985, Seitz et al. 1985, Lauren et al. 2006, Scudamore and Patel in press).

The human consumer however is not exposed directly to mycotoxins in raw maize but to the mycotoxins remaining in the food products manufactured after milling and processing of the raw cereal. Maize processing is a complex technology (e.g. Kent and Evers 1994) and the levels of mycotoxins in the finished product may not reflect the concentrations in raw maize as the amounts surviving through the food processing chain will depend on the properties of the mycotoxin and on the nature of the process(es). The distribution and survival of mycotoxins in products obtained by milling and subsequent processing has been reported but often in a piecemeal fashion and rarely at full commercial production scale. Such studies show that mycotoxin concentrations can vary considerably in different mills.

This paper reports how mycotoxins present in whole maize are distributed in the milling streams obtained in typical commercial UK maize mills and forms part of a more extensive project studying the fate of *Fusarium* mycotoxins occurring during cereal processing. The occurrence of mycotoxins in the maize samples studied here, including the effects of cleaning, has already been reported (Scudamore and Patel 2008a), while their fate during processing is to be reported elsewhere (e.g. Scudamore and Patel 2008b). These studies should assist industry in managing the EC regulations for mycotoxins and provides data to the UK Government and to the EC to assist with assessing current legislation and setting future legislation as necessary.

## Materials and methods

*Maize and milling streams*

82 consignments of cleaned maize collected during the period 2004-2007 from three major UK maize mills were studied following normal commercial milling practice at each mill. The commercially important fractions for food production are the different grades of grits and flour. Thus the flaking grit stream was collected from each consignment of Argentinean maize because this is the ingredient used for cornflake manufacture and the flour from each French maize consignment as the important ingredient in many foods such as snack products. For approximately one in every three consignments, all the main milled fractions were collected to include the flour, polenta (flour), flaking grits, coarse grits, fine grits, grits recovered by reprocessing bran, broken maize, germ, meal, bran and so on.

## Sampling and analysis

*Sampling at maize mills*

Sampling followed the principles described previously for raw and cleaned maize resulting in bulk samples of several kilograms (Scudamore and Patel, 2008a). Samples collected were kept cool and dry out of direct sunlight before sending to the analytical laboratory. These were examined over an approximate 3-year period spanning four different harvest years. The quantity of sample taken was 4 to 10 kg depending on the agreed method of sampling used (2008a) and the mill. Each operated on a different scale, that milling Argentine grain milled on a very large scale, while the throughput of the smaller of the two French mills operated with a much smaller throughput. Each aggregate sample was well mixed and a sub sample sent for analysis. On receipt, the whole sample was ground to pass through a 0.8 mm screen and mixed for 30 minutes in a Gardner horizontal mixer to ensure homogeneity. Samples were stored at -20°C if not analysed immediately.

All mills have in-house sampling protocols at in-take to their mills and supplied samples based on the internal quality control system in place, or adaptations thereof. Mills 1 and 2 sampled 26 and 30 consignments respectively of French maize, supplied by lorry. Mill 3 supplied 30 consignments of raw Argentinean maize discharged from 26 different ships.

***Analysis***

DON (and other related trichothecene mycotoxins) was determined following the method of Patel *et al*. 1996 as used by Scudamore *et al*, 2007. Ground 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 method for fumonisins was a modification of the method of Shephard *et al.* 1990 as used by Scudamore and Patel 2000. A ground sample (25 g) was extracted with 100 ml acetonitrile:water (50:50) and filtered. After adjusting an aliquot of the filtrate to pH 5.8 - 6.5 it was cleaned-up using a Bond-Elut strong anion exchange (SAX) cartridge. The cartridge was washed with methanol:water (75:25), methanol and finally the fumonisins were eluted with 10 ml 1% acetic acid in methanol. The eluate was evaporated to dryness and re-dissolved in acetonitrile:water (50:50). The fumonisins were determined using HPLC with fluorescence detection after forming the ortho-ophthaldialdehyde derivatives.

*Recovery, Limit of Detection and Determination*

All analyses were conducted with a spiked sample, i.e. a known amount of toxin was added to a sample of ground wheat or maize each day prior to extraction, clean-up and HPLC determination for each batch of 1-5 samples. 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 spike level was 200 µg/kg for DON, 50 µg/kg for ZON and 400 µg/kg for each fumonisin.

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 was then spiked at 400 µg/kg. 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 of ZON was spiked at 53 µg/kg. Typical mean recovery was 88% with a coefficient of variability of 7.2 % for 10 replicates.

The limit of detection and limit of determination respectively for each mycotoxin were 5 µg/kg and 10 µg/kg for DON and each fumonisin mycotoxin and 1.5 µg/kg and 3 µg/kg 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, 3 and 10 µg/kg for DON, ZON and each fumonisin. After analysis samples were retained and stored at -20°C.

**Comment [K1]:** This is correct

*Method validation and quality control*

UKAS accreditation ensures that methods are valid for the tasks they are performing, but do not provide an absolute measure of accuracy. Under the Laboratory Quality System a protocol for internal and external proficiency test schemes participation is defined and the laboratory demonstrates on-going precision and accuracy. This laboratory analysis uses in-house reference material, participation in sample testing schemes such as FAPAS (www.fapas.co.uk/fapas.cfm) and Certified Reference Materials (CRM's), intermittently. Acceptable results have been achieved in the FAPAS Scheme for DON, ZON and $FB_1$ and $FB_2$ in cereals during rounds during the past 8 years.

## Results and Discussion

*Background to maize milling technology*

Approximately 1.3 million tonnes of maize are imported into the UK each year for food related purposes, with around 40% of the total being used in UK food production. Maize is both wet-milled and dry milled although the major process is wet milling to produce starch. Imported French maize has an endosperm that produces flour which is suitable for a whole range of food products while Argetinean Flint maize produces grits ideally suited for cornflake manufacture. Dry milling produces milling streams that are the ingredients for a range of food products while co-products are used for animal feed or other industrial purposes.

**INSERT FIGURE 1**

Figure 1 is a schematic diagram that indicates from where the main milled maize fractions originate. The outer layer of a maize seed is the pericarp from which bran is derived, and this encloses the endosperm and a relatively large embryo. The endosperm is composed of both horny parts that contribute most of the large particulate grits (B and C) and softer parts that readily break down to flour of finer composition (D, E and F). The embryo (G) contains high oil content around 20% and does not contribute to the yield of grits. Maize meal, H, can be very variable in composition and for example may consist of any of the materials removed during the process that are not desired in the food products e. g. germ, stalks, bran and pieces of endosperm with some germ still attached. Thus the mycotoxin concentrations found in the milling streams relate principally to how they are originally formed and distributed in the unground individual seeds.

An important initial stage in the maize milling process after cleaning is to de-germ the maize and the efficiency with which this can be carried out affects the yield and composition of the grits produced. As mycotoxins are associated with different parts of cereal seeds the distribution into the milling streams will vary considerably with batch and mill. Complex milling systems also result in overlap between the different fractions. The feed product for example may contain both the bran from the pericarp, extracted germ from the embryo, and some endosperm. The precise composition of the different products can thus vary from mill to mill or mill configurations can be changed on demand to alter the nature of the raw ingredients produced. The use of particle size at least in relation to legislation is thus helpful in attempting to simplify a complex situation. Thus statutory levels can be applied to products with particle size >500 µm and those with particle size ≤500 µm together and to with fractions used for animal feed, see table 1

The products of highest value are grits, which are used to produce breakfast cereals such as cornflakes while maize 'flour', is the basis of many foods and of maize-based snack products. The terminology used to describe the products from dry maize milling is often unclear to users in different countries and comparison of data for the concentrations of mycotoxins in milled maize products is difficult and sometimes conflicting. Recently the EC agreed that maize fractions can be usefully considered on the basis of their particle size and this has simplified comparison of such data. Table 1 gives the current EC limits for *Fusarium* mycotoxins in maize and products derived from maize. Only guideline limits exist for animal feed and the values of these are included for reference for the most sensitive species Legislation is now given partly on the basis of particle size and the table gives examples of the milling streams falling within these ranges. 'Polenta' emphasises the complexity of

the situation where polenta meal is a corn meal, in which case it is not pre-cooked and thus not for human consumption. Depending on particle size range its maximum allowed levels of fumonisin for example could be 1400 µg $kg^{-1}$ or 2000µg $kg^{-1}$. If however, it is pre-cooked/pre-boiled polenta corn meal that can be consumed directly as such as polenta, the level of 1000 µg $kg^{-1}$ is applied.

**INSERT TABLE 1**

Products referred to as bran, germ, meal or mixtures of these can thus vary enormously in the parts and proportions of the maize seed incorporated but in practice, most of these fractions are used for animal feed or other industrial uses and so for practical purposes can be considered together.

Milled products from maize are used in a wide range of processes to give the range of foods consumed by the public. The fate of mycotoxins from raw maize to consumer product thus depends on two distinct phases, the redistribution during milling which is a physical process and changes occurring during food processing. These changes will depend on the chemical properties of the mycotoxin, the conditions of processing including temperature, pressure, moisture, pH and enzyme activity, or dilution effects occurring because of the inclusion of other components.

Small scale or pilot scale studies of cereals mimicking industrial situations do not always reflect accurately what happens in the full commercial situation as for example operating scale, mill set up and commercial product will differ from mill to mill.

**INSERT FIGURE 2**

Previous studies of the distribution of mycotoxins from raw cereals into milling streams have shown in most instances that there is a reduction in concentration in the endosperm-derived milling streams and a concentration in the products derived by the outer layers of the grain seeds (Scudamore 2004).

The distribution pattern of mycotoxins in each maize milling streams produced at the three mills studied here is shown in Figures 2-4. The concentrations of mycotoxins found in the grits and flours, which are mostly derived from the endosperm typically, contain the lowest mycotoxin levels and concentrations are further closely related to particle size (see Table 1). Thus flaking and coarse grits (those with the largest particles) are much reduced in mycotoxin concentration compared with the raw maize, while flour (containing the finest particles) has higher levels. This can be seen clearly e.g. in figure 3 where the mycotoxin concentrations increase from being lowest in the coarse grits, through fine grits, polenta flour and flour as particle size is reduced. However, all these milling streams are much lower in mycotoxins than the bran/meal/germ-derived fractions in which they are concentrated.

This pattern is the same at all 3 mills although the three grit products from mill 3 which used Argentinean flint maize have the lowest mycotoxin levels in the grits of all relative to the intake maize. The proportion of DON found in grits (particle size >500 µm) tended to be higher than the fumonisins while the situation was reversed in flour so that a higher proportion of the fumonisins compared to DON were found in flour (≤500 µm particles). The situation for ZON was unclear, as this was only found intermittently throughout the study and usually in low concentrations in raw maize so could not be detected in a significant number of grit samples.

**INSERT FIGURE 3**

During the study, one 'grit' product (bran grits) from mill 2 did not fit the expected pattern as it contained much higher levels of mycotoxins (particularly fumonisins) despite being composed of particles of a size similar to other grits. In addition, DON, ZON and NIV (not shown in figure 3) were much higher than expected in grits but were still considerably less so than the fumonisins. This product was produced by re-processing bran to extract residual grit particles.

**INSERT FIGURE 4**

The milled products most used for the manufacture of human foods are the flour and grits of various grades as discussed previously. The number of commercial consignments in which the change in mycotoxin concentration from whole maize to ingredient were measured is given in the title for each table. Mycotoxin levels were both reduced and increased in flour compared with the intake maize and the proportions were different in each mill. French maize was examined at mills 1 and 2 and Argentinean flint maize at mill 3. It is likely that these differences are due not only due to the maize type, history and condition of the maize of each consignment but also to different machinery configurations set at each mill. Hard flint maize produces a larger yield of large flaking grits that reduces the yield of flour while the softer varieties of French maize result in a smaller proportion of coarse grits and a higher yield of flour.

The efficiency of the initial de-germing stage also determines the yields of flaking grits and flour. The range of relative amounts of mycotoxins in individual consignments of raw maize and flour processed were also very variable so that sometimes concentrations were increased in the flour while on other occasions

reduced, even in a single mill. The number of consignments examined at each mill was sufficient to be confident that there is a real difference between mills. Each mill also produces a variety of different milled products varying in their individual properties and nature so that differences between mills should not be unexpected.

**INSERT TABLE 2**

Tables 2-4 show the mean, median and range of concentrations of each mycotoxin in the whole maize and flour and also the mean, median and range of values found for the proportion of mycotoxins in the maize flour after milling compared with that in intake maize. Fumonisins were present in virtually every sample in contrast to DON and particularly ZON where concentrations were lower and less frequent. As a result, the number of times when it was possible to determine the change from intake maize to flour varied and this is given by 'n' for each mycotoxin. The % change in concentration for each mycotoxin transferred to the flour is very variable as shown by the values for % SD in Tables 2-5. Within each mill these values are quite similar for the mycotoxins and this perhaps suggests that the variability is in large part due to sampling as the coefficients of variability for the analytical methods are small in comparison. The least variable results were obtained from mill 1 which operates on a much smaller scale than mills 2 and 3 and sampling the milling streams was much easier.

Applying a paired T-test to each of the pairs of mycotoxins DON/$FB_1$, DON/ZON and ZON/FB1 showed that there was no significant difference in the relative concentrations in intake maize and flour for each mycotoxin in the two larger mills, 2

(p=0.8, 0.7 and 0.8) and mill 3 (p= 0.4, 0.7 and 0.7). In the smallest mill 1, there was a significant difference between DON and $FB_1$ but not between DON and ZON (p = <0.05, 0.7 and 0.22). When the relative concentration of each mycotoxin between intake maize and flour were compared between mills the difference was significant between the smaller mill 1 and the larger mills 2/3 (p for each = <0.05, <0.05 and <0.05 for DON, ZON and $FB_1$) but somewhat less so at least for DON and ZON between the two larger mills (p= 0.3, 0.3 and <0.05 for DON, ZON and $FB_1$).

EC legislation has set 1400 µg $kg^{-1}$ as the limit for $FB_1$+$FB_2$ for processed maize with particle size >500 micron whereas the limit for unprocessed maize is 4000 µg $kg^{-1}$. In addition there is a limit of 2000 µg $kg^{-1}$ for processed maize of particle size </= 500 micron that includes maize flour. However on the basis of the mean change calculated from all consignments at each site, this required reduction for flour was not achieved in any of the mills. In mills 2 and 3, Tables 3 and 4, the mean figures showed increased concentration in the flour. It has been speculated that the amount remaining in the various milled fractions might also depend on the actual concentration in the raw maize so that a larger reduction occurs at higher concentrations although it is difficult to find firm evidence for this.

**INSERT TABLE 3**

This relationship is examined in Figure 5 for each mill by plotting the flour : raw maize concentration ratio of $FB_1$ +$FB_2$ using the mean of the highest 5 concentrations, the mean of the highest 10 concentrations and mean of all samples against the ratio of flour : raw maize concentrations. This indicates clearly that the relative amount of $FB_1$+$FB_2$ in flour decreases with higher intake contamination. However even in mill 3 where the five highest intake concentrations in maize consignments gave a mean concentration of 2873 µg $kg^{-1}$, the value of 0.75 would still result in a level of 2155

µg $kg^{-1}$ $FB_1$ +$FB_2$ in maize exceeding the 2000 µg $kg^{-1}$ needed to meet legislation. From the buyer's and the miller's viewpoint, this means that either stricter purchasing requirements must be enforced for the maximum concentrations of fumonisins acceptable, that the subsequent maize flour must be monitored closely or its further use carefully considered.

**INSERT TABLE 4**

A rather lower proportion of DON, ZON and NIV were found in flour than for the fumonisins. In addition, the concentrations of these mycotoxins found usually occurred well below the EC limits, so that if transfer of mycotoxins to flour are less at higher concentrations, any problem appears much less for the fumonisins. During the period of this study no sample approached the legislative limits for these other mycotoxins.

The mycotoxin concentrations remaining in flaking grits after maize milling at mill 3 that uses Argentinean Flint maize, are given in Table 5. The mycotoxin concentrations remaining in flaking grits are much lower than in flour. Thus the mean reduction for all consignments for $FB_1$ +$FB_2$ is 93%. In contrast to the regulations pertaining to flour this is a much greater reduction than is anticipated by the limits set and should present no problem for the miller. Using the approach as for flour by taking the mean of the 5 and 10 most contaminated samples and the mean of all, showed that the reduction achieved for the more contaminated maize increased to 94 % and 95.5 % respectively for the 10 and 5 most highly contaminated samples respectively suggesting that the reduction was again related to concentration although it is not clear whether these small changes are statistical significant (figure not given).

**INSERT TABLE 5**

*Fusarium* mycotoxins other than DON, ZON, NIV and fumonisins were sometimes detected in intake maize. These were low levels of 3-Ac DON, 15-Ac DON, fusarenone-X, HT2 and T2 although T2 and HT2 were never detected in Argentinean maize. When these trace concentrations were found in intake maize, their presence was confirmed after milling by the higher levels in the milling streams such as bran, meal and germ in which mycotoxins are concentrated. 3- and 15- Ac-DON and FUS-X when detected usually correlated to the concentration of DON present.

Because the distribution of mycotoxins into different milling streams from different consignments is very variable, % SD values being typically around 50%, accurate predictive models are extremely difficult to design especially as the distribution pattern of the mycotoxins in the milling streams is related to some extent to the mycotoxin concentration in the raw maize so that care must be taken in extrapolation from one level to another. Variability occurs as the result of many factors such as analysis and sampling. These are well recognised but there are also many other factors that could include the variability in the physical structure and chemical composition of the maize seeds resulting from different growing conditions; temperature, rainfall and drought, inadvertent loss of contaminated dust or physical damage to seeds during transport, handling and storage, variety or to changes in milling machinery settings in order to maximise the production of required maize ingredients.

**INSERT FIGURE 5**

**Conclusions**

Universally applicable predictive models are difficult to design because so many factors influence the mycotoxin distribution pattern in milling streams. However, some relationships are consistent in the mills covered by this study so that maize grits always contain reduced levels of all the mycotoxins studied, and mycotoxins were always concentrated in the feed components such as the maize germ/meal/bran, broken maize and similar products. Mycotoxin concentrations in maize flour could be similar to, reduced or somewhat increased compared with those present in the raw maize. It is concluded that if whole maize complies with legal limits, maize users should in most situations be able to meet those set for maize ingredients destined for food purposes although this might not always be achievable particularly for fumonisins in products with particle size ≤500 micron such as flour

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**References**

Abbas HK, Mirocha CJ, Pawlosky RJ and Pusch DJ. 1985. Effect of cleaning, milling and baking on deoxynivalenol in wheat. Applied Environmental Microbiology 50:482-486.

Anderson HW, Nehring EW, Wichser, WR. 1975. Aflatoxin contamination of corn in the field. Journal of Agricultural Food Chemistry 23:775-782.

Brekke OL, Peplinski AJ and Griffin EL Jr. 1975. Cleaning trials for corn containing aflatoxin. Cereal Chemistry 52:198-204.

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.

European Commission, 2006a, Commission RECOMMENDATION (EC) No 2006/576, of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding Official Journal of the European Union. L229: 7-9.

European Commission, 2007, Commission Regulation (EC) No 1126/2007, of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Official Journal of the European Union L255: 14-17.

Gelderblom WCA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vleggaar R, Kriek NPJ. 1988. Fumonisins -novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme.* Applied and Environmental Microbiology 54:1806-1811.

Gilbert J, Shepherd MJ, Startin JR. 1983. A survey of the occurrence of the trichothecene mycotoxin deoxynivalenol (vomitoxin) in United Kingdom grown barley and in imported maize by combined gas chromatography-mass spectrometry. Journal of the Science of Food and Agriculture 34:86-92.

Kent NL, Evers, AD. 1994. *Kents Technology of Cereals*, 4th Edition (Oxford: Elsevier Science Ltd), 334 pages.

Lauren DR, Jensen DJ, Smith WA. 2006. Mycotoxin contamination in graded

fractions of maize (*Zea mays*) in New Zealand. New Zealand Journal of Crop and Horticultural Science 34: 63–72.

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

Patel S, Hazel CM, Winterton AGM, Gleadle AE. 1997. Survey of fumonisins in maize and maize products. Food Additives and Contaminants 14:187-191.

Ritieni A, Moretti A, Logrieco A, Bottalico A, Randazzo G, Monti SM, Ferracane R. Fogliano V. 1997. Occurrence of Fusaproliferin, Fumonisin $B_1$, and Beauvericin in Maize from Italy. Journal of Agricultural Food Chemistry 45:4011–4016.

Scott PM, Kanhere SR, Dexter JE, Brennan PW and Trenholm HL. 1984. Distribution of the trichothecene mycotoxin deoxynivalenol (vomitoxin) in hard red spring wheat. Food Additives and Contaminants 1:313-323.

Scudamore KA,. 2004. Control of mycotoxins: secondary processing. In Mycotoxins in Food; Detection and Control, .Edited by N. Magan and M.Olsen., Woodhead Publishing Ltd: Cambridge 224-243.

Scudamore KA, Hetmanski MT, Chan HK, Collins S. 1997. Occurrence of mycotoxins in raw ingredients used for animal feeding stuffs in the United Kingdom in 1992. Food Additives and Contaminants, 14:157-173.

Scudamore KA, Nawaz S, Hetmanski MT. 1998. Mycotoxins in ingredients of animal feeding stuffs: II. Determination of mycotoxins in maize and maize products. Food Additives and Contaminants 15:30-55.

Scudamore, KA, Patel S. 2000. Survey for aflatoxins, ochratoxin A, ZON and fumonisins in maize imported into the United Kingdom. Food Additives and Contaminants, 17:407-416.

Scudamore KA, Patel S. 2008a. Occurrence of fusarium mycotoxins in maize imported into the United Kingdom, 2004-7. Food Additives and Contaminants, 25: 000-000 .(

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

Seitz LM, Yamazaki WT, Clements RL, Mohr HE and Andrews L. 1985. Distribution of deoxynivalenol in soft wheat mill streams. Cereal Chemistry 62:467-469.

Sharman M, Gilbert J, Chelkowski J. 1991. A Survey of the occurrence of the mycotoxin moniliformin cereal samples from sources world-wide. Food Additives and Contaminants 8: 459-466.

Shephard GS, Thiel PG, Stockenström S, Sydenham, EW. 1996. World-wide survey of fumonisin contamination of corn and corn--based products. Journal of the Association of Official Analytical Chemists International 79: 671-687.

Shephard, GS, Sydenham EW, Thiel PG, Gelderblom WCA. 1990. Quantitative determination of fumonisin $B_1$ and $B_2$ by high-performance liquid chromatography with fluorescence detection. Journal of Liquid Chromatography 13:2077-2087.

Shotwell OL, Hesseltine CW, Goulden ML, Vandegraft EE. 1971. Survey of corn for aflatoxin, zearalenone, and ochratoxin. Cereal Chemistry 47: 700-707.

Shotwell OL, Hesseltine CW, Goulden ML. 1969. Ochratoxin A: Occurrence as a natural contaminant of a corn sample. Applied Microbiology 17:765-766.

Thompson M, Wood R. 1995. Guidelines on Internal Quality Control in Analytical Chemistry Laboratories. Pure and Applied Chemistry 67: 649-666.

Vesonder RF, Ciegler A, Jensen AH. 1973. Isolation of the emetic principle from

496 Fusarium-infected corn. Applied Microbiology 26:1008-1010.

Table 1. EC, Maximum Permissible limit for mycotoxins in maize, (EC 2006, EC 2007), related to product examples

| | Mycotoxin, µg kg$^{-1}$ | | | Milling stream |
|---|---|---|---|---|
| | DON | ZON | $FB_1+FB_2$ | examples |
| Unprocessed maize with the exception of that intended to be processed by wet milling | 1750 | 350 | 4000 | intake and cleaned maize |
| Milling fractions of maize with particle size >500 µm falling within CN code 1103 13 or 1103 20 40 | 750 | 200 | 1400 | coarse grits, flaking grits |
| Milling fractions of maize with particle size ≤500 µm falling within CN code 1102 20 | 1250 | 300 | 2000 | flour, polenta flour/meal, fine grits |
| Maize or maize-based foods intended for direct human consumption, (with the exception of the 2 categories below) | 750 | 75 | 1000 | pre-cooked/pre-boiled polenta meal |
| Breakfast cereals, etc. | 500 | 100 | 800 | finished products |
| Processed cereal based foods for infants and young children | 200 | 20 | 200 | finished products |
| Animal feed* | 900[a] | 100[b] | 5000[c] | bran, meal, germ, |

* = guideline limits depend on feed/animal. Lowest guideline values cited here (see EC, 2006a for all limits)

[a] = complementary and complete feedingstuffs for pigs,

[b] = complementary and complete feedingstuffs for piglets and gilts

[c] = complementary and complete feedingstuffs for pigs, horses (*Equidae*), rabbits and pet animals

CN= Custom Tariff Code

Table 2: Mycotoxin content of maize flour milled from French maize at mill 1 2004-2007 compared with that in intake maize, n = 25 (DON), n = 12 (ZON), n = 29 ($FB_1$), n = 28 ($FB_2$), n =23 ($FB_3$), n = 29 ($FB_1$+$FB_2$), n = 11 (NIV)

| | | mycotoxin concentration, µg $kg^{-1}$ | | | relative % in flour | | | |
|---|---|---|---|---|---|---|---|---|
| mycotoxin | sample | mean | median | range | mean | median | range | SD, % |
| DON | intake | 139 | 63 | <10-444 | 45 | 40 | 14-85 | 45.0 |
| | flour | 58 | 31 | <10-290 | | | | |
| ZON | intake | 16 | <3 | <3-86 | 43 | 34 | 2-84 | 54.3 |
| | flour | 5 | <3 | <3-24 | | | | |
| $FB_1$ | intake | 320 | 267 | <10-1110 | 75 | 67 | 35-134 | 40.9 |
| | flour | 234 | 215 | 16-654 | | | | |
| $FB_2$ | intake | 80 | 57 | <10-269 | 78 | 71 | 32-177 | 40.5 |
| | flour | 58 | 48 | <10-167 | | | | |
| $FB_3$ | intake | 49 | 30 | <10-180 | 90 | 86 | 46-141 | 31.4 |
| | flour | 40 | 31 | <10-133 | | | | |
| $FB_1$+$FB_2$ | intake | 400 | 324 | <10-1279 | 74 | 67 | 34-143 | 41.2 |
| | flour | 292 | 263 | <10-821 | | | | |
| NIV | intake | 40 | 28 | <10-134 | 43 | 38 | 17-77 | 37.2 |
| | flour | 13 | 5 | <10-55 | | | | |

n= number of pairs on which relative % values are calculated

Table 3: Mycotoxin content of maize flour milled from French maize at mill 2 2004-2007 compared with that in intake maize, n = 24 (DON), n = 19 (ZON), n = 24 ($FB_1$), n = 22 ($FB_2$), n =19 ($FB_3$), n = 24 ($FB_1$+$FB_2$)

| | | mycotoxin concentration, µg $kg^{-1}$ | | | relative % in flour | | | |
|---|---|---|---|---|---|---|---|---|
| mycotoxin | sample | mean | median | range | mean | median | range | SD, % |
| DON | intake | 271 | 254 | 19-932 | 60 | 40 | 22-219 | 63.0 |
| | flour | 182 | 172 | <10-754 | | | | |
| ZON | intake | 37 | 23 | <3-165 | 81 | 64 | 17-225 | 60.9 |
| | flour | 25 | 19 | <2-114 | | | | |
| $FB_1$ | intake | 541 | 439 | 14-2590 | 111 | 104 | 34-336 | 58.7 |
| | flour | 488 | 337 | <10-1530 | | | | |
| $FB_2$ | intake | 155 | 120 | <10-811 | 113 | 107 | 37-348 | 58.5 |
| | flour | 147 | 91 | <10-463 | | | | |
| $FB_3$ | intake | 90 | 67 | <10-501 | 119 | 106 | 50-350 | 55.8 |
| | flour | 86 | 60 | <10-293 | | | | |
| $FB_1$+$FB_2$ | intake | 696 | 543 | 19-3401 | 109 | 104 | 26-310 | 59.0 |
| | flour | 653 | 428 | <10-1993 | | | | |
| NIV | intake | 31 | 22 | <10-170 | 75 | 70 | 27-156 | 53.0 |
| | flour | 21 | 11 | <10-82 | | | | |

n= number of pairs on which relative % values are calculated

Table 4: Mycotoxin content of maize flour milled from Argentine maize at mill 3 2004-2007 compared with that in intake maize, n = 15 (DON), n = 9 (ZON), n = 3 ($FB_1$), n = 13 ($FB_2$), n =13 ($FB_3$), n = 13 ($FB_1$+$FB_2$), n = 7 (NIV)

| | | mycotoxin concentration µg $kg^{-1}$ | | | relative % in flour | | | |
|---|---|---|---|---|---|---|---|---|
| mycotoxin | sample | mean | median | range | mean | median | range | SD, % |
| DON | intake | 89 | 74 | 16-220 | 92 | 79 | 27-188 | 61.5 |
| | flour | 68 | 51 | 22-132 | | | | |
| ZON | intake | 15 | 10 | <3-42 | 127 | 107 | 44-263 | 50.4 |
| | flour | 15 | 7 | <3-101 | | | | |
| $FB_1$ | intake | 1426 | 1405 | 345-3813 | 131 | 99 | 39-288 | 56.6 |
| | flour | 1405 | 1510 | 228-2810 | | | | |
| $FB_2$ | intake | 484 | 408 | 101-1230 | 123 | 113 | 34-244 | 55.0 |
| | flour | 440 | 493 | <10-701 | | | | |
| $FB_3$ | intake | 249 | 229 | 74-711 | 185 | 132 | 49-354 | 61.3 |
| | flour | 385 | 361 | 37-706 | | | | |
| $FB_1$+$FB_2$ | intake | 1990 | 1661 | 505-5002 | 129 | 102 | 34-278 | 56.3 |
| | flour | 1846 | 2003 | 612-3511 | | | | |
| NIV | intake | 53 | 13 | <10-496 | 65 | 45 | 6-118 | 69.6 |
| | flour | 28 | 8 | <10-131 | | | | |

n= number of pairs on which relative % values are calculated

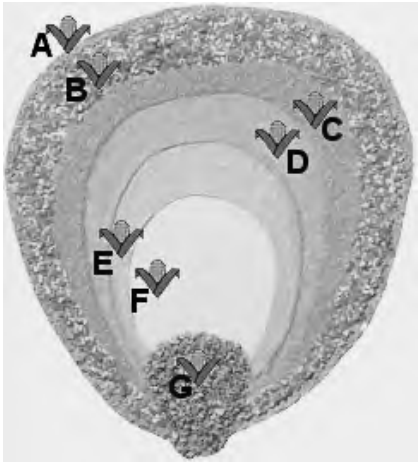
Table 5: Reduction in mycotoxin concentration occurring in preparation of flaking grits from Argentinean flint maize in mill 3, n = 8 (DON), n = * (ZON), n = 19 ($FB_1$), n = 19 ($FB_2$), n =19 ($FB_3$), n = 19 ($FB_1$+$FB_2$)

| | | mycotoxin concentration, µg $kg^{-1}$ | | | Reduction in grits, % | | | |
|---|---|---|---|---|---|---|---|---|
| mycotoxin | sample | mean | median | range | mean | median | range | SD, % |
| DON | intake | 89 | 74 | 16-220 | 73 | 83 | 3-93 | 113.6 |
| | grits | 13 | 5 | <10-87 | | | | |
| ZON | intake | 15 | 10 | <3-42 | >83* | >88* | >65-96* | - |
| | grits | * | * | * | | | | |
| $FB_1$ | intake | 1426 | 1405 | 345-3813 | 93 | 93 | 86-98 | 47.4 |
| | grits | 92 | 90 | 28-222 | | | | |
| $FB_2$ | intake | 484 | 408 | 101-1230 | 94 | 95 | 82-98 | 61.5 |
| | grits | 24 | 23 | <10-72 | | | | |
| $FB_3$ | intake | 249 | 229 | 74-711 | 92 | 92 | 79-98 | 59.4 |
| | grits | 15 | 15 | <10-27 | | | | |
| $FB_1$+$FB_2$ | intake | 1990 | 1661 | 505-5002 | 93 | 93 | 85-98 | 49.4 |
| | grits | 116 | 115 | 33-206 | | | | |
| NIV | intake | 53 | 13 | <10-496 | >70 | >64 | >50-97 | - |
| | grits | 57 | 13 | <10-31 | | | | |

* mean concentration of ZON in flaking grits below limit of detection in all samples except one. Values based on those samples where ZON was detected in whole maize compared to half the limit of detection in grits to enable the least value of reduction to be calculated

n= number of pairs on which reduction values are calculated

534
535




A= bran B= flaking grits C= coarse grits
D= fine grits E= polenta F= flour
G= germ H= maize meal (not labelled as it is of variable composition)

Figure 1: schematic view of a maize seed. Origin of different milled products

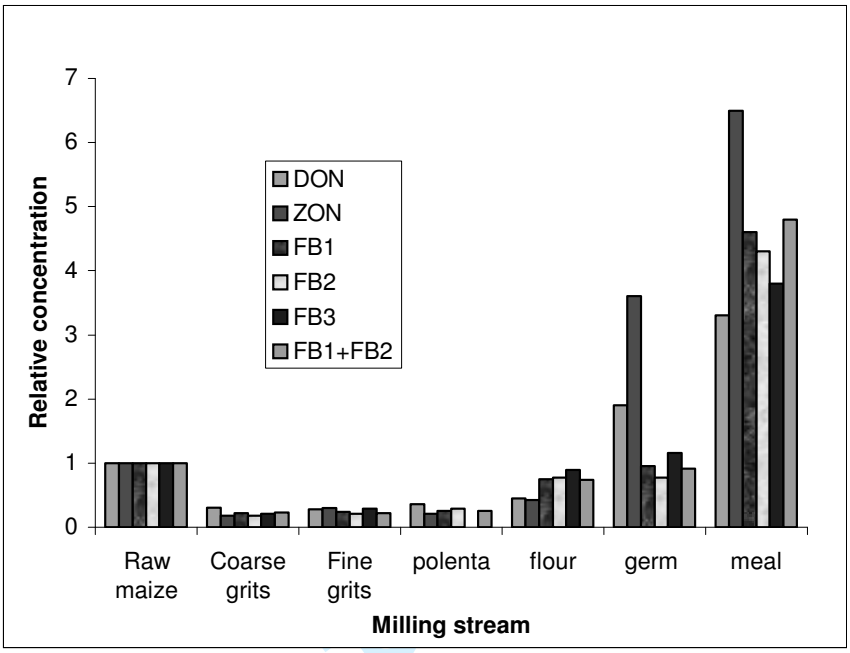



Figure 2: Relative amount of mycotoxins in each milling stream compared to whole maize (=1) in French maize used at Mill 1.

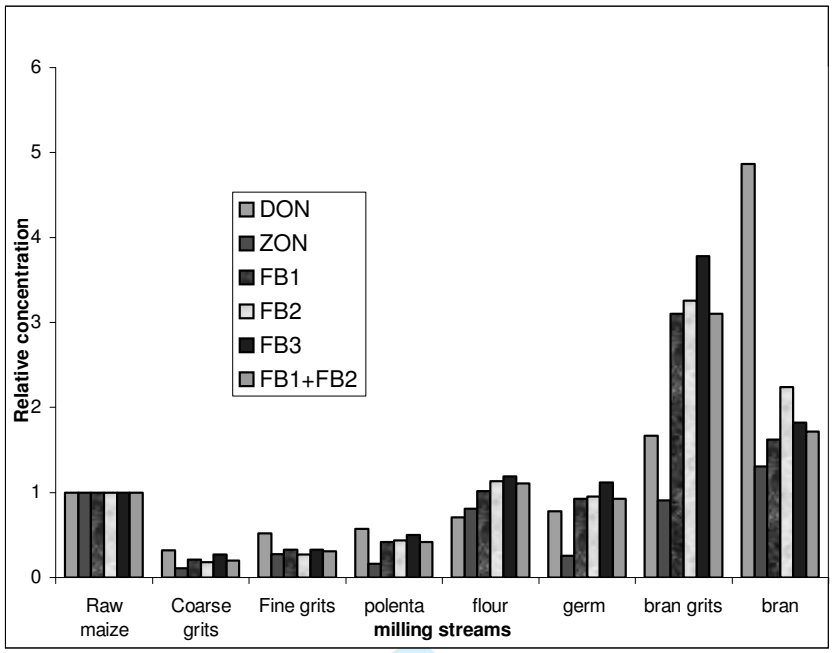



Figure 3: Relative amount of mycotoxins in each milling stream compared to whole maize (=1) in French maize used at Mill 2.

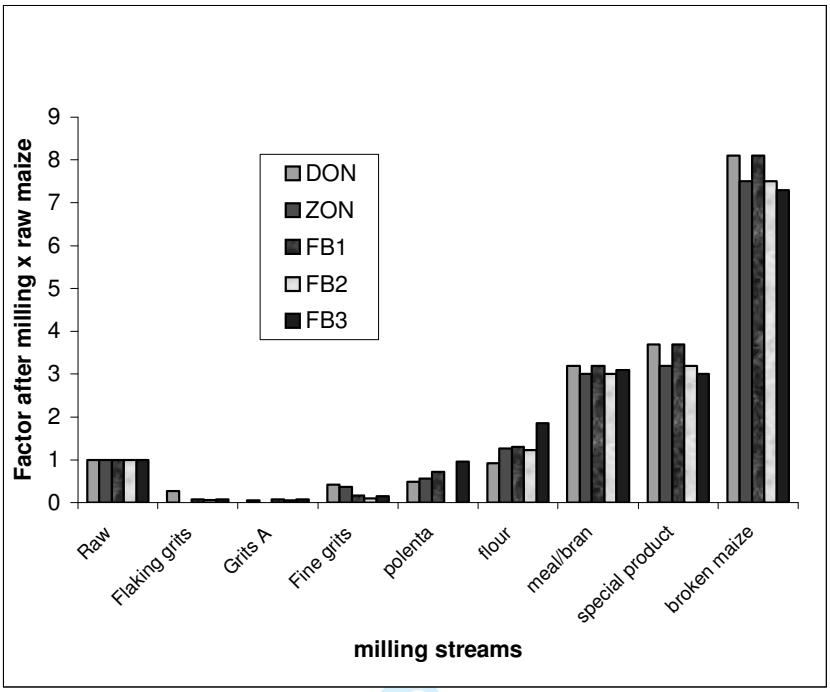



Figure 4: Relative amount of mycotoxins in each milling stream compared to whole maize (=1) in Argentinean Flint maize used at Mill 3.

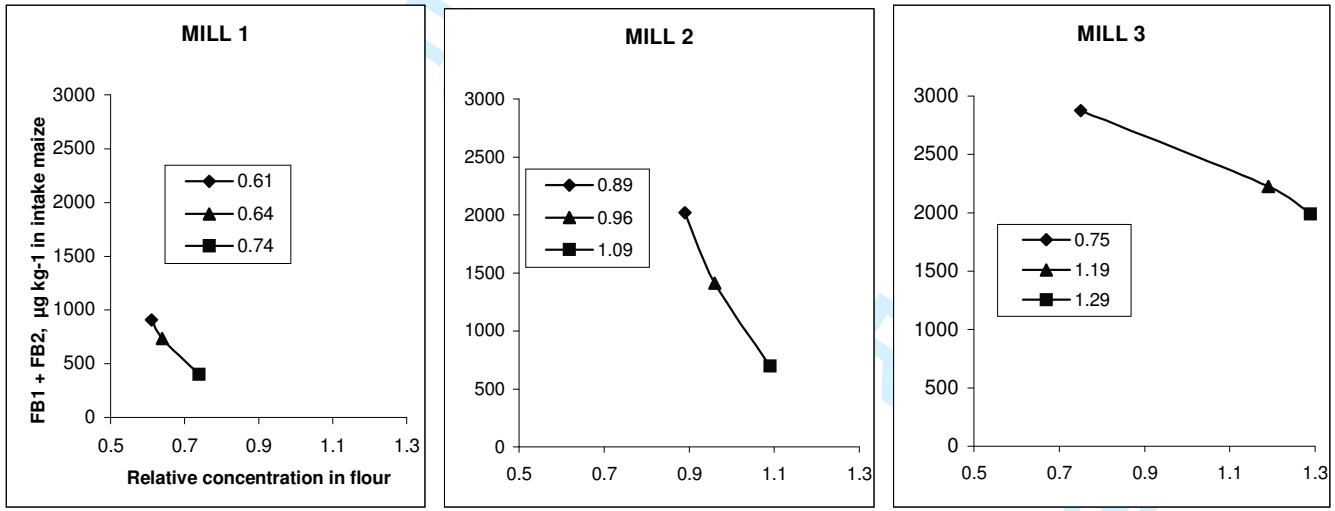



Figure 5: Change in the proportion of $FB_1 + FB_2$ with concentration in intake maize ◆ = based on consignments with the 5 highest concentrations in intake maize , ▲= based on 10 highest, ■ = based on all consignments