

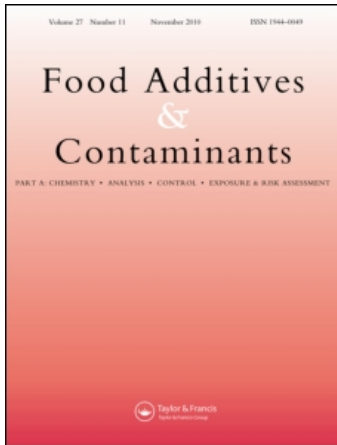
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## Stability of the mycotoxin deoxynivalenol (DON) during the production of flour-based foods and wheat flake cereal

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Deoxynivalenol (DON) is a mycotoxin found in cereal grains and cereal-based foods. DON concentrations in finished products are reduced under some processing conditions, but not others. DON concentrations in flour, wheat and selected foods made from them under commercially relevant conditions were compared by GC with electron capture detection. Average concentrations ( $n=9/\text{item}$ ) in cookies, crackers and pretzels ranged from 61% (cookies) to 111% (pretzels) compared with flour ( $100\% = 0.46 \mu\text{g g}^{-1}$ ). Lesser amounts were found in donuts and bread: their respective DON concentrations were 44% and 30% that of flour. Mass balance estimates for DON ( $\mu\text{g g}^{-1}$  flour equivalents) ranged from 50% (bread =  $0.23 \mu\text{g g}^{-1}$  flour equivalents) to 120% (donuts), indicating that dilution by recipe ingredients contributed to DON reductions in bread and accounted for all of the apparent reduction in donuts. Mass balance estimates averaged 76% (crackers) to 107% (pretzels) for the other flour products. DON concentrations were higher in cereal flakes ( $0.55 \mu\text{g g}^{-1}$  in the finished product and  $0.58 \mu\text{g g}^{-1}$  on a mass balance basis) than in wheat ( $0.40 \mu\text{g g}^{-1}$ ), suggesting that DON concentrations might increase during processing of wheat cereals under some conditions. In summary, DON concentrations of finished food products were reduced  $\geq 50\%$  only in bread and donuts. Reduction in bread resulted from a combination of DON 'loss' and dilution by recipe ingredients whereas the reduction in donuts was due entirely to dilution. These results are further evidence of DON stability during the preparation of popular flour or wheat-based products.

**Keywords:** GC; GC/MS; mycotoxins; trichothecenes; bakery products; bread; cereals; snack products

### Introduction

Deoxynivalenol (DON) is a *Fusarium* mycotoxin found in cereals including wheat, barley and maize (Canaday et al. 2001). Also known by the trivial name vomitoxin, DON causes a spectrum of toxic effects in animals that vary depending on dose and species (Canaday et al. 2001; Pestka 2010). These include emesis, feed refusal, decreased weight gain, altered immune function, IgA nephropathy in mice and various adverse effects on the gastrointestinal tissues. Human exposure is potentially extensive. DON occurs in a wide range of cereal-based foods and beverages (Canaday et al. 2001; Ok et al. 2009) and urinary DON excretion, a biomarker of exposure, has been correlated with the consumption of cereal-based foods in the United Kingdom (Turner et al. 2009). The health implications of DON for humans are not well understood. However, consumption of highly contaminated foods has been associated with outbreaks of gastrointestinal illness (Canaday et al. 2001, *passim*). Growth suppression (Iverson et al. 1995; Amouzie and Pestka 2010) from chronic exposure is considered the critical effect for risk assessment (Tritscher and Page 2004)

and the Commission of European Communities (2005) has set lower allowable concentrations of DON for infant and baby foods ( $0.2 \mu\text{g g}^{-1}$ ) than for other cereal products ( $0.50\text{--}0.75 \mu\text{g g}^{-1}$ ).

Investigations on the stability of DON during food processing have been reviewed by Canaday et al. (2001), Kushiro (2008) and Samar et al. (2001). DON concentration in pasta or noodles before cooking is significantly reduced by boiling (Nowicki et al. 1988; Visconti et al. 2004) and alkaline cooking (nixtamalization) reduces DON in maize (Abbas et al. 1988). Extrusion cooking, which involves high heat and pressure, had little effect on DON in wheat (Scudamore et al. 2008), although a significant reduction was found if the contaminated wheat was soaked in 5% aqueous sodium bisulfite beforehand (Accerbi et al. 1999).

DON in wheat is considered stable under most other processing conditions including bread baking. However, results of individual experiments have been variable (reviewed by Canaday et al. 2001; Samar et al. 2001; Hazel and Patel 2004; Kushiro 2008). Reductions of up to 64% (Abbas et al. 1985; Neira et al. 1997),

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flour-equivalent basis, were found in some experiments, whereas increased DON concentrations relative to the flour before baking were found in others (El-Banna et al. 1983; Scott et al. 1984; Seitz et al. 1986; Boyacioglu et al. 1993; Sugiyama et al. 2009). Reasons for these inconsistent results are unknown, but it is likely that the specific baking conditions including temperature, time, moisture content of the dough, type and quality of flour, and recipe influence mycotoxin stability. DON stability during the preparation of popular baked or fried cereal products other than bread made under conditions common to large-scale establishments has received little attention. Therefore, the stability of DON during the preparation of selected wheat flour-based food items, including bread made using a 'sponge and dough' recipe, and of flake cereal made from cut wheat using commercially relevant recipes and conditions was investigated.

## Materials and methods

### Solvents

Hexane and acetonitrile (Burdick and Jackson, Muskegon, MI, USA) and methanol (Fisher Scientific, Fair Lawn, NJ, USA) were of analytical grade or better.

### Flour and flour products

Crisp sugar cookies, snack crackers, white pan 'sponge and dough' bread, and cake donuts were prepared from wheat flour by AIB, International (Manhattan, KS, USA) using commercial recipes and conditions. Baking or, for donuts, frying temperatures and times were: cookies, 163°C (325°F) for 18 min; crackers, temperature varied by oven zone from 193 to 252°C (380–485°F) over a total time of 6 min; donuts, 190°C (375°F) for 45 s per side, total time of 90 s; and bread, 227°C (440°F) for 18 min. Pretzels were prepared from the flour at the Frito-Lay Technology Processing Center (Duncanville, TX, USA). Representative portions of flour and each food product were shipped to the Toxicology and Mycotoxin Research Unit (Athens, GA, USA) and stored frozen until processed for analysis.

### Wheat and cereal flakes

Whole wheat flakes were manufactured under proprietary commercial conditions at General Mills (Minneapolis, MN, USA). Samples of the wheat, the intermediate (dough and extruded pellets) and the final (wheat cereal flakes) products were handled as described above.

### Sample preparation

Three 300 g samples of each flour product, cut wheat, and the wheat cereal products were freeze-dried and ground using a Wiley Mill (1 mm mesh) (Thomas Scientific, Swedesboro, NJ, USA). Each sample was divided into three 100 g portions, the portions defatted with 500 ml of hexane, the hexane removed by gravimetric filtration (Whatman No. 4 filter paper), the material air-dried, and the portions recombined to reconstitute the original 300 g sample. Each was then blended overnight in a long roll jar mill (US Stoneware, East Palestine, OH, USA) fitted with a 1 L ceramic milling jar with a cylindrical high-density burundum grinding medium. Flour was processed as described, except that it was not ground prior to freeze-drying and defatting. All items were stored frozen in sealed plastic bags until processed further.

Sample extraction and clean-up for GC-EC analyses were done using a modification of the method of Neira et al. (1997). Each of the three flour, flour product, wheat and wheat product samples were subsampled (25 g) in triplicate for analyses so that there were  $n=9$  analyses per item. The subsamples were extracted with 100 ml acetonitrile:water (84:16). For clean-up, 8 ml of extract were loaded onto a MycoSep<sup>®</sup> 227 Trich+ (Romer Laboratories, Union, MO, USA) and the first 4 ml coming off the column were collected and dried under a gentle stream of nitrogen at 35°C. The dried residues were derivatized by adding 200 µl of a 3:3:2 mixture of trimethylsilylimidazole (TMSI), *N,O*-bis-(trimethylsilyl)acetamide (BSA) and trimethylchlorosilane (TMCS) (Tri-Sil<sup>®</sup> TBT, Pierce, Rockford, IL, USA) and heating (75°C) for 2 h.

### Quantification

The derivatized flour, wheat and product subsamples (1 µl) were injected (two to three injections/subsample) onto a Thermo Finnigan (Austin, TX, USA) Trace GC fitted with an AS2000 autoinjector, <sup>63</sup>Ni electron capture detector, and a J&W DB-5 column (30 m × 0.25 mm × 0.25 µ i.d.). Conditions were: injection port temperature = 120°C; detector temperature = 350°C; carrier gas = helium at a flow rate of 1.5 ml/min; makeup gas = 30 ml/min 5% methane:95% argon; and column temperature programme = 120°C for 1 min, increasing at 6°C/min for 30 min and hold at 300°C for 10 min. The retention time of DON was 12.7 min. Quantification was by comparison with an external standard curve of DON (Sigma, St. Louis, MO, USA). The results were adjusted for recovery using factors determined by spike and recovery studies.

### Spike and recovery

A total of 25 g portions of flour, flour products, wheat and wheat products ( $n=2-3$ /item) were spiked with 2.75 (bread) or  $5.5 \mu\text{g g}^{-1}$  DON (Sigma) dissolved in 250  $\mu\text{l}$  of methanol (equal to  $0.11 \mu\text{g g}^{-1}$  (bread) or  $0.22 \mu\text{g g}^{-1}$  equivalents, respectively). The spiked materials were allowed to dry overnight and DON quantified as described. Unspiked portions ( $n=2-3$ ) of each item were simultaneously analysed and DON recovery (%) calculated by the formula:

$$\left[ \frac{\text{Mean } \mu\text{g g}^{-1} \text{ DON in spiked portion} - \text{mean } \mu\text{g g}^{-1} \text{ DON in unspiked portion}}{\mu\text{g g}^{-1} \text{ DON equivalents spiked}} \right] * 100.$$

### GC-MS confirmation of DON

Extracts of selected items (crackers, bread, wheat and cereal flakes) were qualitatively analysed by GC-MS (SIM) to confirm the identity of the peak as DON. After clean-up and derivatization, 1  $\mu\text{l}$  was injected onto a gas chromatograph-mass spectrometer (GC-MS; Series 5890 GC fitted with Model 5970 MS Detector, Hewlett Packard, Wilmington, DE, USA). Chromatography conditions were: column: 25 m  $\times$  0.2 mm HP-5ms 5% phenyl methyl silicone; injection temperature = 250°C; column temperature gradient = 80°C for 2 min, increase to 245°C at 60°C/min, hold for 3 min, increase to 270°C at 10°C/min, hold for 7 min (end of run); carrier gas: helium; flow rate = 2 kPa/min; and detector mode = selected ion monitoring (SIM). The retention time of the derivatized DON under these conditions was 13.8 min. Mass spectra were verified by comparison with GC-MS spectra ( $m/z=512, 422$  and 235) of genuine DON (Sigma).

### Statistical analysis

DON concentrations in uncooked flour and wheat were compared with those of their respective products ( $n=9$  subsamples/item) by means of paired  $t$ -tests (SigmaStat® software, Systat Software, San Jose, CA, USA). Significance was determined at  $p < 0.05$ .

### Results and discussion

Recovery of DON from the spiked flour and its products averaged  $80\% \pm 19\%$  (SD) and ranged from 52% (donuts) to 108% (crackers). Recoveries of DON from the flour, cookies and crackers were in the range of 68–91%. Because the pretzels were made at a different facility than the other products, it was considered that transport and differing storage conditions could influence recovery. Recovery from the flour used for pretzels was indeed lower (68%) than recovery

from the flour used for the other products (86%). However, DON concentrations of both flours after correction for recovery were in agreement: their respective values were 0.46 and  $0.47 \mu\text{g g}^{-1}$ . Recoveries of DON from spiked wheat and the wheat flake cereal products averaged  $90\% \pm 5\%$  and ranged from 82% to 95%. The identity of the peak as DON was corroborated by its co-elution with genuine DON (GC-EC) as well by GC-MS.

DON concentrations were modestly reduced ( $p < 0.05$ ) in the finished cookies (39% reduction) and crackers (30% reduction), whereas no reduction was found in the pretzels (Table 1 and Figure 1). When considered on a mass balance basis, DON concentrations ( $\mu\text{g g}^{-1}$  flour equivalents) of the cookies, crackers and pretzels were 76–107% relative to flour (Table 1 and Figure 2). The difference was statistically significant only for crackers. This result indicates that dilution of the flour by other recipe ingredients from partially to completely accounts for the decreased DON concentrations in the crackers and cookies. A greater reduction (56%) of DON concentration was found in the finished donuts. The addition of other recipe ingredients together with the uptake of oil during frying (>50% of the donuts' mass was lost during defatting) resulted in the high yield of 2.8 kg donuts per kg of flour and, as a result, the estimated amount of DON ( $\mu\text{g g}^{-1}$  flour equivalents) recovered when considering mass balance was 120% (not statistically significant) than the amount in flour. The results for cookies compared favourably with those of Young et al. (1984) and Scott et al. (1984) who found no evidence of DON instability. DON concentrations (flour-equivalent basis) in cookies ranged from 65% to 110% compared with flour in these earlier experiments. While we found more DON in donuts than did Scott et al. (1984) (66% relative to flour, flour equivalent basis), both experiments nonetheless show that DON is stable during donut preparation.

Among the products tested, DON was least stable in bread. Its concentration was significantly reduced, about 70%, in the finished loaves: mean concentrations in flour and bread were 0.46 and  $0.14 \mu\text{g g}^{-1}$ , respectively (Table 1 and Figure 1). Much of the reduction resulted from dilution as an estimated 1.6 kg of finished bread was produced per kg of flour. Accordingly, 'loss' of DON through thermal decomposition, reaction with matrix constituents or other means was about 50% as indicated by mass balance estimations (Table 1 and Figure 2). Results of earlier experiments on DON stability during bread baking have been comprehensively reviewed (Canaday et al. 2001; Samar et al. 2001; Hazel and Patel 2004; Kushiro 2008). The majority of reports indicate that DON is stable; however, as illustrated by the following examples, results have not been consistent, indicating that the fate of DON in baked breads should be evaluated

Table 1. DON in flour and food products made from the flour.

Flour or product	DON concentration		Yield <sup>a</sup>	Mass balance	
	( $\mu\text{g g}^{-1}$ item)	Relative amount (%)		( $\mu\text{g g}^{-1}$ flour equivalents) <sup>b</sup>	Relative Amount (%)
Flour	0.46 <sup>c</sup> $\pm 0.06$	100	1	0.46 <sup>c</sup> $\pm 0.06$	100
Cookie	0.28 <sup>d</sup> $\pm 0.03$	61	1.6	0.44 $\pm 0.05$	96
Cracker	0.32 <sup>d</sup> $\pm 0.04$	70	1.1	0.35 <sup>d</sup> $\pm 0.05$	76
Pretzel	0.51 $\pm 0.07$	111	0.96	0.49 $\pm 0.07$	107
Donut	0.20 <sup>d</sup> $\pm 0.05$	44	2.8	0.55 $\pm 0.15$	120
Bread	0.14 <sup>d</sup> $\pm 0.01$	30	1.6	0.23 <sup>d</sup> $\pm 0.02$	50

Notes: <sup>a</sup>Amount (kg) of product yielded per kg of flour in the recipe.

<sup>b</sup>( $\mu\text{g DON g}^{-1}$  product)/(1/ $x$ ), where  $x$  is the yield (kg) of product per 1 kg of flour in the recipe.

<sup>c</sup>Values indicate the mean  $\pm$  standard deviation (SD),  $n=9$  subsamples per flour or product.

<sup>d</sup>Significantly different from flour,  $p < 0.05$ .

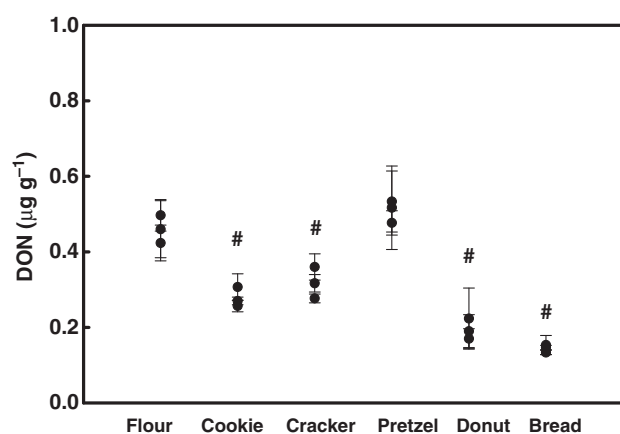


Figure 1. DON concentrations ( $\mu\text{g g}^{-1}$  wet weight) of the flour and finished flour-based products. Three samples of each were processed for quantification. Symbols represent the mean value for triplicate (three subsamples) analyses per sample. Error bars indicate the standard deviation (SD). Results differing from the flour ( $p < 0.05$ ; see text) are indicated by the hash symbol '#

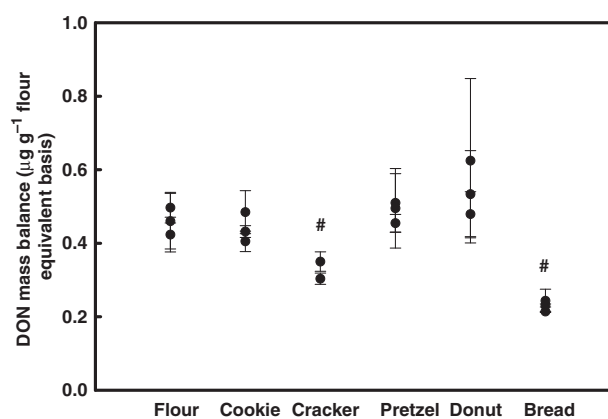


Figure 2. Mass balance estimates of DON ( $\mu\text{g g}^{-1}$  flour equivalent basis) in flour and the finished flour-based products. Three samples of each were processed for quantification. The symbols represent the mean value for triplicate (three subsamples) analyses per sample. Error bars indicate standard deviation (SD). Results differing from the flour ( $p < 0.05$ ; see text) are indicated by the hash symbol '#

on a case-by-case basis. Sugiyama et al. (2009) surveyed a series of flours and breads collected from nine commercial Japanese bakeries and found that the amount of DON remaining in 35 breads baked over a range of temperatures (160–220°C) for approximately 30 min varied considerably. Initial concentrations in the flours averaged  $31.3 \pm 28.9$  (SD)  $\text{ng g}^{-1}$  with individual values ranging from  $<5.0$  (LOQ) to  $113.2 \text{ ng g}^{-1}$ . DON concentration in the breads averaged  $74.4\% \pm 84.8\%$  (mass balance basis) that of flour with a range of 10% to over 300% for individual breads. It is noteworthy that DON was present in their bread samples only at low levels,  $\leq 37 \text{ ng g}^{-1}$  (mean  $8.6 \pm 5.1 \text{ ng g}^{-1}$ ), and that all breads having the highest

amounts of DON (176–355%) relative to flour originated from a single location. In this case,  $8.8\text{--}17.8 \text{ ng g}^{-1}$  DON were found in the baked product although, enigmatically, DON was not detected in the flours.

Lancova et al. (2008) reported that DON was stable based on their finding that its concentrations in bread (baked at 210°C for 14 min) were 94–100% compared with the flour (40 or  $1.2 \mu\text{g g}^{-1}$ ), whereas Abbas et al. (1985) found evidence of modest instability. In the latter study,  $435.3 \pm 0.95$  (SD) (16% reduction) and  $108.9 \pm 7.05 \text{ ng g}^{-1}$  (64% reduction) DON was found in whole wheat bread baked from flours with respective concentrations of  $520.9 \pm 14.0$

and  $305.9 \pm 27.2 \text{ ng g}^{-1}$ . There are also indications that DON concentration might increase slightly under some conditions: its concentration in breads baked at 205 or 212°C for 28–30 min averaged 107–108% that of the flour (Scott et al. 1984) while the findings of Seitz et al. (1986) were more variable. Like Sugiyama et al. (2009), they reported variable results with reductions (up to 47%) in some breads but increases (up to 25%) in another.

The results are in general agreement with those of Abbas et al. (1985) and fall within the range of results reported by Seitz et al. (1986) and Sugiyama et al. (2009). However, the 50% DON reduction in our bread was greater than the 6% reduction to approximate 8% increase reported by Scott et al. (1984) and Lancova et al. (2008). Factors influencing DON stability during baking are not well understood and should be the subject of further study. The relatively higher temperature (227°C=440°F) compared with that of Lancova et al. (2008), 210°C, and Scott et al. (1984),  $\leq 212^\circ\text{C}$ , or slightly longer baking time (18 min) in this experiment compared with that (14 min) used by Lancova et al. (2008) might have promoted DON decomposition or matrix binding. The relatively high temperatures (up to 325°C) used for baking crackers, the only other flour product showing loss of DON on the basis of both concentration and mass balance (Table 1 and Figures 1 and 2), are noteworthy. In addition, Neira et al. (1997) noted a correlation between increased baking time at 210°C and DON reductions during bakery processing. Recipe might also be a factor and, in this regard, L-cysteine (Boyacioglu et al. 1993) and fermentation (Neira et al. 1997; Samar et al. 2001) have been shown to promote DON reductions. Interestingly, the bread recipe was the only one in our study to include fermentation, in this case, a 4-h fermentation component with compressed baker's yeast (2.5% w/w). Fermenting and baking under conditions found in Argentinean bakeries reduced DON concentrations by 44% on average (Neira et al. 1997), a result that is consistent with our findings.

Determining the presence of DON decomposition or matrix-binding products in the flour, wheat or finished items was not attempted. It is plausible that one or more of the several known DON decomposition products (Young et al. 1986; Berthiller et al. 2005; Bretz et al. 2006; Berthiller, Dall'asta et al. 2009; Berthiller, Schumacher et al. 2009) were present in variable amounts in the flour products and could account, at least in part, for the lower mass balance results obtained for crackers and bread. Three decomposition products, norDON A, norDON B and norDON C, have indeed been found in low amounts ( $\leq 36 \text{ ng g}^{-1}$ ) in cookies, crackers and breads purchased from German markets (Bretz et al. 2006). The most prevalent, norDON A, was detected in up to 66%

of the items and its concentration in the positive samples averaged from 10% to 27% that of DON. Preliminary indications are that thermal degradation of DON can be beneficial. This is because norDON A, norDON B and norDON C displayed no toxicity to immortalized human kidney epithelial cells at a concentration of  $100 \mu\text{mol l}^{-1}$  whereas the  $\text{EC}_{50}$  for DON toxicity in the same in vitro bioassay was  $1.1 \mu\text{mol l}^{-1}$ .

To our knowledge, this is the first report on DON stability during the production of wheat flake cereal. The process involves mixing whole wheat and other ingredients into a moist dough, extrusion cooking of the dough to form pellets and then rolling the extruded pellets into flakes. DON concentrations in the intermediate dough and pellet products averaged 15–27% lower than in wheat (Table 2 and Figure 3) and the difference was statistically significant only for the extruded pellets. When considering mass balance (Table 2 and Figure 4), no differences between wheat and the intermediate products were found, although the average DON concentrations of the dough and pellets relative to wheat tended to be slightly higher. The mean DON concentration in finished flakes cereal ( $0.55 \mu\text{g g}^{-1}$ ) was about 35% higher than in the wheat ( $0.40 \mu\text{g g}^{-1}$ ), but the difference was not statistically different. DON concentrations in the flakes were similar ( $0.58 \mu\text{g g}^{-1}$ ) when calculated on a wheat equivalent basis but, in this case the difference was statistically significant ( $p < 0.04$ ).

A possible explanation for the trend toward higher DON concentrations in cereal flakes, donuts ( $\mu\text{g g}^{-1}$  flour equivalent basis), and wheat or flour products evaluated by others is the liberation of DON from DON-glycosides or other DON-conjugates (reviewed by Berthiller, Schumacher et al. 2009) during processing. Glucoside conjugates occur naturally and have been found in wheat at relative concentrations (compared with DON) of up to 12% in one study (Berthiller et al. 2005), up to 46% in a second (Berthiller, Dall'asta et al. 2009) and slightly more than 100% in a third (Sasanya et al. 2008). More extensive surveys are clearly needed to determine the prevalence of conjugated and other masked forms of DON in wheat and their contribution to DON content of processed foods. In this regard, DON-3-glucoside was detected in 100% of wheat ( $n=23$ ) and maize ( $n=54$ ) samples collected from the field in Europe (Berthiller, Dall'asta et al. 2009). It is also possible that the apparent increase in DON found in the wheat flakes was an incidental consequence of heterogeneous mycotoxin distribution in the sampled wheat kernels. This possibility is supported by the large relative standard deviations of up to 35% and 47% obtained when the mean values and variance obtained for the three wheat and cereal flake samples (each was subsampled for triplicate analyses; Figures 3 and 4).

Table 2. DON in wheat, intermediate products (dough and pellets) and finished flake cereal.

Wheat or product	DON concentration		Yield <sup>a</sup>	Mass balance	
	( $\mu\text{g g}^{-1}$ item)	Relative amount (%)		( $\mu\text{g g}^{-1}$ wheat equivalents) <sup>b</sup>	Relative amount (%)
Wheat	0.40 <sup>c</sup> $\pm 0.11$	100	1	0.40 <sup>c</sup> $\pm 0.11$	100
Dough	0.34 $\pm 0.10$	85	1.47	0.50 $\pm 0.14$	125
Pellets	0.29 <sup>d</sup> $\pm 0.08$	73	1.47	0.42 $\pm 0.12$	105
Finished flakes	0.55 $\pm 0.17$	138	1.06	0.58 <sup>d</sup> $\pm 0.18$	145

Notes: <sup>a</sup>Amount (g) of product yielded per g of wheat in the recipe.

<sup>b</sup>( $\mu\text{g DON g}^{-1}$  product)/(1/ $x$ ), where  $x$  is the yield (kg) of product per 1 kg of wheat in the recipe.

<sup>c</sup>Values indicate the mean  $\pm$  standard deviation (SD),  $n=9$  subsamples per wheat or product.

<sup>d</sup>Significantly different from wheat,  $p < 0.05$ .

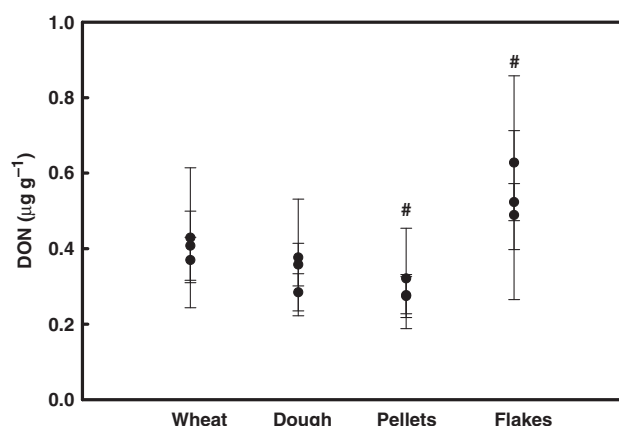


Figure 3. DON concentrations ( $\mu\text{g g}^{-1}$  wet weight) of the wheat, dough, and pellet intermediate products and finished flake cereal. Three samples of each were processed for quantification. The symbols represent the mean value for triplicate (three subsamples) analyses per sample. Error bars indicate standard deviation (SD). Results differing from the flour ( $p < 0.05$ ; see text) are indicated by the hash symbol '#'.

In summary, the findings are further evidence that DON is generally stable during the production of popular flour-based food items and wheat flake cereal prepared under industry relevant conditions. Its concentration in the finished products was reduced 50% or more only in donuts and bread. The reduction in donuts resulted entirely from dilution of the flour by other ingredients whereas, in addition to dilution, a modest 'loss' of DON was demonstrated in the baked bread. Further investigations are warranted to determine the conditions that optimize reduction of DON during secondary processing. Studies should include identification and quantification of thermal degradation and DON-food matrix binding products that form, as well as the contribution of DON from naturally occurring conjugates present in wheat and flour.

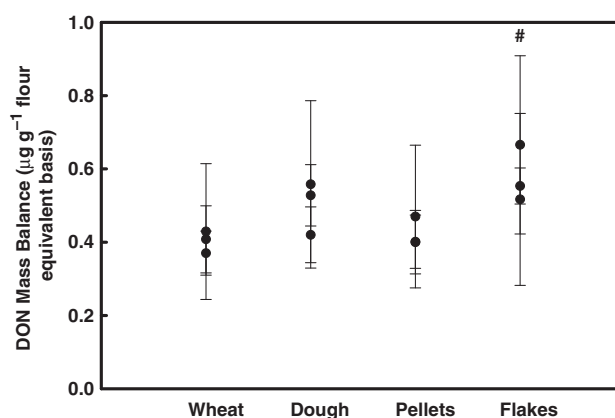


Figure 4. Mass balance estimates of DON ( $\mu\text{g g}^{-1}$  flour equivalent basis) in wheat, dough, and pellet intermediate products and the finished cereal flakes. Three samples of each were processed for quantification. The symbols represent the mean value for triplicate (three subsamples) analyses per sample. Error bars indicate standard deviation (SD). Results differing from the flour ( $p < 0.05$ ; see text) are indicated by the hash symbol '#'.

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