Distribution of Fusarium mycotoxins in UK wheat mill fractions

Simon G Edwards, Keith Scudamore, Susan Macdonald, Sue Patel, Clare Hazel, Dereck Buttler, Edward Dickin

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The EU has set maximum limits for the Fusarium mycotoxins deoxynivalenol (DON) and zearalenone (ZON). The maximum permitted level decreases from unprocessed wheat, through intermediary products e.g. flour, to finished products such as bakery goods and breakfast cereals. It is therefore important to understand the effects of processing on the mycotoxin distribution in mill fractions. Between 2004 and 2007, samples were taken at commercial flour mills at various points in the milling process and analysed for trichotheccenes and ZON. Samples with a range of mycotoxin concentrations harvested in 2004 and 2005 were processed in a pilot mill and the mycotoxins in the different mill fractions quantified. In the commercial samples, DON was the predominant mycotoxin with highest levels detected in the bran fraction. Analysis of the pilot mill fractions identified a significant difference between the two years and between mycotoxins. The proportion of DON and nivalenol in the mill fractions varied between years. DON and nivalenol were higher in flour fractions and lower in bran and offal in samples from 2004 compared to samples from 2005. This may be a consequence of high
rainfall pre-harvest in 2004 resulting in movement of these mycotoxins within grains before harvest. There was no significant difference in the distribution of ZON within mill fractions between the two years. For DON, higher concentrations in the grain resulted in a greater proportion of DON within the flour fractions. Understanding the factors that impact on the fractionation of mycotoxins during milling will help cereal processors to manufacture products within legislative limits.
Distribution of *Fusarium* mycotoxins in UK wheat mill fractions

S.G. EDWARDS¹, E.T. DICKIN¹, S. MACDONALD², D. BUTTLER³, C M HAZEL⁴, and S. PATEL⁴, K. A. SCUDAMORE⁵

¹Harper Adams University College, Newport, Shropshire, TF10 8NB, UK
²Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK;
³Campden BRI, Station Road, Chipping Campden, GL55 6LD, UK
⁴Premier Analytical Services, The Lord Rank Centre, Lincoln Road, High Wycombe, HP12 3QR, UK
⁵KAS Mycotoxins, 6 Fern Drive, Taplow, Maidenhead, SL6 0JS, UK.

Corresponding author. E-mail: sedwards@harper-adams.ac.uk

**Keywords:** deoxynivalenol, zearalenone, nivalenol, wheat, milling

**Abstract**

The EU has set maximum limits for the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZON). The maximum permitted level decreases from unprocessed wheat, through intermediary products e.g. flour, to finished products such as bakery goods and breakfast cereals. It is therefore important to understand the effects of processing on the mycotoxin distribution in mill fractions. Between 2004 and 2007, samples were taken at commercial flour mills at various points in the milling process and analysed for trichothecenes and ZON. Samples with a range of mycotoxin concentrations harvested in 2004 and 2005 were processed in a pilot mill and the mycotoxins in the different mill fractions quantified. In the commercial samples, DON was the predominant mycotoxin with highest levels detected in the bran fraction. Analysis of the pilot mill fractions identified a significant difference between the two years and between mycotoxins. The proportion of DON and nivalenol in the mill fractions varied between years. DON and nivalenol were higher in flour fractions and lower in bran and offal in samples from 2004 compared to samples from 2005. This may be a consequence of high rainfall pre-harvest in 2004 resulting in movement of these mycotoxins.
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the flour fractions. Understanding the factors that impact on the fractionation of
mycotoxins during milling will help cereal processors to manufacture products
within legislative limits.

Introduction

Mycotoxins are secondary fungal metabolites that can develop on a range of
important food commodities. Several mycotoxins often occur in wheat and other
cereals; those commonly found in UK grown cereals being deoxynivalenol
(DON), nivalenol (NIV), HT-2 toxin (HT2), T-2 toxin (T2) and zearalenone
(ZON), although DON occurs most frequently in wheat and at the highest levels
(MacDonald et al. 2004; Edwards 2009). The toxicology of the most important
mycotoxins have been assessed internationally in order to protect the consumer
against mycotoxins in the food supply. Maximum permitted limits have been set
by European Union legislation for DON and ZON (Anon. 2006). EC regulations
for DON and ZON apply not only to the unprocessed wheat both before or after
cleaning (1250 and 100 µg kg\(^{-1}\) respectively) but to milled intermediate products
eg flour (750 and 75 µg kg\(^{-1}\) respectively), finished products (500 and 50 µg kg\(^{-1}\)
respectively) and to infant food (200 and 20 µg kg\(^{-1}\) respectively).

There are more than 50 flour mills in the UK that produce many types and
grades of flour for bread, cakes, biscuits, breakfast cereals and other foods. Wheat
is the industry’s main raw material, with approximately 6.8 million tonnes milled
in 2009. Flour production was approximately 4.9 million tonnes in 2009, of
which 49% was for white bread production, 6% for wholemeal bread baking and
12% for biscuits (Anon 2010c)

Bran is used as a source of dietary fibre and may be used directly by, for
example, sprinkling on breakfast cereals, in high-fibre breakfast cereals or mixed
in recipes. Because mycotoxins tend to be higher in the bran this has implications
for its use for human food and ensuring that concentrations meet EU regulations.
Bran and other co-products, such as offal, are known collectively as wheat feed
and are fed to animals. It is generally accepted that policy should be to prevent or
minimise the exposure of the consumer to mycotoxins and that this is best achieved by limiting their formation as far as is possible. To this end, research on cereal agronomy (Edwards 2004) has enabled working practices to be developed to assist with achieving this, such as the “Guidelines to minimise risk of fusarium mycotoxins in cereals” (Anon 2010b). However, despite following these practices, mycotoxins still develop and information is thus required to determine how these mycotoxins are reduced by cleaning and distributed during milling. The factors that influence this distribution need to be understood so that the miller can predict, at least in part, the concentration of mycotoxins in the resulting mill fractions, based on the concentration within the original grain consignment before milling. This will help mill managers and secondary processors ensure end products conform to legislation based on mycotoxin testing of grain at intake.

Several studies have reported the distribution of fusarium mycotoxins in a limited number of samples using laboratory or pilot scale mills. This information is largely restricted to DON and is limited to other countries (Kushiro 2008) where the distribution may be different to the UK situation due to factors such as wheat variety and climate. These studies have identified that fusarium mycotoxins are reduced during milling of white flour with higher concentrations occurring in the bran and germ mill fractions (Kushiro 2008). The concentration of DON in white flour compared to that in the wheat grain has ranged from 21-123% (Abbas et al. 1985; Seitz et al. 1985). But they more typically range from 50-80% (Scott et al. 1984; Young et al. 1984; L’Vova et al. 1998). Factors that cause this variation have not been determined.

The aim of this study was to identify the distribution of fusarium mycotoxins within UK commercial flour mills and in a pilot scale mill. The pilot scale study used wheat samples from two different years and with a range of fusarium mycotoxin concentrations to identify if different years and the concentration of mycotoxins present had an impact on the distribution of each fusarium mycotoxin within each mill fraction.

**Materials and Methods**

*Commercial milling*

Samples were collected at various points during wheat milling from several representative UK wheat mills. Figure 1 shows a schematic view of the main
operations and the points where samples were taken. At each mill, wheat from different origins was delivered by lorries containing ca. 27 tonnes into a silo that held approximately 400 tonnes. Each lorry at intake was sampled for quality purposes using automatic sampling probes following the ISO 6190 standard of eight spear dips (full length) which combine to provide a 6-8 kg composite sample. In view of reports that cleaning can be extremely variable the starting point for the commercial milling study was the cleaned wheat. Specific bins/silos were allocated to this study. Grain then underwent a comprehensive cleaning procedure, involving considerable mixing, before return to a holding silo from where it was withdrawn for milling. The cleaned wheat was sampled to give a representative sample of the grain entering the milling process. For each sampling point before and after milling, five composite 1 kg grab portions of each fraction were taken to provide a 5 kg total at the different stages through the milling process (e.g. post break rolls, post reduction rolls) and the final mill streams (five composite grab samples: 5 kg total) were sampled e.g. bran, germ and finished white flour (Figure 1).

In total, 35 consignments of wheat were sampled from the harvests 2004-2007. A full set of samples that comprised cleaned wheat (1), conditioned wheat (2), break flour (3), white flour (4), semolina (5), wheat flour (6), bran (7) and germ (8) was collected from six of the consignments. For the remaining 29 commercial milling consignments only samples of cleaned wheat, finished flour, germ and bran were collected to determine the distribution of mycotoxins in these streams. Samples were despatched to Premier Analytical Services (PAS), High Wycombe where they were analysed for trichothecenes and ZON.

Pilot scale milling

Samples (ca. 25 kg) from 2004 and 2005 were sourced from wheat in store from samples analysed at harvest as part of a previous project (Edwards, 2009). The aim was to select both bread and biscuit milling varieties from fields of known agronomy with a range of DON contamination from 200-20000 µg kg⁻¹. Each sample was thoroughly mixed before 40 x 50 g sub-samples were removed to form a 2 kg whole-wheat sample. Each 2 kg sample was milled, mixed in a tumbler mixer and two 200 g laboratory samples collected. One set of samples was sent for analysis of trichothecenes and ZON, the other was archived at -20°C.
Based on the mycotoxin concentrations found, ten samples from each year were selected.

For each sample selected 12-15 kg were delivered to Campden BRI where the moisture content was measured, samples were cleaned using a dockage tester (Carter-Day International, Minneapolis, USA) (the weights of screenings and clean wheat were recorded), conditioned to 16% moisture content (weights and moisture content measured again) and milled using a pilot scale mill (MLU-202, Bühler AG, Uzwil, Switzerland) with a standard setting for high starch damage/hard wheats. The samples were milled in order of mycotoxin contamination (lowest first) and clean wheat was passed through the mill between each sample to minimise possible cross-contamination of samples within the mill. The fractions examined were screenings, cleaned wheat, reduction flour, break flour, bran flour, finished bran, offal flour and finished offal. Reduction and break flour were removed directly from the mill, and combined are referred to as Straight Run Flour (SRF). SRF is the sole or dominant component of commercial white flour (sample 4 in Figure 1). Bran and offal fractions were cleaned in a laboratory impact finisher (MLU-302, Bühler). Bran is coarse cleaned bran separated from break flour after the break rollers. Offal is a mixture of germ and fine bran (mainly fine bran) which is separated from reduction flour after the reduction rollers; this product is termed shorts in US mills. Bran and offal finisher flours are produced during the cleaning of the bran and offal fractions respectively. Each fraction was weighed, mixed, sub-samples collected (200 g or total sample supplied if less than 200 g) and delivered to the Food and Environment Research Agency (FERA), York for trichothecone and ZON analysis.

“Whole wheat” is used to describe the original wheat sample as collected.
“Cleaned wheat” is used to describe the samples after cleaning. Within the legislation, both of these samples are defined as unprocessed wheat.

Mycotoxin analysis
Analysis of samples from commercial mills was carried out at PAS, High Wycombe and samples associated with the pilot mill were analysed at FERA, York. The methods used by the laboratories are similar, have compared favourably over a number of years in quality check trials and collaborative testing
and are UKAS accredited. All methods are published (Patel et al. 1996; MacDonald et al. 2005) and were described in detail recently (Scudamore et al. 2007; Scudamore et al. 2009).

**Trichothecenes**

For analysis of commercial mill samples at PAS a 20 g portion of ground cereal sample was extracted with 100 ml acetonitrile/water (84/16) by shaking for 2 hours on a wrist action shaker. A 5 or 10 ml aliquot of the extract was applied to a pre-washed charcoal/alumina column and then washed with 40 ml acetonitrile/water (84/16). The total eluate was taken to dryness and transferred to a vial with acetonitrile. The sample extracts were again taken to dryness and derivatised to form the trichothecene -trimethyl silyl (TMS) derivatives and determined by GC/MS operating in selected ion mode, using up to four ions for confirmation. Samples spiked with 200 µg kg⁻¹ of each trichothecene were run in each batch. All results were corrected for recovery with acceptable recoveries in the range 70-110%. The LoQ was 10 µg kg⁻¹ and the RSD for DON was 8% (n=35). Analysis of pilot mill samples was conducted by FERA as detailed above except that Trichothecene P clean-up columns (R:Bio pharm Rhone) were used, and samples were spiked with 500 µg kg⁻¹ of each trichothecene. The LoQ was 20 µg kg⁻¹ and the RSD for DON was 12% (n=38).

**Zearalenone**

For analysis of commercial mill samples a 25 g portion of ground cereal sample was extracted with 125 ml of acetonitrile/water (75/25) by shaking for 30 minutes. The extract was filtered and 10 ml of the filtered extract (equivalent to 2 g of sample) was diluted with 40 ml of Phosphate Buffered Saline (PBS) solution. The diluted extract was passed through an EASI-EXTRACT ZON immunoaffinity (R:Biopharm Rhone) column under gravity. The column was washed with 10 ml water and then ZON was eluted under gravity with 2 ml of acetonitrile. The eluate was evaporated to dryness and transferred to a vial with chloroform which was then evaporated to dryness and dissolved in 1 ml of the HPLC mobile phase of 1% acetic acid:acetonitrile (55:45). Liquid chromatography was performed on a Kromasil C8 (Hichrom) 5µ particle size 25 cm x 3.2 mm i.d. column, with mobile phase operated at 0.5 ml/min. The excitation wavelength of the detector was set at 274 nm and the
emission wavelength was 440 nm. Sample volumes of 20 µl were injected. Samples spiked with 50 µg kg\(^{-1}\) ZON were run in each batch. All results were corrected for recovery with acceptable recoveries in the range 70-110%. The LoQ was 3 µg kg\(^{-1}\) and the RSD was 10% (n=35). Pilot mill samples were analysed by FERA as above except extracts were blended for 3-5 minutes instead of shaking and the immunoaffinity column clean-up and HPLC injection were performed automatically using an ASPEC system. The LoQ was 5 µg kg\(^{-1}\) and the RSD was 16% (n=16).

**Statistical analysis**

All statistical analysis was completed with Genstat (version 10, Lawes Agricultural Trust). All concentrations were logarithmically transformed to normalise the residuals for regression analysis and analysis of variance (ANOVA). Mass balance calculations to compare the combined mycotoxin content of mill fractions to the concentration of the whole wheat were performed by regression analysis. Correlation of the DON content of SRF as a percentage of the cleaned wheat concentration against the cleaned wheat concentration was also performed by regression analysis. The relative concentration of each mycotoxin in mill fractions were analysed by paired t-tests and factorial ANOVA with mycotoxin and year as factors. Individual values were compared by LSD (p=0.05).

**Results and discussion**

**Commercial samples**

It was not possible to conduct accurate mass balance checks on the commercial samples as the mass of each fraction within a continuous flow process can not be determined. For this reason, mass balance checks were conducted for each sample assuming a split of mill fractions of 74:12:5 for white flour, bran and germ, which are typical yields of a commercial mill. Two samples had a mass balance difference of over 50% and results from these samples were not included in the analysis. The probable cause of this inconsistency in the mass balance relates to the difficulty of sampling an industrial scale continuous flow process.

The mean concentrations of DON in UK wheat found in commercial wheat samples at mills after cleaning following the harvests of 2004-2007 are
detailed in Table 1. DON was quantifiable (>10 µg kg⁻¹) in every sample over this period, although the maximum concentration of 481 µg kg⁻¹ found was well below the EU maximum of 1250 µg kg⁻¹, while the mean value was 87 µg kg⁻¹. Despite the total number of consignments being small, with only two consignments examined from the 2004 harvest, the results for the other years show a marked seasonal variation almost certainly related principally to the climate. Unbalanced ANOVA identified that significantly higher levels of DON occurred in 2007, which was a year with a wet June, while the dry summer of 2006 resulted in lowest levels of DON occurring. This result agrees with national surveys of fusarium mycotoxin in UK wheat at harvest (Anon 2010a).

While the dominant mycotoxin was DON, other *Fusarium* mycotoxins were detected (Table 2). NIV occurred in 43% of consignments with a maximum of 43 µg kg⁻¹, ZON in 21%, maximum 34 µg kg⁻¹, and HT2 and T2 in only 11% of consignments and these at only just above the limits of quantification. In contrast to the higher levels of DON occurring in 2007, no HT2 or T2 was found in the samples examined that year. However, that year was the only one in which ZON was detected.

**Commercial mill fractions**

When the wheat consignments referred to in Table 1 were milled in commercial mills DON was lower in the white flour by an average of 30% compared to the level in the original cleaned wheat, while bran was higher by 282% (ca. three-fold higher) (Figure 2). DON concentration in the germ was approximately equivalent to the cleaned wheat. Results for six consignments for which more detailed sampling identified similar DON concentrations in all white flour fractions (results not shown). The distribution pattern between all consignments was variable, particularly for bran, this in part will be a consequence of sampling mill fractions during industrial scale continuous flow process. Little information was obtained on the distribution of mycotoxins other than DON during commercial operations because of the low incidence and levels at which they occurred. However, ZON was quantifiable in six consignments (Table 3) from which it appears that ZON is similar to DON in being higher in the bran and lower in the white flour. There are too few data to confirm the situation with the germ fraction.
Pilot scale samples

Table 4 provides data for the 20 samples used for pilot scale milling from 2004 and 2005. These samples were selected to provide wheat with a range of DON concentrations and other Fusarium mycotoxins (where present). They had a DON range of 193 to 15095 µg kg$^{-1}$, NIV from <20-384 µg kg$^{-1}$ and ZON from <5-768 µg kg$^{-1}$. Although most selected samples were intended for milling, the specific weights of many samples were too low to meet quality specifications for commercial flour mills. This was typical for the 2004 harvest, which due to a wet period in the late summer, was generally of poor quality.

Cleaning is a separate stage to milling and the proportion of screenings is variable, being largely dependent on the quality of wheat before harvest (grain size distribution), grain moisture at harvest, the combine harvester settings and if the wheat was cleaned before entering the farm store. Screenings ranged from 0.2 to 3.5% with a mean of 1.4% of whole wheat by weight. The mean recovery after milling was 98%. The two percent loss was probably a result of moisture loss during milling. Straight run flour (SRF) was equivalent to 72.5% of the whole wheat by weight, which is an acceptable recovery for SRF. Cleaned offal and bran accounted for 11.0 and 9.3% of the whole wheat respectively while their finisher flours accounted for 2.5 and 1.6% respectively.

Pilot scale mill fractions

DON was detected in all mill fractions of all ten samples in each year. NIV was detectable in all mill fractions in five and six samples in 2004 and 2005 respectively. ZON was only detectable in all mill fractions in four and two samples in 2004 and 2005 respectively. For each mycotoxin, samples were only included in the analysis if the toxin was detectable in all fractions, thus allowing mass balance calculations to be performed. Mycotoxin concentration of the whole wheat and screenings were adjusted to the conditioned moisture content (average = 16.0%) and the actual amount of each mycotoxin in each fraction was calculated based on its concentration and the weight of each fraction. These amounts were combined and compared to the mycotoxin concentration of the original whole wheat sample (mass balance calculation). Regression analysis of the combined content of mycotoxins in the mill fractions against the whole wheat
concentrations showed a good correlation for DON and ZON with a gradient close to one, a constant close to zero and an $r^2$ close to one (Figure 3 a, b). This indicates that the combined calculated content of all the fractions is close to that of the original sample. This shows that the mycotoxin analysis has acceptable accuracy, calculations are correct and there is no evidence of loss or gain of mycotoxin during processing. The equivalent correlation for NIV is not as good with an $r^2$ of 0.71 (Figure 3 c). When the NIV regression is forced through the origin (constant equals zero) then the gradient is close to one. This is most likely the true relationship (ie sum of NIV in mill fractions equals NIV content of whole wheat). This mass balance therefore indicates the analysis was not as accurate for NIV ($r^2 = 0.31$) compared to DON and ZON. This may in part be due to the smaller range of NIV at lower concentrations present within the samples. One sample was not included in the analysis of NIV as the mass balance was not acceptable (Figure 3 d).

The mass balance calculations conducted in this study strongly suggest that the differences in fusarium mycotoxin concentrations before and after milling are purely due to fractionation with no evidence of any loss or gain of any mycotoxin quantified during milling. Few previous studies have conducted mass balance determinations (Abbas et al. 1985), but where mill fractions’ mass and DON concentrations are provided, calculations show a good correlation between the mycotoxin concentration in the wheat grain and the sum of mycotoxin within the mill fractions (Lancova et al. 2008). Mass balance calculations are important quality control checks but require mycotoxins to be quantified in all mill fractions. If not all fractions are analysed eg Hart et al. (1998) or the mass balance equation is not balanced eg Table 2 in Scott et al. (1984) then the data should be treated with caution.

As the impact of cleaning has previously been shown to be highly variable (Seitz et al. 1985) the percentage reduction of mycotoxins by cleaning was calculated and then the effect of milling of cleaned wheat was determined separately. A paired one-sided t-test was used to compare mycotoxin concentration in cleaned wheat to whole wheat for DON, NIV and ZON. There was significantly ($p<0.001$) less mycotoxin in cleaned wheat compared to the whole wheat for each mycotoxin. Unbalanced ANOVA with mycotoxin and year as factors was used to compare the mycotoxin concentration of cleaned
wheat (as a percentage of the whole wheat). There was no significant differences between mycotoxins (p=0.061) or years (p=0.564) and there was no significant interaction (p=0.643). The average mycotoxin content for DON, NIV and ZON as a percentage of the whole wheat concentration was 93.4%. Previous studies have identified that cleaning is highly variable in its ability to reduce DON. This is because the “cleanliness” of the wheat before milling can be highly variable. Mycotoxins are routinely higher in the contents of screenings (eg light grains (Tkachuk et al. 1991), chaff (Sinha and Savard 1997) and dust (Lancova et al. 2008)) and therefore the greater quantity of screenings within a wheat sample, then the greater the reduction achievable. Also the greater the stringency of the cleaning process then the greater the reduction achievable. For example, in a process plant manufacturing whole-wheat breakfast cereals, a mean value of about 50% was achieved with a particularly vigorous cleaning regime (Scudamore and Patel 2008). With screenings ranging from 1 to 15% by weight, Seitz (1985) found reductions in DON ranged from -30 to +40% (average 16%). The reduction observed in this study was only 7% but this was with fewer screenings (average 1.4% w/w). This is the same DON reduction as reported by Young et al. (1984) with cleaning within an industrial mill.

ANOVA identified a significant difference between mycotoxins (p<0.001) and between years (p=0.002) for the relative concentration of mycotoxins in the screenings (as percentage of the whole wheat concentration). There was no significant interaction (p=0.763). The relative concentration for each mycotoxin in each year is shown in Figure 4. The relative concentrations of DON and ZON were significantly higher than that of NIV in screenings and the relative concentration of all mycotoxins was higher in 2004 compared to 2005.

A paired t-test identified there was no significant difference (p=0.96) in the mycotoxin content of break flour and reduction flour. The content of these two flours were combined and referred to as Straight Run Flour (SRF). The mycotoxin content of bran and offal and bran and offal finisher flours were significantly different to one another (p<0.001 for both pairs) and were analysed separately.

For each sample with quantifiable mycotoxins in each mill fraction concentrations were converted to percentage of cleaned wheat concentration and analysed as an unbalanced factorial ANOVA with factors mycotoxin and year for
each mill fraction (SRF, bran, offal, bran finisher flour and offal finisher flour).
There were significant differences between mycotoxins and between years. For
SRF (Figure 5a) both mycotoxin (p<0.001) and year (p<0.001) were significant,
and there was no significant interaction (p=0.238). The relative concentration in
SRF was similar for DON and NIV in each year, ca. 102% in 2004 and
significantly lower in 2005 at ca. 80%. The relative concentration of ZON is
significantly lower than DON and NIV and the relative concentration of ZON
was not significantly different between years (ca. 40%). For bran (Figure 5b),
both mycotoxin (p<0.001) and year (p<0.001) were significant, and there was a
significant interaction (p=0.004). The trends for bran were the opposite for SRF
with significantly lower levels in 2004 (ca. 77%) than 2005 (ca. 167%) for DON
and NIV. The relative concentration of ZON did not differ significantly between
years and was significantly higher than DON and NIV with a relative
concentration of ca. 285%. For offal (Figure 5c), both mycotoxin (p<0.001) and
year (p<0.001) were significant, and there was no significant interaction
(p=0.225). The trends for offal were similar to bran except the relative
concentrations for NIV were not significantly different between years whereas
ZON was significantly higher in 2005 (380%) compared to 2004 (257%).

For bran finisher flour (Figure 5d), mycotoxin (p<0.001) and the
interaction were significant (p<0.001) but not year (p=0.343). The relative
concentration of NIV was higher in 2005 (131%) compared to 2004 (61%). The
relative concentration of DON (ca. 56%) and ZON (ca. 120%) were not
significantly different between years. For offal finisher flour (Figure 5e) only the
interaction was significant (p=0.03). The only significant difference for the
relative concentration of mycotoxins was that DON in 2005 (96%) was lower
than DON in 2004 (126%) and ZON in 2005 (150%). As finisher flours are only
a small proportion of the total mill fractions they would have little effect on the
final mycotoxin concentration of any final flour blends.

Regression analysis of the percentage of DON in SRF against the original DON
content of cleaned wheat was highly significant (p<0.001) and best fitted by
parallel lines for each year (Figure 6). The regression accounted for 74% of the
variance. The equation for each line showed that a 10-fold increase in DON in
the cleaned wheat resulted in a 10% increase in the relative concentration of DON
in the SRF as a percentage of the cleaned wheat concentration. The relative
concentration of DON in SRF was 25% higher in 2004 than 2005. Nishio et al. (2010) reported consistent relative concentration of DON in white flour (60%) and bran (180%) irrespective of year (samples from 4 years) or DON concentration in the wheat grain in Japan. The consistency between years maybe a result of similar growing conditions during the grain development/ripening. Observation of the linear regressions of DON concentration in flour and bran to the DON concentration in the grain shows that as the DON concentration in grain exceeds 1000 µg kg⁻¹ then both flour and bran relative concentrations move towards 100%.

There were no significant equivalent regressions for NIV (p=0.113) or ZON (p=0.777). A lack of a significant regression for these mycotoxins may be a result of the lower number of samples with quantifiable NIV and ZON and the lower concentration range detected for NIV and ZON.

The fractionation of wheat was analysed based on the cleaned wheat concentration due to the variation between samples during cleaning. In the commercial mills the DON content as a percentage of the cleaned wheat concentration was 70% and 282% in SRF and bran respectively and ca. 100% in the germ fraction. In the pilot scale mill the distribution varied between years with 103 and 71% in SRF and bran respectively in 2004 and 78% and 162% in SRF and bran respectively in 2005. Previous authors have published a wide range of relative DON concentrations in flour with averages ranging from ca. 50% (TrigoStockli et al. 1996) to ca. 90% (Seitz et al. 1985). Seitz et al. had the most variable range of 47-123% of the cleaned DON wheat concentration. Most previous studies report distributions similar to the commercial mills and the 2005 pilot mill samples in this study (Kushiro 2008). Only one study, (Scott et al. 1983), reported a distribution similar to the 2004 pilot scale mill samples in this study, with percentage relative DON concentrations of 89% and 99% for SRF and bran compared to the wheat grain. This was a single grain consignment that had sprouted grains and a Hagberg falling number of 115 seconds. Both of which are indications of a wet, delayed harvest. For NIV there are few previous studies, but of those published the distribution between mill fractions appears similar to DON (Lee et al. 1987; Thammawong et al. 2010). There are also few previous studies for ZON. In these studies, the percentage relative ZON concentration in SRF has been consistently lower than DON or NIV, with values reported between 11 and
40% with corresponding high concentrations in bran and offal (ca. 200%) (Lee et al. 1987; TrigoStockli et al. 1996; L'Vova et al. 1998). Limited results for NIV and ZON in this paper are in agreement with those published previously.

Conclusions

All wheat grain and mill fractions analysed from UK commercial mills had low levels of fusarium mycotoxins. Only one bran sample was close to a European maximum level (71 µg ZON kg\(^{-1}\)).

Previous studies have not had a suitable range of samples to compare differences between samples harvested in different years or samples with a range of DON concentrations. The pilot scale study identified significant differences in DON concentration between years with less DON within the bran fraction in 2004, which was a wet harvest in the UK. Results indicated that the DON concentration in SRF was equivalent to that of the cleaned wheat, with a lower concentration in bran. This has not been reported previously, although DON is known to be highly water soluble. DON can be removed from wheat grains by soaking in water (Accerbi et al. 1999) or from wheat products by cooking in water (Visconti et al. 2004).

It is assumed that more severely *Fusarium* infected consignments of grain have deeper infections and will therefore have a greater proportion of DON within the endosperm and hence in the SRF fraction after milling. Analysis of data from wheat fractions separated by a gravity table showed that, for a single grain consignment, fractions with the lightest density had the greatest number of *Fusarium* damaged grains, the highest DON concentrations and the greatest proportion of DON within the white flour fraction (Tkachuk et al. 1991). The pilot scale study described in this paper confirmed that the proportion of DON within SRF increased as the concentration of DON in grain consignments increased.

Further experimental studies are required to prove and further quantify the relationship between pre-harvest rainfall and fusarium mycotoxin distribution within mill fractions. Information on the impact of the pre-harvest rainfall and the DON concentration of the wheat grain will help processors predict mycotoxin concentrations of mill fractions. Such predictions will reduce the need for
mycotoxin testing of intermediary products and provide evidence for rational
limits for fusarium mycotoxins at mill intake.

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Quality and Safety LINK Programme (FQS64).

References
Abbas HK, Mirocha CJ, Pawlosky RJ, Pusch DJ. 1985. Effect of cleaning,
50:482-486.

Accerbi M, Rinaldi VEA, Ng PKW. 1999. Utilization of highly deoxynivalenol-

Anon. Fusarium mycotoxin incidence and levels [Internet]. 2010a. London:

Anon. Guidelines to minimise risk of fusarium mycotoxins in cereals (2nd
from [http://www.hgca.com](http://www.hgca.com)

Anon. Industry Stats - Flour Production [Internet]. 2010c. London: Nabim; [cited


Edwards SG. 2004. Influence of agricultural practices on fusarium infection of
cereals and subsequent contamination of grain by trichothecene

Edwards SG. 2009. Fusarium mycotoxin content of UK organic and conventional

chromatography electron capture and enzyme-linked immunosorbent
assay for deoxynivalenol in milled fractions of naturally contaminated

Kushiro M. 2008. Effects of milling and cooking processes on the deoxynivalenol


Table 1. Occurrence of DON in commercial wheat samples from harvests 2004-2007.

<table>
<thead>
<tr>
<th>Harvest year</th>
<th>No of samples</th>
<th>DON (µg kg⁻¹)</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Median</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>2</td>
<td>91</td>
<td>-</td>
<td>63-118</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>12</td>
<td>140</td>
<td>109</td>
<td>22-481</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>15</td>
<td>45</td>
<td>43</td>
<td>16-113</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>6</td>
<td>292</td>
<td>280</td>
<td>208-382</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>35</td>
<td>87</td>
<td>61</td>
<td>16-481</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Occurrence of *Fusarium* mycotoxin other than DON in commercial wheat samples, 2004-2007.

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Incidence, % (n=35)</th>
<th>Mean of quantifiable samples</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZON</td>
<td>21*</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td>NIV</td>
<td>43</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>T2</td>
<td>11</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>HT2</td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

* One sample not analysed for ZON
Table 3: Concentration of ZON in commercial wheat mill fractions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ZON (µg kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cleaned wheat</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
</tr>
</tbody>
</table>

Mean % difference to cleaned wheat

<table>
<thead>
<tr>
<th></th>
<th>44</th>
<th>170</th>
<th>360</th>
</tr>
</thead>
</table>

For Peer Review Only
Table 4. Details of wheat samples selected for pilot scale milling.

<table>
<thead>
<tr>
<th>Year</th>
<th>Variety</th>
<th>NABIM Group</th>
<th>Specific Weight (kg hl(^{-1}))</th>
<th>Mycotoxin (µg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DON</td>
</tr>
<tr>
<td>2004</td>
<td>Malacca</td>
<td>1</td>
<td>74.9</td>
<td>193</td>
</tr>
<tr>
<td>2004</td>
<td>Malacca</td>
<td>1</td>
<td>73.3</td>
<td>1280</td>
</tr>
<tr>
<td>2004</td>
<td>Nijinsky</td>
<td>3</td>
<td>72.2</td>
<td>15095</td>
</tr>
<tr>
<td>2004</td>
<td>Xi19</td>
<td>1</td>
<td>71.4</td>
<td>299</td>
</tr>
<tr>
<td>2004</td>
<td>Xi19</td>
<td>1</td>
<td>78.5</td>
<td>658</td>
</tr>
<tr>
<td>2004</td>
<td>Xi19</td>
<td>1</td>
<td>71.4</td>
<td>1160</td>
</tr>
<tr>
<td>2004</td>
<td>Xi19</td>
<td>1</td>
<td>70.4</td>
<td>3155</td>
</tr>
<tr>
<td>2004</td>
<td>Claire</td>
<td>3</td>
<td>70.2</td>
<td>433</td>
</tr>
<tr>
<td>2004</td>
<td>Robigus</td>
<td>3</td>
<td>73.9</td>
<td>813</td>
</tr>
<tr>
<td>2004</td>
<td>Claire</td>
<td>3</td>
<td>73.1</td>
<td>1625</td>
</tr>
<tr>
<td>2005</td>
<td>Paragon</td>
<td>1</td>
<td>72.9</td>
<td>249</td>
</tr>
<tr>
<td>2005</td>
<td>Claire</td>
<td>3</td>
<td>63.3</td>
<td>2014</td>
</tr>
<tr>
<td>2005</td>
<td>Xi19</td>
<td>1</td>
<td>76.7</td>
<td>548</td>
</tr>
<tr>
<td>2005</td>
<td>Paragon</td>
<td>1</td>
<td>72.4</td>
<td>1161</td>
</tr>
<tr>
<td>2005</td>
<td>Deben</td>
<td>3</td>
<td>72.7</td>
<td>418</td>
</tr>
<tr>
<td>2005</td>
<td>Nijinsky</td>
<td>3</td>
<td>69.2</td>
<td>5688</td>
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<tr>
<td>2005</td>
<td>Hereward</td>
<td>1</td>
<td>75.1</td>
<td>831</td>
</tr>
<tr>
<td>2005</td>
<td>Deben</td>
<td>3</td>
<td>76.4</td>
<td>406</td>
</tr>
<tr>
<td>2005</td>
<td>Xi19</td>
<td>1</td>
<td>73.1</td>
<td>416</td>
</tr>
<tr>
<td>2005</td>
<td>Malacca</td>
<td>1</td>
<td>74.1</td>
<td>355</td>
</tr>
</tbody>
</table>

All other trichothecenes analysed were either very low or absent. NABIM group is the milling quality of the variety; group 1 are bread wheats, group 3 are biscuit wheats.
Figure 1. Schematic flow diagram of a commercial wheat flour mill with sampling points identified.
Figure 2. Relative concentration of DON in wheat milling streams from commercial UK wheat flour mills. Values are reported as a percentage of the DON concentration of the cleaned whole wheat before milling. Bars represent 95% confidence limits.
Figure 3. Regression analysis of the sum of the mycotoxin content of mill fractions to the mycotoxin concentration of the whole wheat (log log plot). a) DON, b) ZON, c) NIV. d) NIV (with regression line forced through origin). Square data point in NIV plot excluded as an outlier.
Figure 4. Relative concentration of mycotoxins DON, NIV and ZON in screenings after pilot scale cleaning. Columns with the same letter were not significantly different (LSD, p=0.05).
Figure 5. Relative concentration of mycotoxins DON, NIV and ZON in each mill fraction from the pilot scale mill. a) straight run flour, b) bran, c) offal, d) bran finisher flour; e) offal finisher flour. Columns with the same letter were not significantly different (LSD, p=0.05).
Figure 6. Regression analysis of the relative DON content of straight run flour (SRF) against the DON concentration of the cleaned wheat.
\[ y = 1.02x - 0.035 \]
\[ R^2 = 0.99 \]

\[ y = 0.98x + 0.020 \]
\[ R^2 = 0.96 \]

\[ y = 0.57x + 0.87 \]
\[ R^2 = 0.71 \]

\[ y = 1.01x \]
\[ R^2 = 0.31 \]
Log(ZON in raw wheat µg kg\(^{-1}\))

Log(NIV in raw wheat µg kg\(^{-1}\))

2.1 2.2 2.3 2.4 2.5
Mycotoxin content (as percentage of clean wheat)

DON  NIV  ZON

Mycotoxin content (as percentage of clean wheat)

2004  2005

DON  NIV  ZON