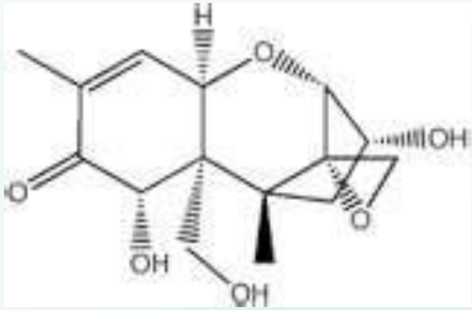
Stability of the Trichothecene, Deoxynivalenol in Processed Foods and Wheat Flake Cereal



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Contributors

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DON Toxicity in Animals

- ✓ **Pigs [Schlatter, Toxicol. Lett, (2004)]**
  - **Feed refusal, vomiting, poor production**
  - **NOAEL 40 – 60 μg/kg BWt**

- ✓ **Mice [Iverson *et al*., Teratog. Carcinog. Mutag. (1995)]**
  - **Reduced feed intake; males fed ≥ 5 ppm**
  - **Decreased body weight; ≥ 1 ppm**
  - **Immunoglobulin levels altered**
  - **NOAEL = 100 μg/kg BWt/day**

- ✓ **Mice [Pestka *et al.*; Tryphonas *et al.;* others] & rats**
  - **Decreased protein synthesis (ribotoxic response)**
  - **Disrupted signaling for growth**
  - **Immune (IgA) nephropathy in mice**
  - **Disrupted signaling for immune functions, cell survival & replication**

DON & Humans

- ✓ **Effects uncertain**
  - **Reports of acute illness, particularly from Asia**
    - **> Gastrointestinal symptoms**
    - **> General discomfort, dizziness etc.**
- ✓ **People are exposed:**
  - **DON excretion in urine & consumption of cereal products correlated (Turner et al. EHP 116, 2008)**
  - **Some exposures exceed EU TDI of 1 µg/kg BWt/day**
  - **DON found in cereal products (breads, pasta, beer, etc)**
- ✓ **Concern for infants and adolescents**

Guidelines / Regulations for Food

- ✓ US FDA Guidance for Industry = 1 ppm (finished products)
- ✓ Japan = 1.1 ppm in unpolished wheat
- ✓ EU = Maximum allowed levels are variable:

Item	Max ppm
• Unprocessed durum wheat	1.75
• Unprocessed wheat	1.25
• Cereal flour, pasta	0.75
• Various products (bread)	0.50
• Processed foods for infants	0.20

Rationale

- ✓ **Minimizing DON in foods desirable**
  -- food safety
  -- guidances / limits
- ✓ **DON is water soluble and boiling reduces amount in noodles**
- ✓ **DON is generally heat stable under routine baking conditions**
  BUT reported results for breads vary: specific conditions likely important
- ✓ **Use conditions that are relevant to US food industry**
- ✓ **Other products not well studied**

Purpose: *to determine the chemical stability of DON during the production of common cooked wheat products under commercially-relevant conditions.*

Flour

- ✓ **Sugar Cookies (AIB)**
- ✓ **Snack Crackers (AIB)**
- ✓ **White Pan Bread (AIB) (sponge & dough)**
- ✓ **Pretzels (FL)**
- ✓ **Cake Donuts (AIB)**

Whole Wheat

- ✓ **Cereal Flakes**
- ✓ **Intermediate products**
  - **Dough**
  - **Pellets**

*** Provided by General Mills**

Flour Products: Cooking Conditions

- ✓ **Cookies: Bake 25-30 min at 163 ^{o}C**
- ✓ **Crackers: Bake ~6 min at 227-252 ^{o}C (top), 193-232 ^{o}C (bottom)**
- ✓ **Pretzels: Proprietary (dough, extrusion, bake, brown in caustic bath, bake)**
- ✓ **Donuts: Fried 45 sec/side at 190 ^{o}C**
- ✓ **Bread: Fermentation (4 hr at RT); Bake 18 min at 227 ^{o}C.**

Wheat Series: Product Stream (proprietary)

Wheat

Grind, mix ingredients

Dough

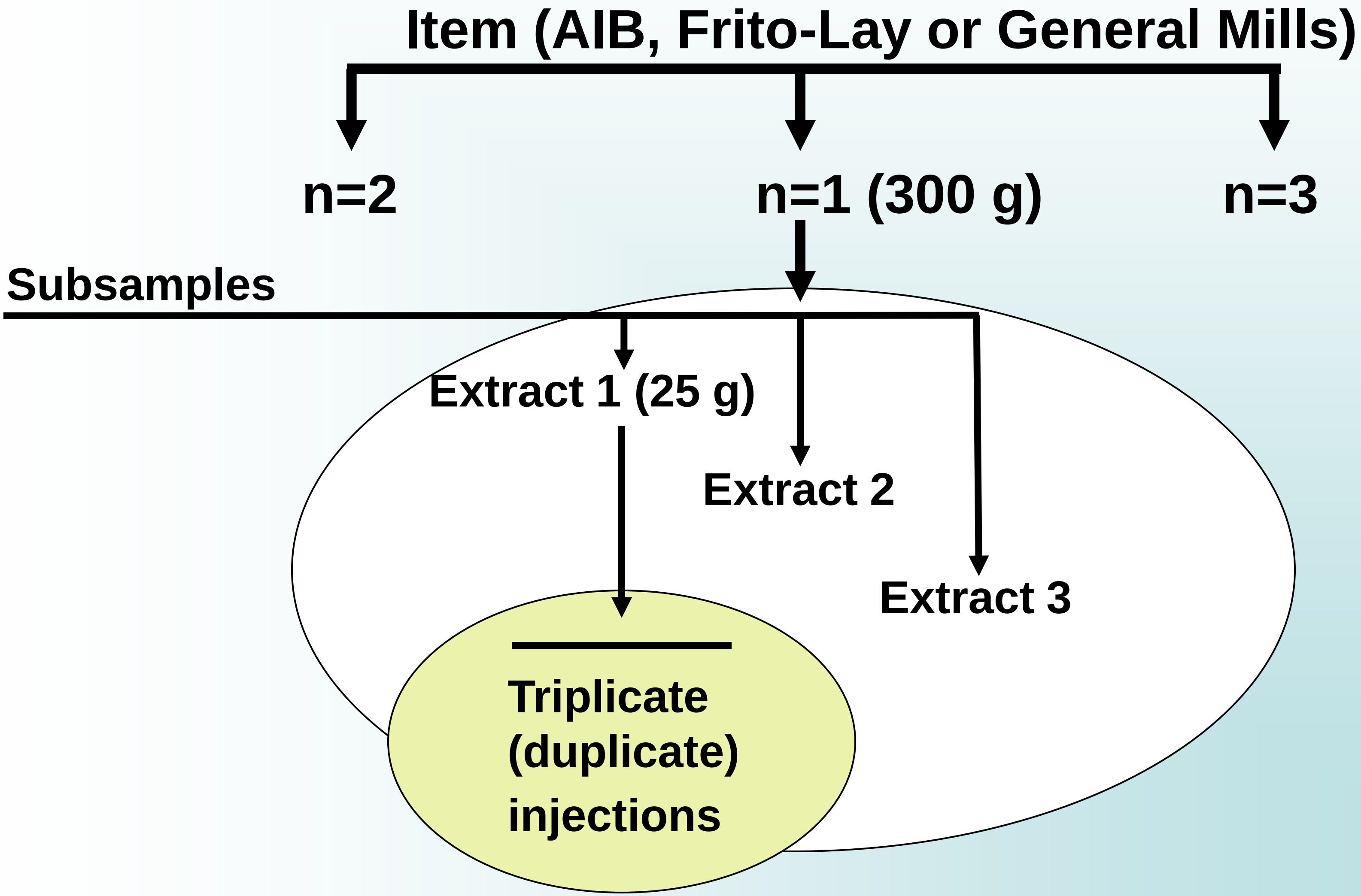
Extrude

Pellets

Roll

Cereal Flakes

EXPERIMENTAL OVERVIEW



ANALYSIS PROTOCOL

300 g Flour, Wheat & Products

↓

1. **Freeze-dry - defat with hexane – dry**
2. **Mix & reduce - ball mill - recombine**

↓

25 g Sample

↓

3. Extract with CH_3N:$H_2$0 (84:16) (100 ml)
4. Cleanup: Romer MycoSep® 227 Trich+
5. Dry & reconstitute with TMS derivitizing agent

↓

6. Quantification (ppm) : Gas chromatography - EC

↓

Mass balance estimation

Spike/Recovery & Product Yield (Flour Products)

Spike/Recovery*	Yield ***
All flour based items = 80%	
Flour = 86% (AIB) 68% (FL)**	1.0
Cookies = 91%	1.6
Crackers = 108%	1.1
Pretzels = 68%	0.96
Donuts = 52%	2.8 (fat uptake)
Bread = 82%	1.6

* triplicate

** same result obtained after correction for recovery

*** relative amount of product produced per g flour in recipe

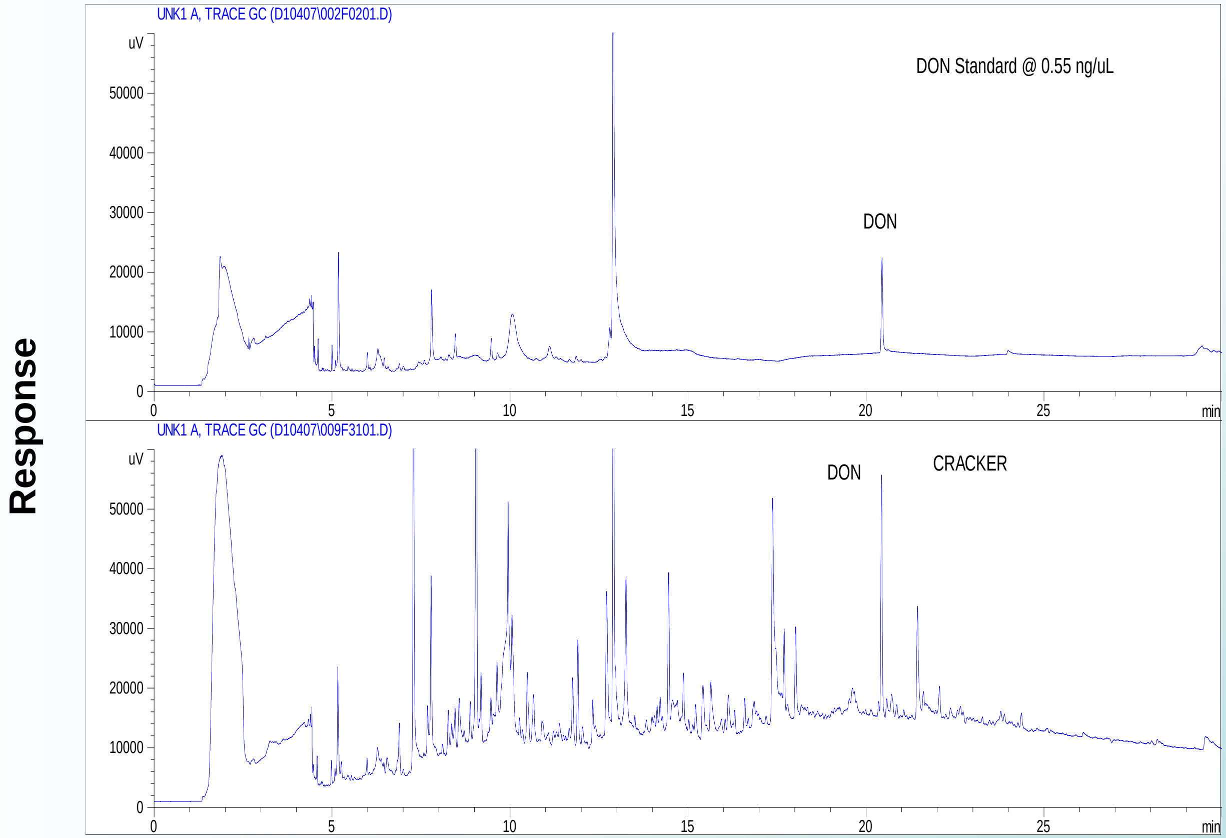
Spike/Recovery & Product Yield (Wheat Series)

Spike/Recovery *	Yield **
All items = 90%	
Wheat = 91%	1.0
Dough = 82%	1.47
Pellets = 90%	1.47
Flakes = 95%	1.06

* triplicate

** relative amount of product produced per g flour in recipe

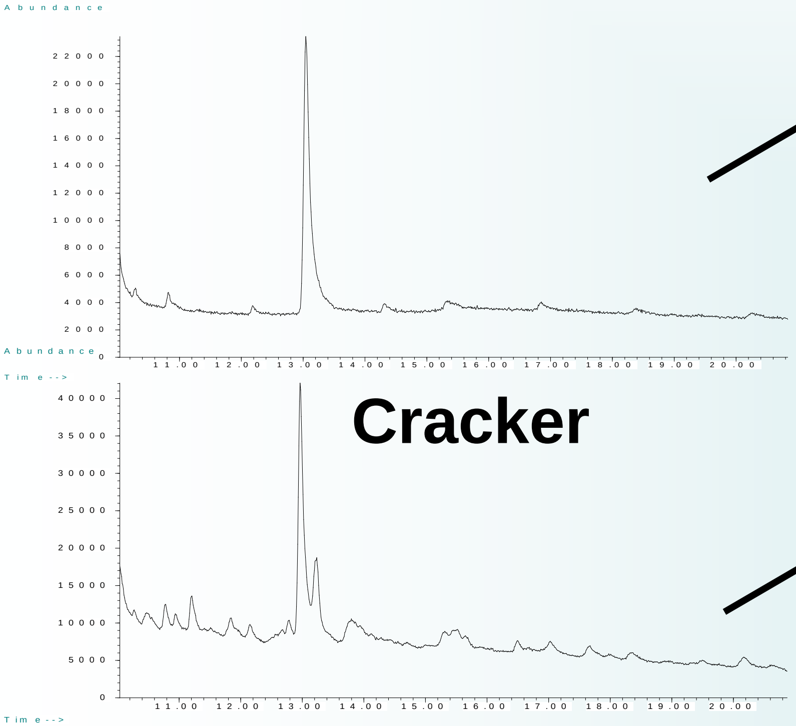
GC-EC CHROMATOGRAMS

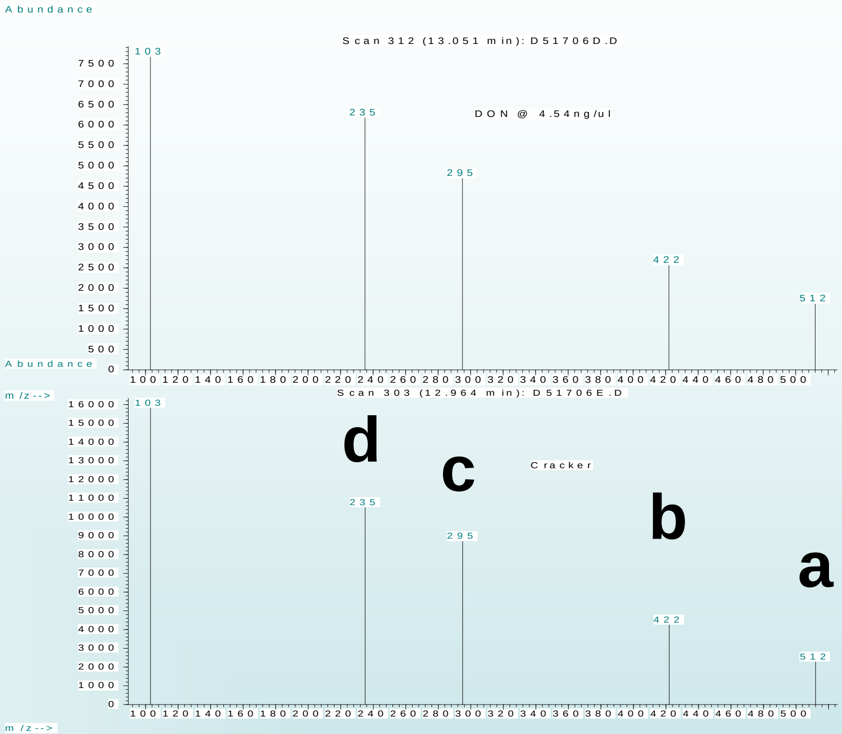


“Spiking” & mass spectral analysis of peak: results consistent with DON

GC-MASS SPECTROMETRY

DON Standard

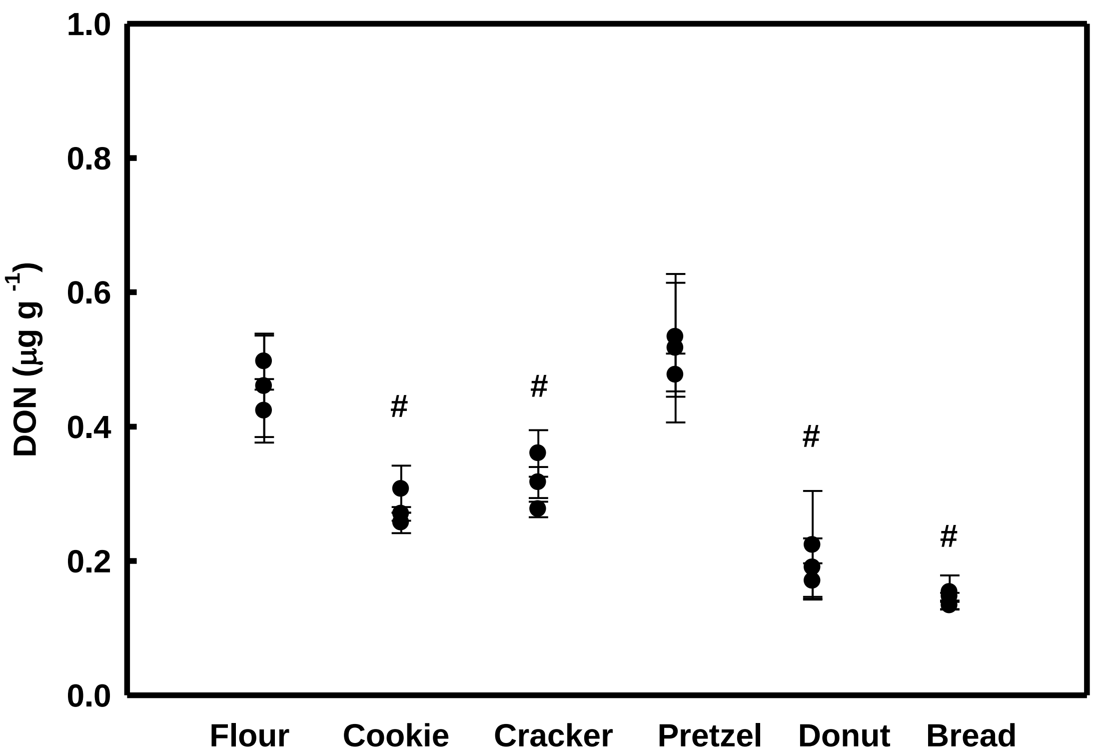




m/z
a; =512
b; =422
c; =295
d; =235

GC-MS (SIM)

DON CONCENTRATIONS
(Finished Flour Products)



Each value = mean + SD, n = 3

ppm (= µg g^{-1})

Flour = 0.46 + 0.06

Cookie = 0.28 + 0.03

Cracker = 0.32 + 0.04

Pretzel = 0.51 + 0.05

Donut = 0.20 + 0.07

Bread = 0.14 + 0.01

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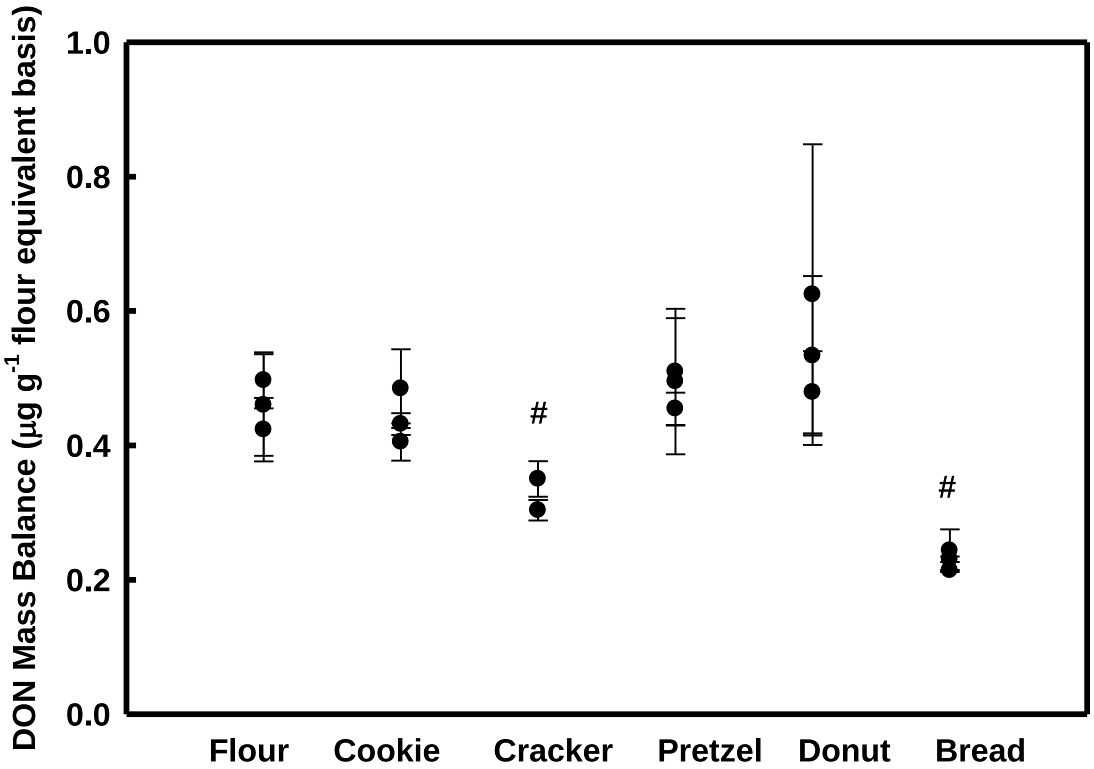
n = 9

MASS BALANCE ESTIMATE
(Finished Flour Products)



Each value = mean ± SD, n = 3

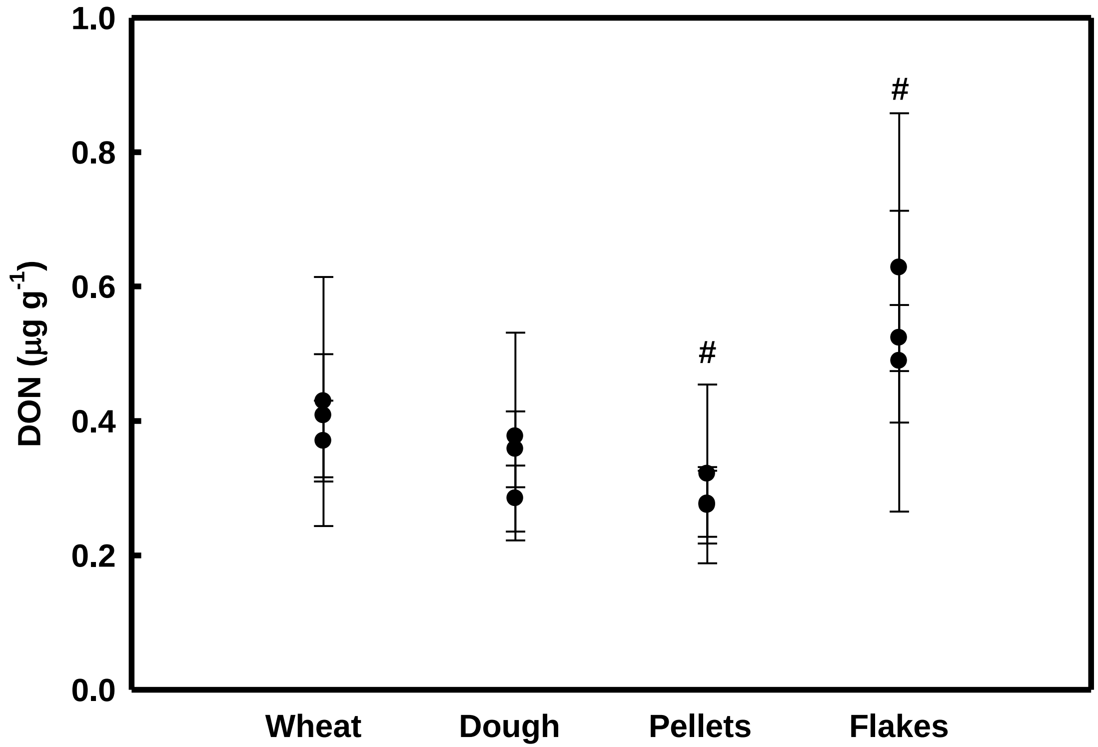
ppm (= µg g^{-1})

Flour = 0.46 ± 0.06
Cookie = 0.44 ± 0.05
Cracker = 0.35 ± 0.05
Pretzel = 0.49 ± 0.07
Donut = 0.55 ± 0.15
Bread = 0.23 ± 0.02

----

n = 9

DON CONCENTRATIONS (Wheat Series)



Each value = mean ± SD, n = 3

ppm (= µg g^{-1})

Wheat = 0.40 ± 0.11

Dough = 0.34 ± 0.10

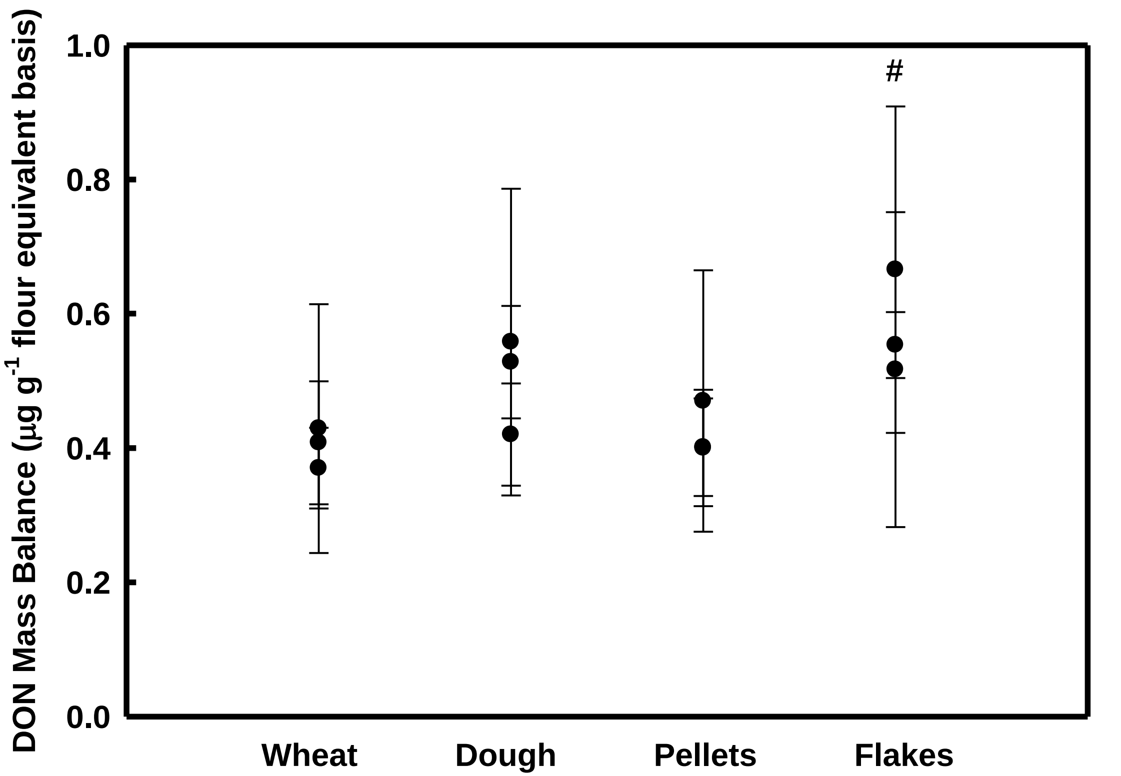
Pellets = 0.29 ± 0.08

Cereal Flakes = 0.55 ± 0.17

----

n = 9

MASS BALANCE ESTIMATE (Wheat Series)



Each value = mean ± SD, n = 3

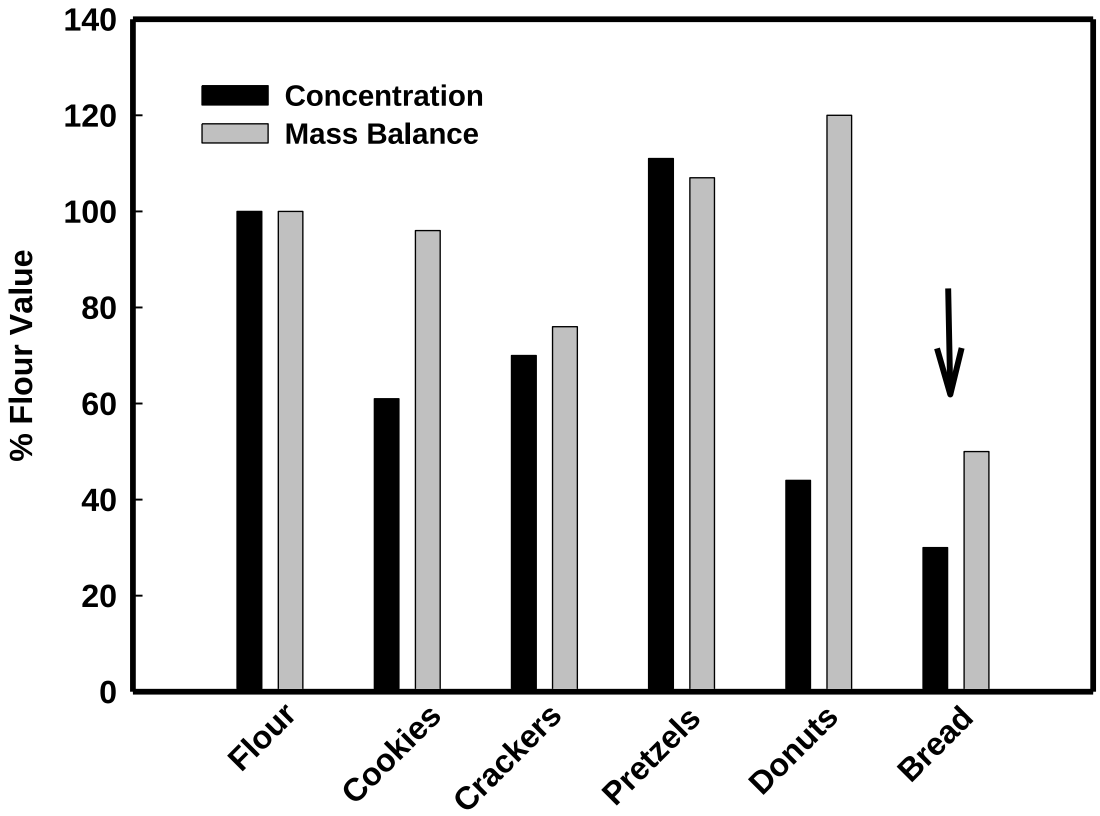
ppm (= µg g^{-1})

Wheat = 0.40 ± 0.11
Dough = 0.50 ± 0.14
Pellets = 0.42 ± 0.12
Cereal
Flakes = 0.58 ± 0.18

----

n = 9

Summary: Finished Flour Products



What Happened in Bread?

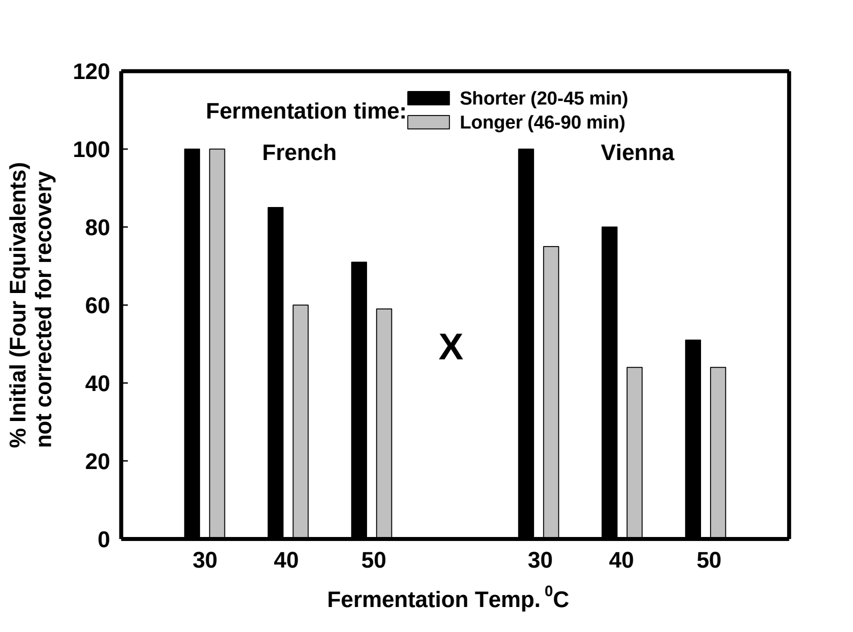
- ✓ **Don't know: chemical fate was not pursued**
- ✓ **Thermal decomposition likely**
  - **-- norDON A**
  - **-- norDON B** | **(Bretz *et al*., 2006)**
  - **-- norDON C**
- ✓ **Found in cookies, crackers, breads (Germany)**
  - **-- norDON A most prevalent; found in 66% of the items at concentrations ~ 10–25 % of DON**
  - **-- norDON compounds not toxic in an *in vitro* assay using IHKE cells at 100 x EC_{50} of DON**
- ✓ **Suggests that – at least in this case – degradation might be beneficial**
- ✓ **Does not rule out production of unknown toxins**

What Happened in Bread?

- ✓ **Fermentation step also unique to bread in the series of flour products examined**

  -- 2.5 % baker's yeast, 4 hr at RT

- ✓ **Neira *et al.* (1997), Samar *et al.* (2001) reported that fermenting contributed to reducing DON in bread (41 – 56 %).**



X = our result

What Happened in Bread?

✓**Bergamini *et al.* (2010) studied baking and fermentation effects on DON during bread making**

1. Fermentation time 2. Fermentation temperature

3. Baking time 4. Baking temperature

-- Fermentation increased DON in the dough

-- Baking time/temperature determined reduction ⬅

-- Initial DON concentration mattered; more reduction was found at higher initial concentrations **

✓ **Industrial experiment: flour DON = 0.33 µg g^{-1}; fermentation conditions held constant (32 ^{o}C/56 min); varied baking time/temperature from 170 – 225 ^{o}C for 3 – 13 min.**

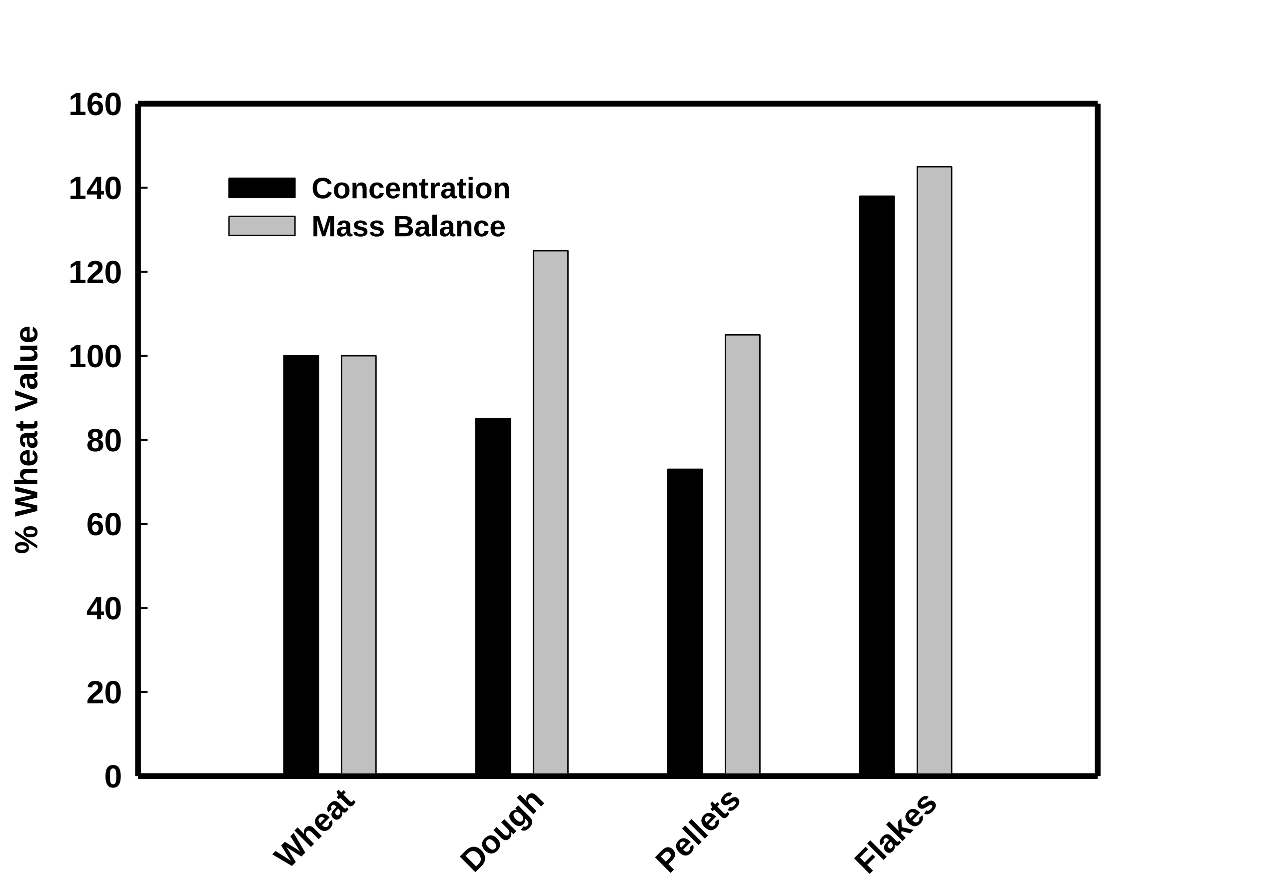
-- Final DON in breads ranged from 0.27 -0.32 µg g^{-1}; corresponded to 81 to 95 % of initial DON amount

**** conflicts conclusion of Neira *et al.* 1997**

Bread Studies Comparison

	% Remaining
✓ El-Banna et al. (1983):	115
✓ Scott et al. (1983,84):	102, 107
✓ Abbas et al. (1985):	36, 84
✓ Seitz et al. (1986):	53-125
✓ Neira et al. (1997):	56
✓ Konishi et al. (2006):	108
✓ Sugiyama et al. (2009):	74 (10 - >300)
✓ Valle-Agarra et al. (2009):	52 (spiked wheat)
✓ Bergamini et al. (2010):	81-96
✓ This study (2010):	50

Summary: Wheat Series



What Happened in Cereal?

- ✓ **Don't know: chemical fate was not pursued**
- ✓ **Release of DON from conjugates is possible**
  - **-- DON-glucoside (Berthiller *et al.*, 2009)**
- ✓ **Occur naturally in wheat (23/23 samples positive)**
  - **-- variable amounts reported**
  - **-- 12%-46% relative to DON (Berthiller *et al.* 2005; 2009)**
- ✓ **Hypothesis: DON can be released from conjugates under some conditions – should be tested.**
- ✓ **CAUTION: The variation (large SD) found in our analysis does not rule out that the increase was incidental (heterogeneity of DON in wheat or from sampling protocol) – needs more work**

SUMMARY & CONCLUSIONS

- ✓ Significant amounts of DON survive during production of most products
- ✓ Reduced DON in bread
  - Concentration (ppm) = ~ 70% reduction
  - Mass balance = 50% reduction
  - Both “loss” and dilution contribute
- ✓ Reduced DON (ppm) in finished donuts due entirely to dilution and fat uptake
- ✓ DON stable or increased somewhat in finished wheat flake cereal

We have a lot of work to do to understand

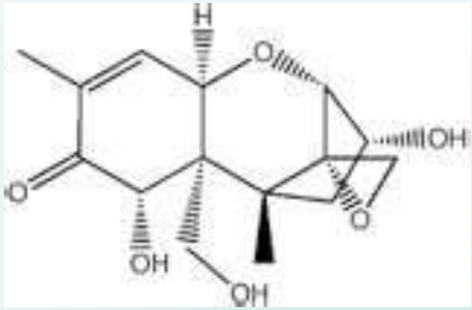
- DON-food matrix interactions
- identity, occurrence, toxicity of decomposition products

Working Hypothesis

DON 3-glucoside

Other conjugates, bound forms

Free DON



norDONs

Matrix binding?

Other decomposition products

Bioavailability & Toxicity

ACKNOWLEDGEMENTS

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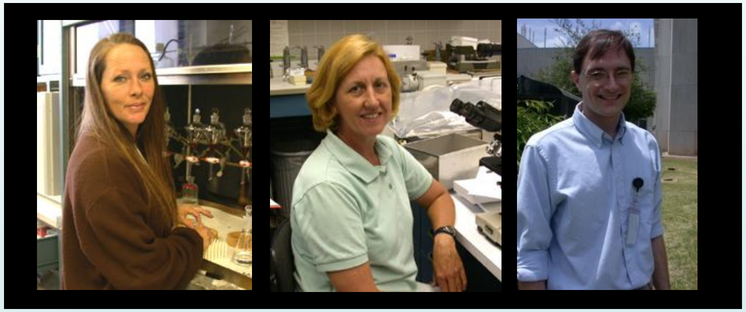
- Emma (Crickett) Wray
- Norma Stewart
- Trevor Mitchell



✓ Technical Personnel

- American Institute of Baking, Manhattan, KS
- Frito-Lay Inc., Plano, TX
- General Mills Inc., Minneapolis, MN

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THANKS
FOR YOUR ATTENTION

Voss KA, Snook ME (2010)
***Food Additives and Contaminants* 27:1694-1700**

USDA

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