Fate of *Fusarium* Mycotoxins in Cereal Food Chain

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Presentation Aim

To contribute data addressing the fate of *Fusarium* mycotoxins during commercial food processing so assisting with an appraisal of legislative limits

Fate of *Fusarium* Mycotoxins in Cereal Food Chain

Consortium

Unique combination of government, food manufacturers and academic experts

Industrial	■ PepsiCo (Quaker Oats) ■ RHM ■ Smiths Flour Millers ■ United Biscuits ■ R-Biopharm	■ DACSA ■ Maizecor ■ Kelloggs ■ Cereal Partners
Academic	■ KAS Mycotoxins ■ Campden and Chorleywood Food Research Association ■ Harper Adams University College	■ University of Bristol ■ Central Science Laboratory ■ Prof Ron Walker
Advisors	■ Food Standards Agency ■ DEFRA ■ SNACMA	■ Home Grown Cereals Authority ■ National Association of British and Irish Millers

Funding

- **UK Government**
- **UK Cereal Industry and Food Manufacturers**

FOOD STANDARDS AGENCY

defra
Department for Environment Food and Rural Affairs

Overall Project Aim

UK recognised that:

- *Fusarium* mycotoxins do occur in UK and imported cereals
- Significant gaps in our knowledge e.g.
  - Fate in full scale commercial processing
  - Breakdown products, hidden metabolites and potential toxicological significance

Project aim:

To assist in the management of key mycotoxins in the cereal processing chain so as to best comply with future regulation and reduce the exposure of consumers to these contaminants

Fusarium Mycotoxins

- **Focus on:**
  - **Commonly occurring mycotoxins in UK cereals**
    - *Fusarium* toxins
      - Trichothecenes DON/NIV/T2/HT2
        - Possibly acetylated DON derivatives
      - Zearalenone (ZON)
      - Fumonisins (maize only)

Processes included

Focus on:

Production of frequently consumed foods

MAIZE	WHEAT	OATS
■ Milling (Dry) ■ Breakfast cereals ■ Extruded snacks ■ Tortilla snacks	■ Milling ■ Baking (bread, cakes, biscuits, crackers) ■ Breakfast cereals ■ Extruded snacks	■ Milling ■ Oat flakes

Timescales

- Project began late 2004
- Schedule to run for just over three years
- Covers multiple harvest years
  - Allows differences in levels and nature of *Fusarium* infection to be studied
- Interim results now available

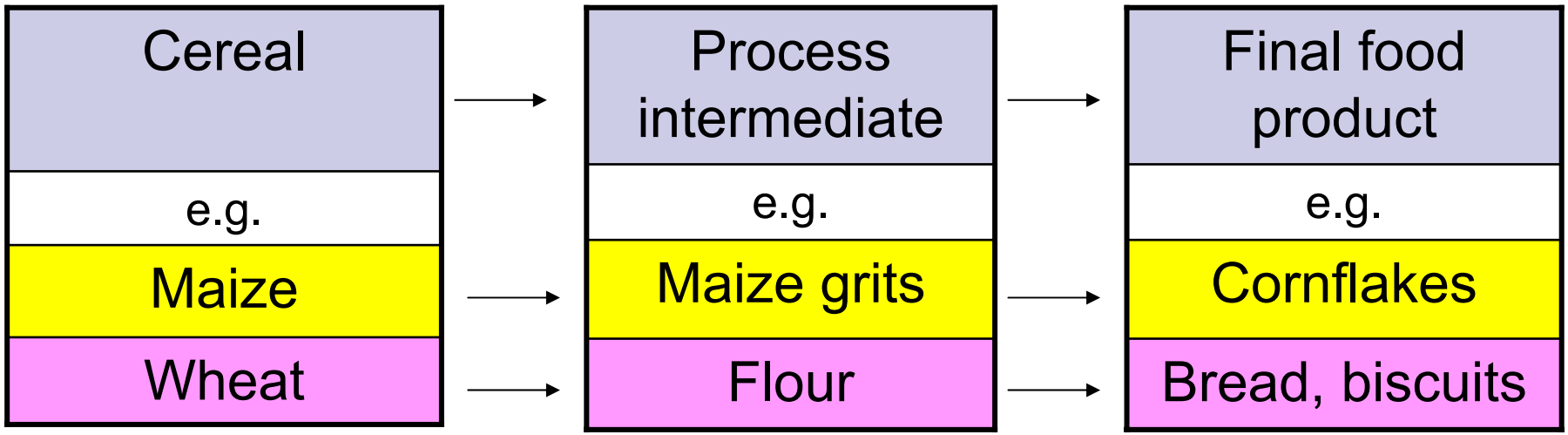
Sampling and Analysis

Sampling and Traceability	**Critical that:** ■ the samples taken are representative of the lot being processed ■ the raw material lot can be traced through the process ➢ Sample points based on chemical and physical processes that may affect mycotoxins ➢ Sampling plans agreed with the UK FSA for each process ➢ Plans are as close as possible to EU sampling plans
Mycotoxin Analysis	Two analytical labs participating Steps taken to ensure all results are accurate, comparable with no bias

CONSIDERATIONS	
Standard quantification	**Sample treatment at the laboratory to achieve homogeneity (grinding/particle size/mixing)**
Extraction (-solvent/time)	**Clean-Up**
End determination techniques	**Method performance parameters**
Accreditation status – all ISO 17025	**Proficiency testing carried out (FAPAS)**

Industrial Processes

For each process aim to demonstrate the levels of *Fusarium* mycotoxins occurring in the raw cereal (maize, oats and wheat) the process the raw cereal undergoes and effect on mycotoxin levels



Key question: Are all toxins presented in the raw cereal accounted for at the end of processing?= **Process mass balance**

Fate of *Fusarium* Mycotoxins in Cereal Food Chain

MAIZE

Occurrence in Raw Maize

µg/kg		Origin = FRENCH 1 (n=17)	Origin = FRENCH 2 (n=14)	Origin = ARGENT. (n=14)
DON	Range	13-519	27-420	16-166
	Mean	112	150	87
	Median	38	92	72
FB1	Range	73-482	19-1027	237-3813*
	Mean	262	316	1563
	Median	220	225	1362

***B1+B2 = 5202**

Occurrence in Raw Maize

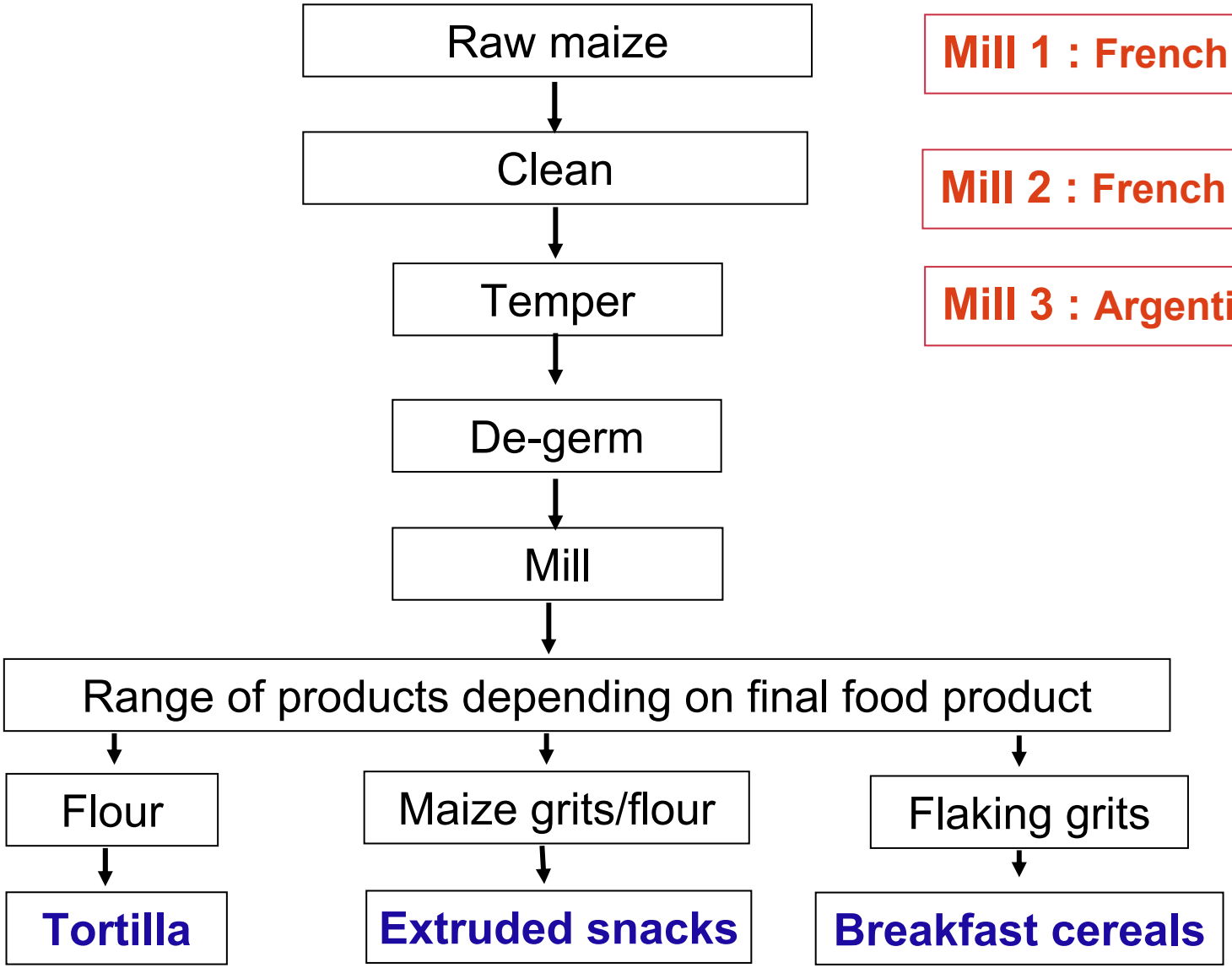
µg/kg		Origin = FRENCH 1 (n=17)	Origin = FRENCH 2 (n=14)	Origin = ARGENT. (n=14)
ZON	Range	<3-86	<3-52	<3-42
	Mean	18	17	14
	Median	<3	12.6	9

Data also for:	**Nivalenol**	**HT-2 toxin**	**T2-toxin**	**3Ac-DON**
	15 Ac-DON	**Fus X**	**Neosolaniol**	**DAS**

Mycotoxins in Raw Maize Summary

- Maize subject to contamination with a range of mycotoxins
- Occurrence varies depending on:
  - Origin
  - Season
- Field control is difficult
- French consignments from 2004 had higher DON and ZON levels that samples from 2005
- DON and FB1 occurred in all consignments, T2 and HT2 not detected in South American maize
- Fumonisins higher in South American maize than French maize

Dry Milling of Maize



Mill 1 : French (SW)

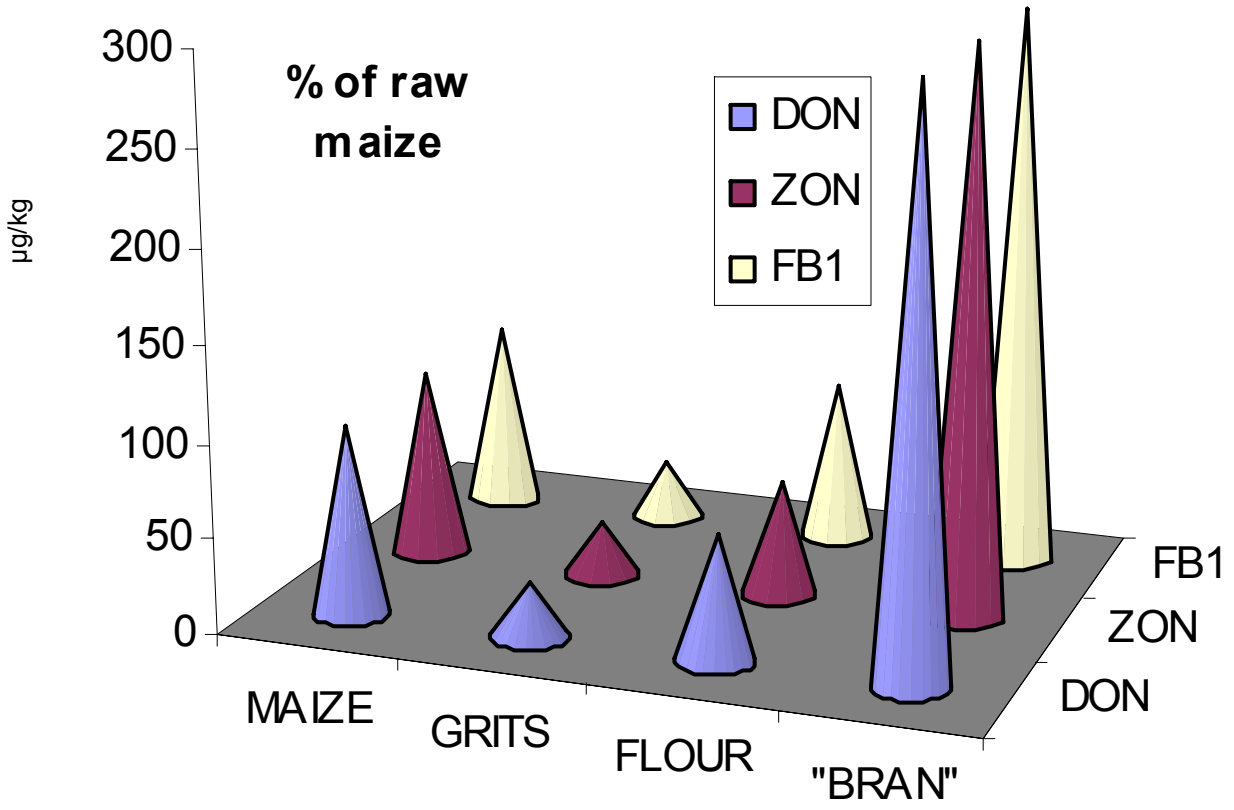
Mill 2 : French (Loire)

Mill 3 : Argentinean

Definitions - Maize Products

- Whole maize
- Coarse and flaking grits <1000-5000µ
- Medium to fine grits 300-1000µ
- Polenta 200-300µ
- Flour 200µ and less
- Other products (maize meal, germ, bran, broken grains

Mycotoxin Concentrations in Milling Streams



Mycotoxins in Milling Streams

Considerable variability from consignment to consignment making prediction difficult due to:

- Source of maize (geographic origin)
- Variety?
- Year/climate
- Condition of seeds
- Treatment prior to receipt at mill
- Mill machinery set up
- Precise properties required by the miller for products (including particle size and oil content)

Maize Milling- Key Points

- Mycotoxins are not lost during milling but merely redistributed in the different milling streams

- In relation to raw maize, concentrations are:
  - Considerably lowered in flaking grits
  - Slightly reduced in flour
  - Much increased in bran, meal etc.

- The distribution in milling streams is very variable

Maize Processes Studied

- Cornflakes- ‘cooking method’
- Snack 1- using maize grits
- Snack 2- using maize flour
- Tortilla- using maize flour
- Pilot scale extrusion-maize grits and flour*

*Maize flour extrusion study commences January 2007

Processing

For simplicity processes can be divided into:

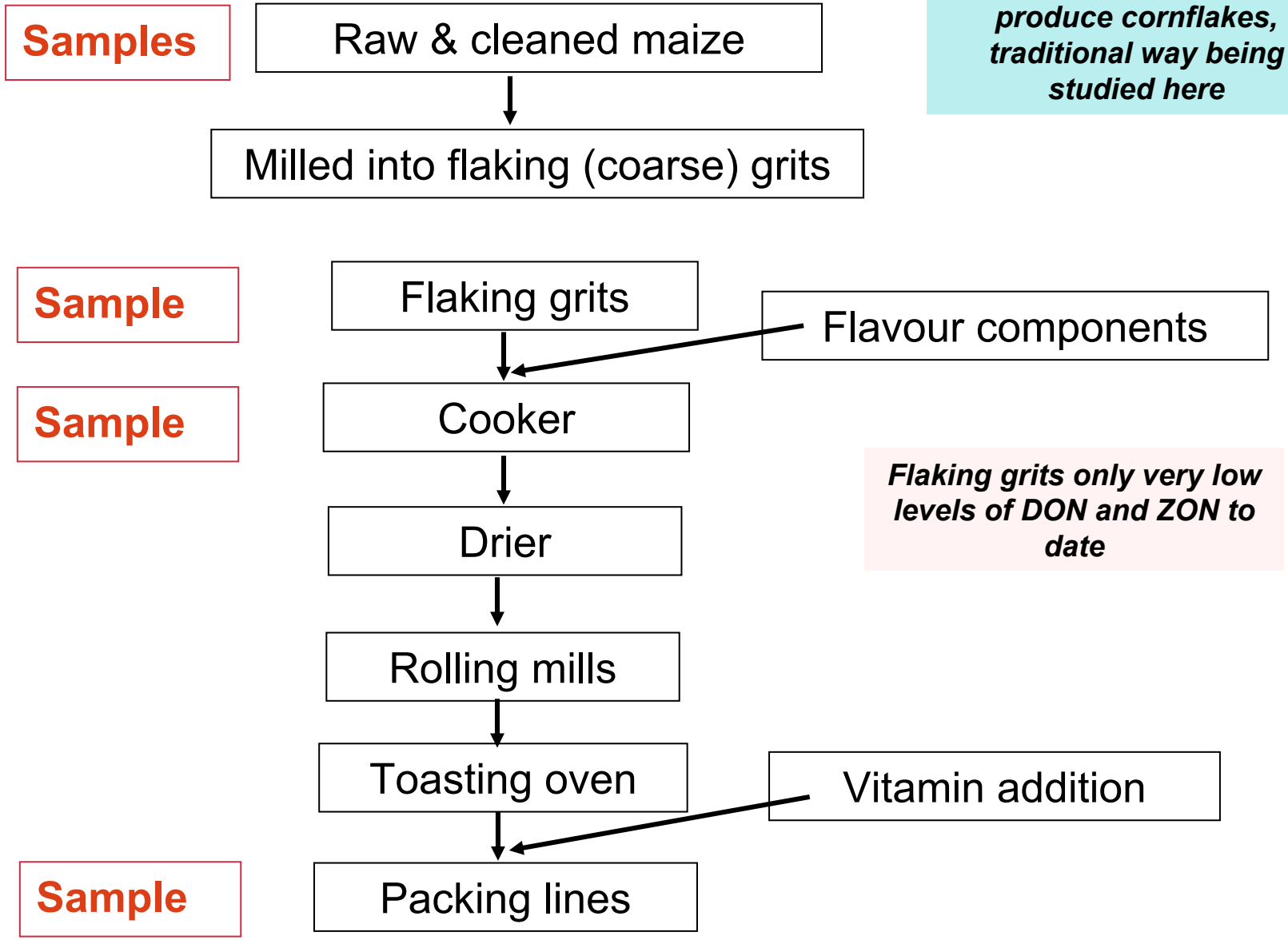
- Cooking with water under temperature and pressure
- Drying, toasting and baking
- Fermentation
- Frying in oil
- Extrusion

(this is short time flow process where raw material is forced through a die under temperature, pressure and shearing forces emerging to give a wide range of materials)

Cornflakes

- There are two ways of manufacturing cornflakes
- One uses maize flour and extrusion
- The other, maize grits and a cooking process
- The effect on mycotoxins is very different
- This study has examined the grit/cooking traditional process

Cornflake Production



DON and Fumonisin B1 in Cornflakes (n=14)

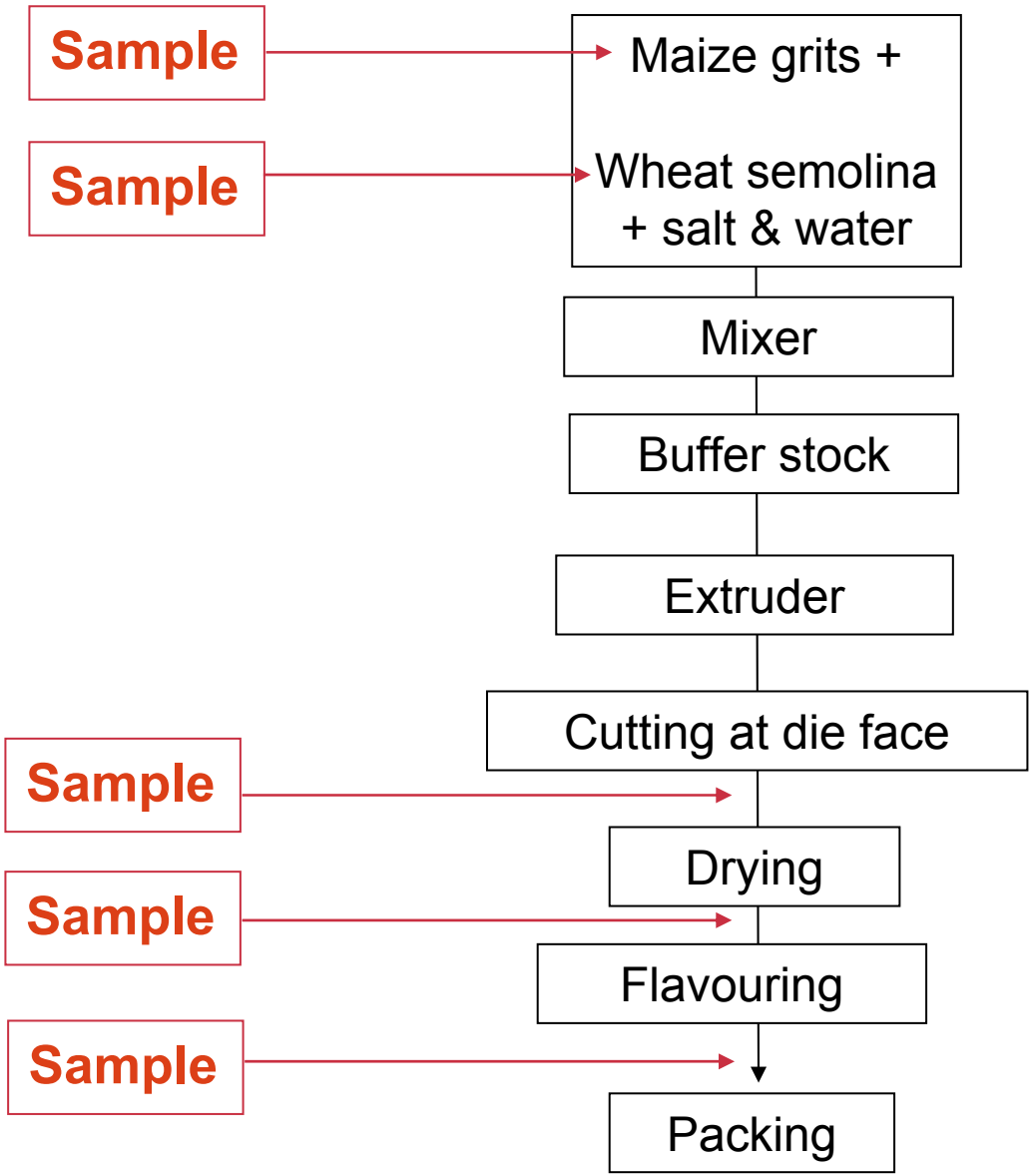
µg/kg		Grits	Cornflakes
DON	Mean	13	18
	Median	<10	14
	Above LoQ	*7*	*12*
	Range	<10-43	<10-44
FB1	Mean	105	<10
	Median	102	<10
	Above LoQ	*14*	*1*
	Range	49-181	<10-13

Traditional cooking method

Cornflakes Summary-Traditional Production Process

- Raw maize to flaking grits reduces mycotoxins by at least 70%
- Hence high levels in grits unusual
- Fumonisins destroyed in the cooker, but to what?
- Appears to be an small increase in DON in cornflakes: is this significant?
- Evidence suggests that mycotoxins in cornflakes made from maize flour by extrusion will be much higher
  - Mycotoxins in flour at higher concentrations than in grits
  - Pilot scale extrusion studies within current project
  - Limited market surveys (visual assessment)

Snack Product by Extrusion of Maize Grits

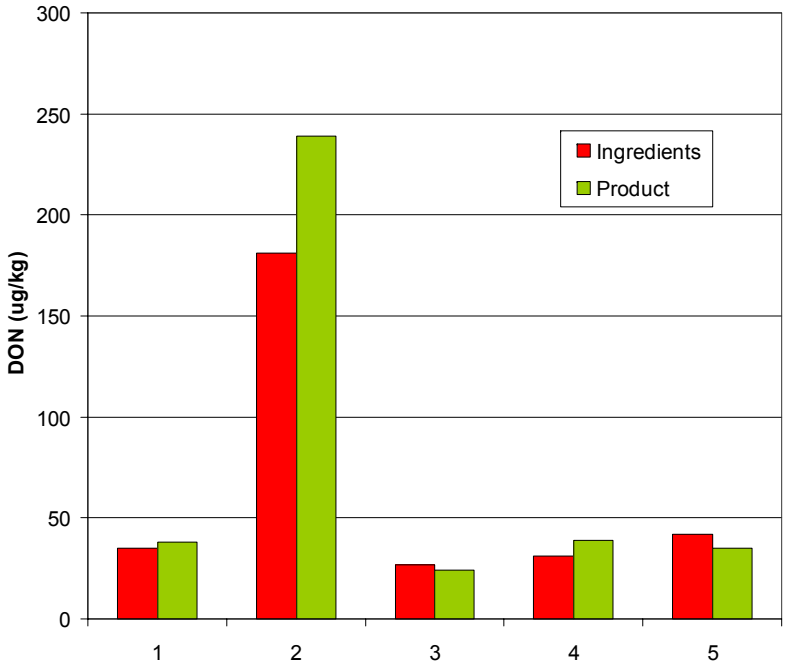


Snack Extrusion of Grits

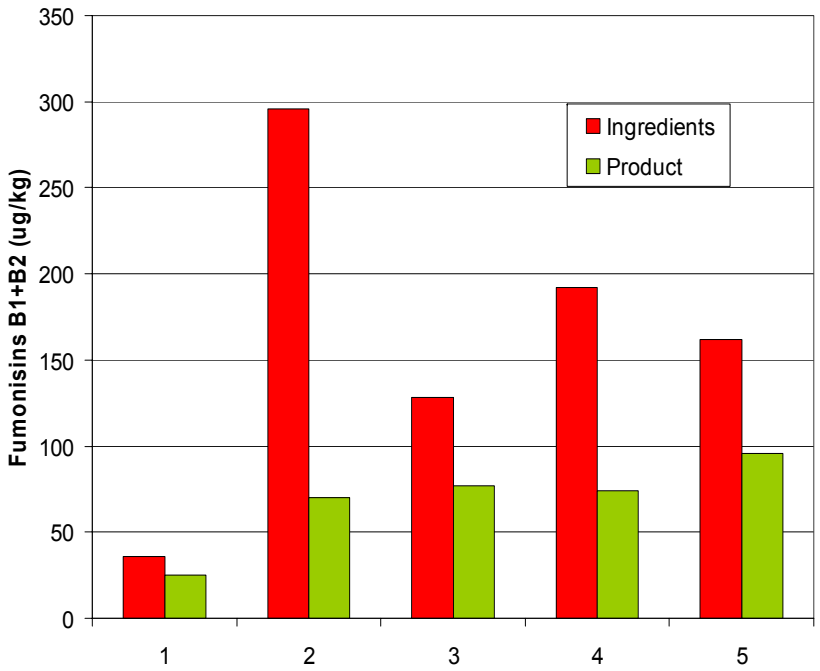
Occurrence of DON and Fumonisins in Cereal Ingredients and Extruded Snacks

corrected for moisture and cereal content)

DON



FB1 +FB2



Key findings:

- **No loss of DON**
- **Fumonisins, variable decrease range -30 to -70%**
- **(Direct extrusion has less effect on fumonisins – 14-27%)**

Pilot Scale Extrusion, Maize Grits-DON

Temp, C°	Moisture, %	*DON, mean	% reduction
140	17	129.6	9.6
160	17	128.0	10.7
180	17	124.1	13.4
140	21	138.6	3.3
160	21	136.5	4.8
180	21	139.2	2.9

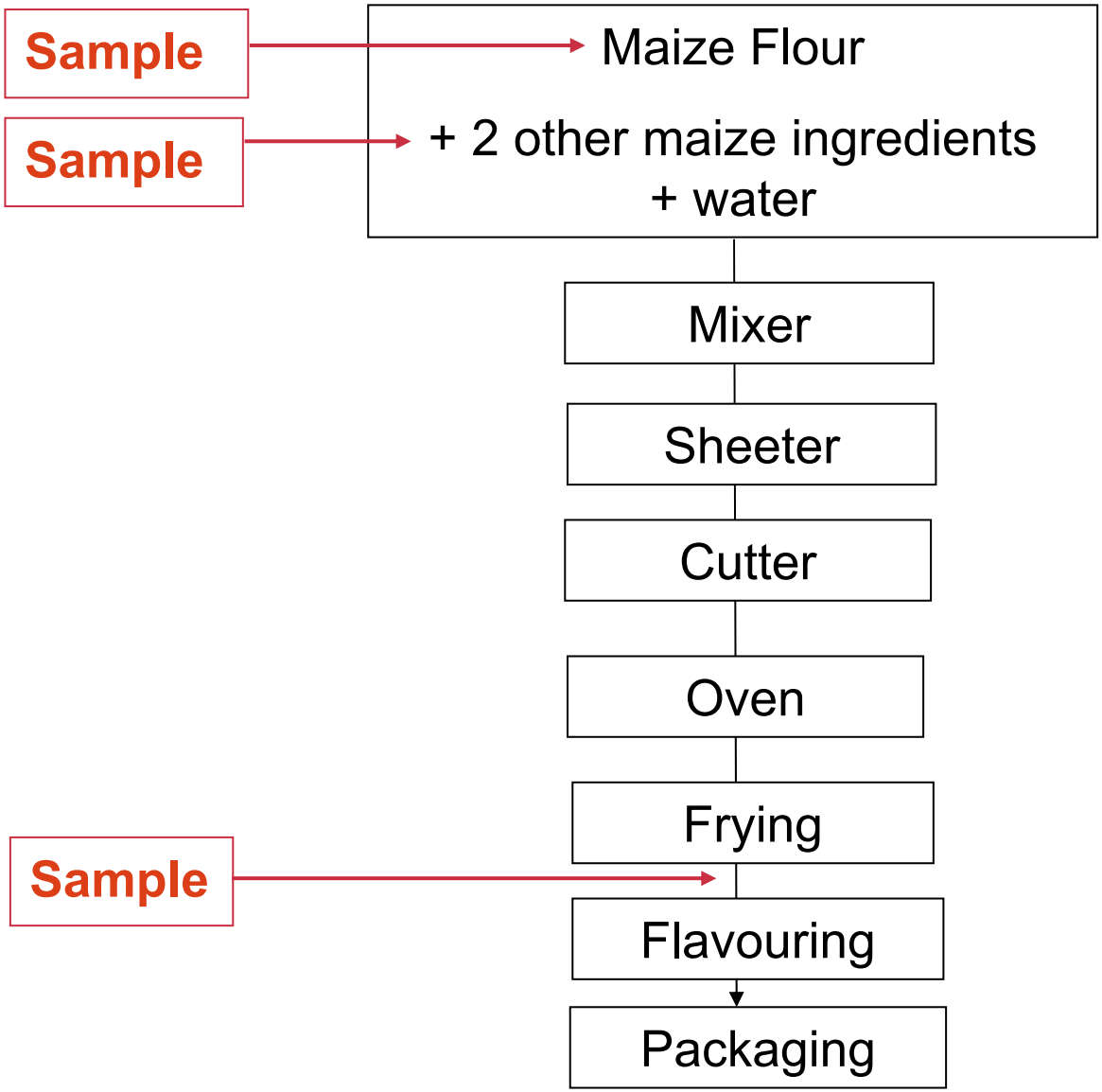
*DON in maize grits used was 143.3 µg/kg

Pilot Scale Extrusion, Maize Grits- FB1

Temp, C°	Moisture, %	*FB1, mean	% reduction
140	17	202.6	26.1
160	17	171.5	37.4
180	17	174.1	36.5
140	21	243.3	11.2
160	21	234.6	14.4
180	21	232.8	15.1

*FB1 in maize grits used was 274.1 µg/kg

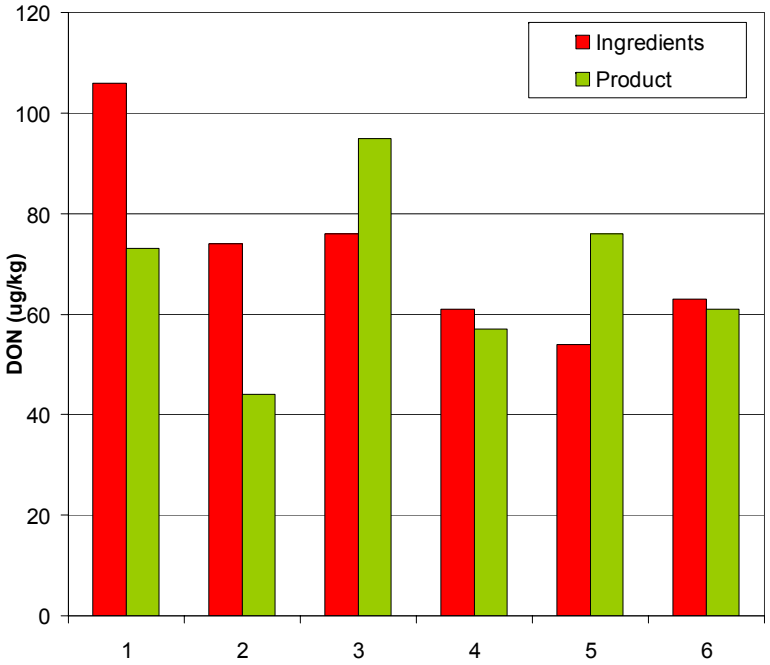
Tortilla Chip Process (Maize Flour)



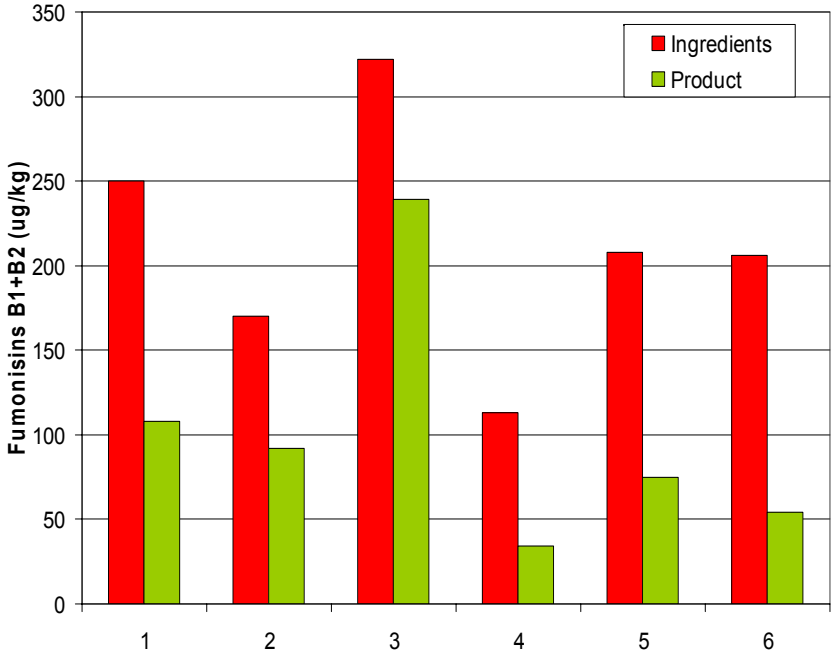
Tortilla Snacks

Occurrence of DON and Fumonisins in Maize Ingredients and Tortilla Snacks (corrected for moisture and cereal content)

DON



FB1 +FB2



Key findings:

- **No loss of DON**
- **Fumonisins, decrease --33 to -76%**

Pilot Scale Extrusion, Wholemeal Wheat-ZON

Temp, C°	Moisture, %	% reduction
140	21	17.4
140	17	2.9
140	15	+15.4
160	21	+2.5
160	17	+6.1
160	15	+4.8
180	21	+2.4
180	17	+10.2
180	15	+7.8

Wholemeal wheat used: †ZON=337, no data for ZON in maize

Maize Processing Summary

- **Effect on mycotoxins depends on the process**
- Ingredients may include several cereal products with different mycotoxin loading
- Fumonisins partially destroyed
- DON and ZON can be very stable
- Limited loss of mycotoxins during extrusion
- Cooking processes are more effective in reducing fumonisins
- Concentrations in products can be lowered because of the dilution effect of other ingredients

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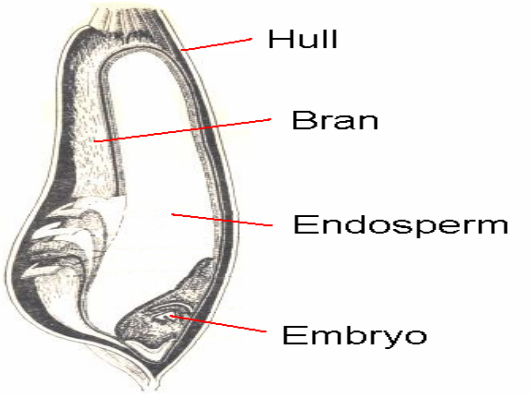
OATS

Oat Processing



Raw Oats ← Sample

↓

Cleaning

↓

Dehulling ← Sample

↓

Sample → Groat

Husks/By-product

↓

Sample → Kilning

Pellet ← Sample

↓

Colour sorted

↓

Sample → Light

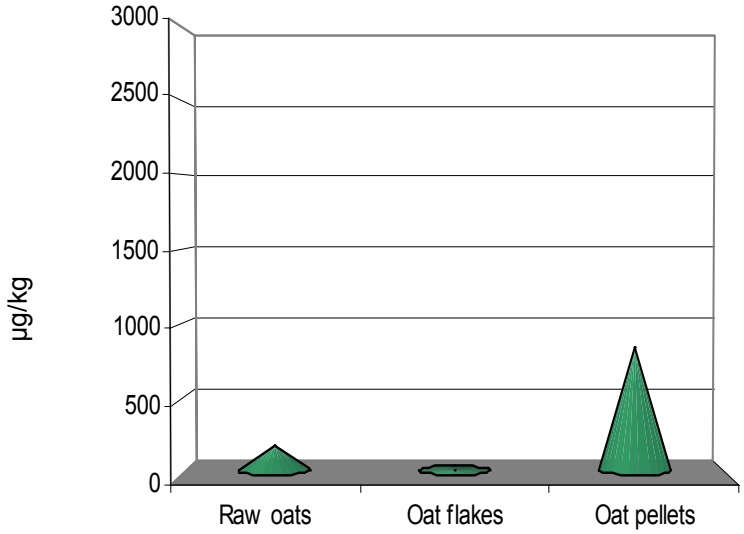
Dark

↓

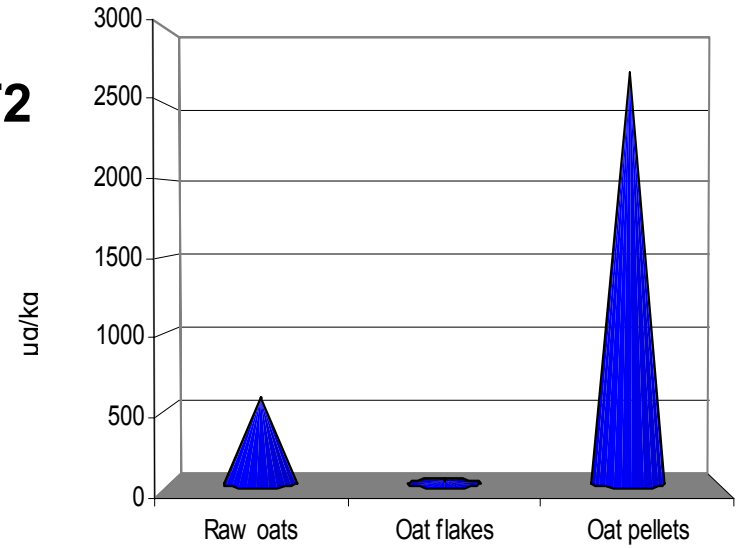
Oat Flakes ← Sample

Oat Processing (n=18)

T2



HT2



	(µg/kg)	Raw oats	Oat flakes	Oat pellets
T2	Mean	174	11	843
	Range	41-958	<10-33	160-6120
HT2	Mean	578	21	2700
	Range	115-3570	<10-45	324-23580

Oat Summary

RAW OATS

- T2 and HT2 occur in all samples
- Maximum 4,500µg/kg combined
- DON and NIV occur but at lower levels, DON higher in Scandinavian oats than UK oats
- Very low ZON

PROCESSING

- Processing of oats highly effective in reducing the level of all trichothecenes in the oat flakes
- **Reductions of >95% consistently achieved**
- Oat flakes maximum HT2=45µg/kg
- Mycotoxins concentrated in the pellet by-products for animal feed
- All toxins accounted for in process streams (mass balance)

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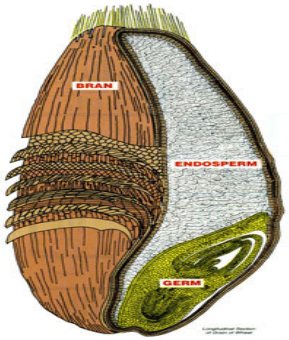
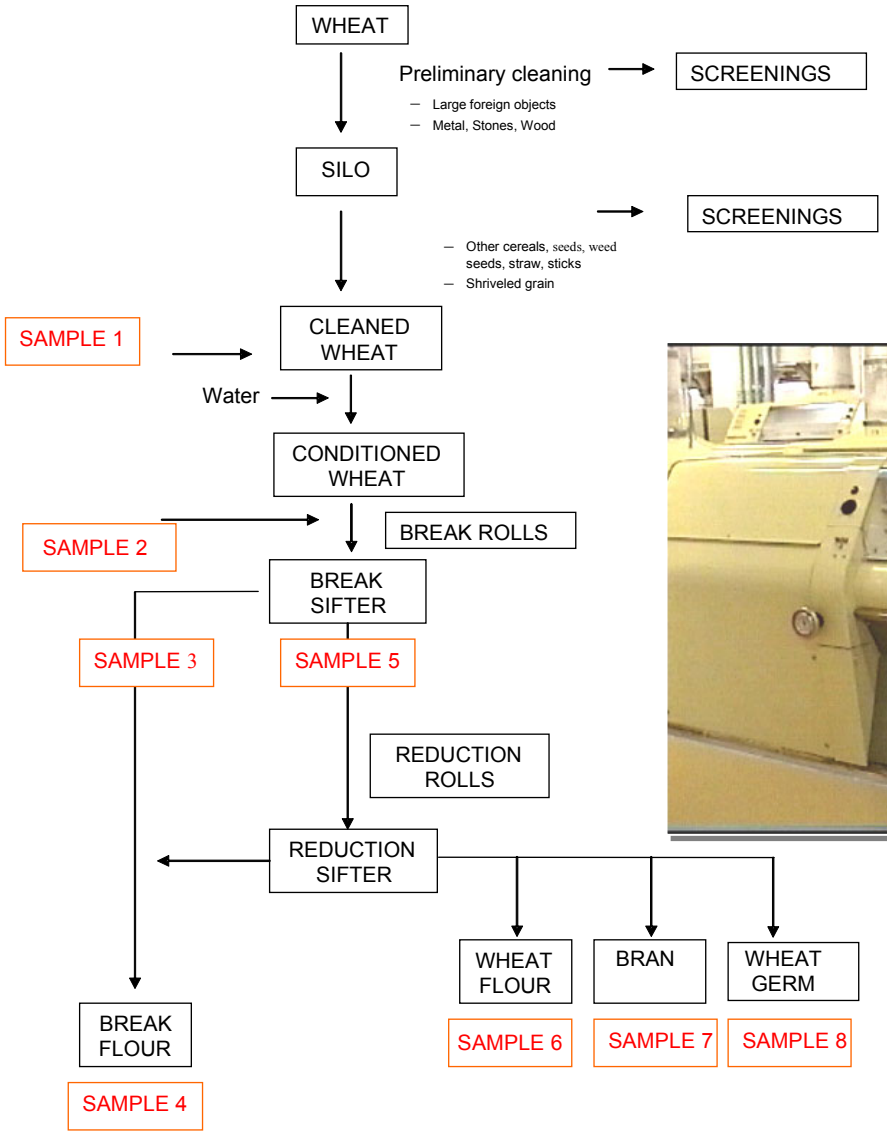
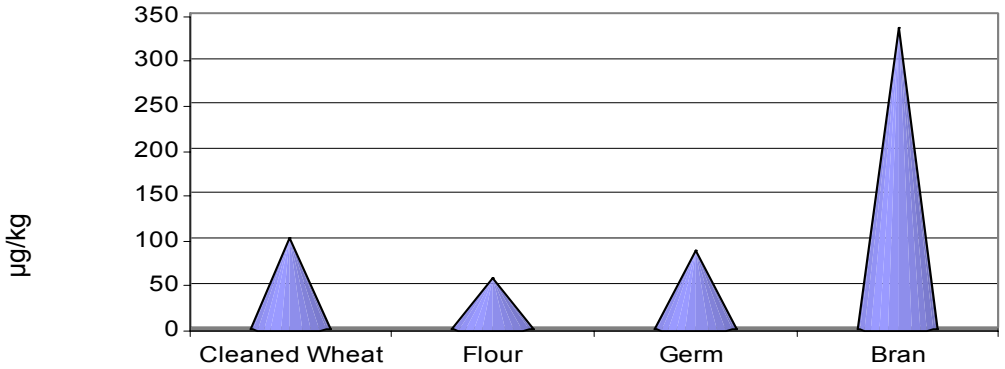
WHEAT

Wheat Milling





Wheat Milling: DON Occurrence in Milled Fractions (n=8)



DON (µg/kg)	Cleaned Wheat	White Flour	Germ	Bran
Mean	101	57	87	335
Range	19-481	15-288	21-375	42-1080

Key findings:

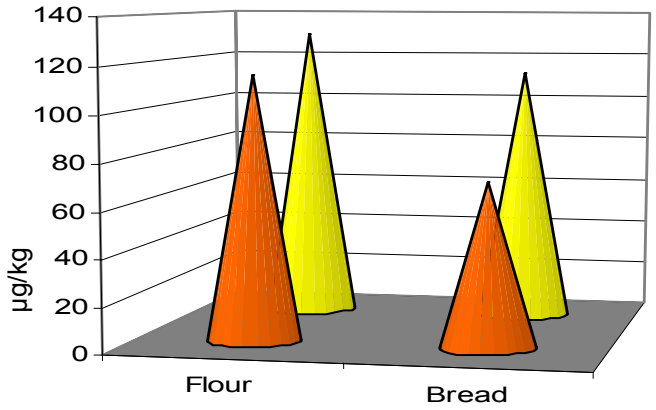
- **Mean DON reduction (cleaned wheat to white flour) = 30% (range 0-67%)**

Bread Baking

Occurrence of DON in flour and bread (as is and moisture corrected)

Oven temp= 244°C

WHITE BREAD (n=10)



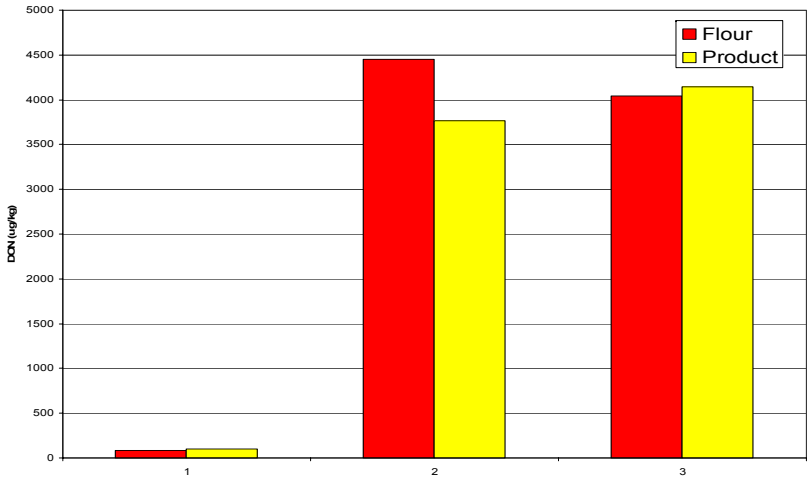
WHOLEMEAL BREAD (n=5)

DON (µg/kg)		White Bread			Wholemeal Bread		
		Flour	Bread	Reduction	Flour	Bread	Reduction
Mean	As is	115	71	**61%**	83	52	**63%**
	Moisture corrected	130	113	**87%**	94	84	**89%**
Range	As is	61-284	31-186		51-110	28-71	
	Moisture corrected	72-322	63-285		58-125	48-118	

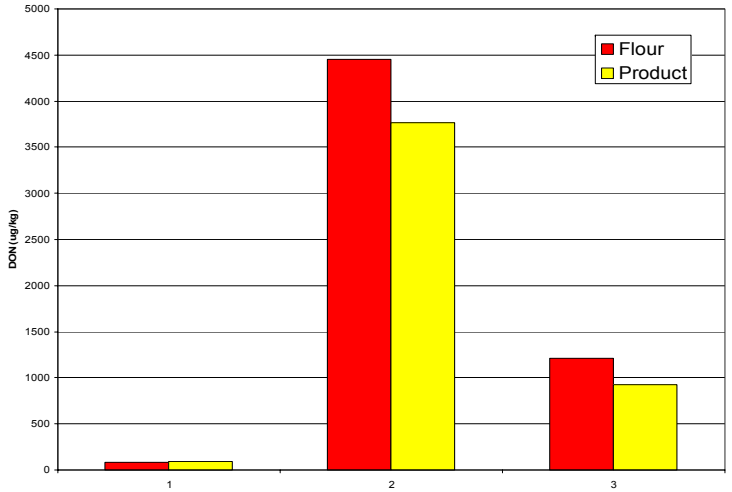
Biscuit and Cracker Baking

Occurrence of DON in wheat flour and biscuits and crackers (corrected for moisture and cereal content)

BISCUITS



CRACKERS



Sample 2 and 3 non commercial/trial samples (high DON)

Key findings:

- **No loss of DON during biscuit baking (oven temp approx 230°C)**
- **Small average decrease during crackers bake (-10%)**
- **No difference in fate from commercial and trial flour**

Wheat Summary

CLEANED WHEAT

- DON detected in all samples, ZON infrequently detected
- Levels of both toxins below EU regulatory limits

EFFECT OF MILLING

- DON is reduced in white flour (mean 30%, range 0-67%)
- Increased in bran
- Other trichothecenes show the same pattern
- All toxins accounted for in the milling stream

BREAD BAKING

- After allowing for the dilution effect between flour and bread there is a small (approx 12%) decrease in DON

BISCUIT and CRACKER PRODUCTION

- No loss of DON during biscuit making, small decrease in crackers

Future Plans

- Complete studies
  - Extrusion-pilot scale
  - Wholewheat breakfast cereals
  - Cake making
- Identification of any breakdown products
- Investigation of presence of any bound toxins
- Effects of climate, harvest conditions and agronomic practice on mycotoxin in wheat

Conclusions

- Appreciation of the effects of processing on mycotoxin level (and nature) is crucial in estimating (and controlling) human exposure
- Important to study food processing on a commercial scale using naturally contaminated raw materials
- Mycotoxins are generally quite stable and survive many processes
- Oats = consistent high reduction
- For other cereals processing cannot be relied on to consistently reduce the mycotoxin levels in intermediates and final food products

Acknowledgements

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