Ochratoxin A In Freshly Harvested and Stored Durum and Hard Red Spring Wheat

Julie Kuruc, Ph.D. Candidate
NDSU – Great Plains Institute of Food Safety
February 12, 2013
Outline

- Background
- Objectives
- Results
- Discussion
- Research Significance
- Future Research

Vincent van Gogh, *Wheat Field*, 1888
Background

- Mycotoxins - secondary metabolites produced largely by *Aspergillus*, *Fusarium*, and *Penicillium* species
  - Produced on stored foods under sub-optimal storage conditions

- Ochratoxin A (OTA) - most common and potent
  - Toxic to kidney, liver, nerves, nerve cells
  - Immunosuppressant, possible human carcinogen
    (Khoury and Atoui, 2010)

- OTA is produced by:
  - *Penicillium* (*P. verrucosum*, *P. nordicum*)
    - Temperate climates (<30°C)
    - Cereals/cereal products
  - *Aspergillus* (*A. ochraceus*, *A. niger*, *A. carbonarius*, others)
    - Tropical climates
    - Coffee, wine, grape juice
      (Bogs et al, 2006; Geisen et al, 2006; Mateo et al, 2011)
OTA contamination can occur prior to harvest
- Environmental conditions
- OTA-producing strain
- Commodity
  (Imperato et al, 2011; Sánchez-Hervás et al, 2008)

Grains are stored for a length of time prior to being sold and processed
- Insufficient drying
- Improper air circulation
- Age of the grain
  (Ozden et al, 2012)
Background

- First report of OTA in foods – corn (Shotwell et al, 1969)
- OTA is present in many types of commodities and foods

<table>
<thead>
<tr>
<th>Country</th>
<th>Product</th>
<th>OTA Limit (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU, Turkey, Canada</td>
<td>Unprocessed cereals</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Products from unprocessed cereals, intended for human consumption</td>
<td>3</td>
</tr>
<tr>
<td>India</td>
<td>Raw wheat, barley and rye</td>
<td>20</td>
</tr>
<tr>
<td>China</td>
<td>Cereals</td>
<td>5</td>
</tr>
<tr>
<td>Israel</td>
<td>Cereals, cereal products</td>
<td>50</td>
</tr>
<tr>
<td>Iran</td>
<td>Wheat</td>
<td>5</td>
</tr>
<tr>
<td>USA, Japan, Mexico, Australia</td>
<td>No advisory limits or action levels in any commodity</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from: Canadian Grain Commission, 2011; European Commission, 2006; European Mycotoxins Awareness Network, 2012
Provide data to assess the risk of OTA exposure from a variety of foods in the U.S.

**USDA NIFA grant**
- Identify primary sources of OTA exposure
- National survey - commodities & domestic/imported products

<table>
<thead>
<tr>
<th>Location</th>
<th>Commodity/Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDSU/Fargo, ND</td>
<td>Wheat, barley</td>
</tr>
<tr>
<td>UNL/Lincoln, NE</td>
<td>Corn, coffee, cocoa, meat</td>
</tr>
<tr>
<td>UI/Moscow, ID</td>
<td>Breakfast cereals</td>
</tr>
<tr>
<td>IIT-NCFST/Bedford Park, IL</td>
<td>Wine, beer, milk, infant formula, baby cereal</td>
</tr>
<tr>
<td>USDA/Albany, CA</td>
<td>Dried fruits, nuts</td>
</tr>
</tbody>
</table>
Why wheat?

- Cereals are the major dietary source of OTA in the human diet
  - Durum – pasta products
  - HRS – bread and other baked goods
    (Kuiper-Goodman et al, 2010)

- In 2000, the average person in the U.S. consumed ~146 lb (~66 kg) of wheat flour
  (USDA, 2000)
Objectives

1. Determine the prevalence of and quantify OTA in *freshly harvested* durum and HRS wheat.

2. Determine the prevalence of and quantify OTA in *stored* durum and HRS wheat.
Freshly Harvested Wheat

- 2011 U.S. regional wheat surveys
  - 100 g sub-samples
  - Stored at -18°C until analysis

- Detection and quantification of OTA
  - AOAC Official Method 991.44
  - HPLC with fluorescence detection
    - LOD = 0.06 ppb (durum)
    - 0.09 ppb (HRS)

<table>
<thead>
<tr>
<th>Wheat Type</th>
<th># Samples</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Durum</td>
<td>103</td>
<td>ND, MT</td>
</tr>
<tr>
<td>HRS</td>
<td>457</td>
<td>MN, MT, ND, SD</td>
</tr>
</tbody>
</table>
Methodology

Stored Wheat

- Collected from processing companies and elevators
  - 2011 & 2012
  - Same regions as freshly-harvested samples
  - 150 g per sample, collected upon receipt
  - Storage length = length of time between the first full month of harvest in the given crop year and the date sampled at processor/elevator
  - Stored at -18°C until analysis

- Detection and quantification of OTA
  - AOAC Official Method 991.44
Stored Wheat – Samples Collected

- Location
- Crop Year (2011/2012)
- Storage Time

<table>
<thead>
<tr>
<th>State</th>
<th>Durum N (%)</th>
<th>HRS N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>0 (0)</td>
<td>5 (3.5)</td>
</tr>
<tr>
<td>Montana</td>
<td>0 (0)</td>
<td>13 (9.1)</td>
</tr>
<tr>
<td>North Dakota</td>
<td>58 (100)</td>
<td>102 (71.3)</td>
</tr>
<tr>
<td>South Dakota</td>
<td>0 (0)</td>
<td>18 (12.6)</td>
</tr>
<tr>
<td>Washington</td>
<td>0 (0)</td>
<td>5 (3.5)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>58</strong></td>
<td><strong>143</strong></td>
</tr>
</tbody>
</table>
Results

Freshly Harvested Wheat

- **Durum**
  - 1 positive; 1/103 = 1.0%
  - 5.56 ppb

- **HRS**
  - 0 positive out of 457

Stored Wheat

- **Durum**
  - 13 positive; 13/58 = 22.4%
  - Range = 0.17-14.94 ppb
  - Median = 1.87 ppb
  - Mean = 3.30 ppb
  - Storage length = 4-12 months

- **HRS**
  - 13 positive; 13/143 = 9.1%
  - Range = 0.31-49.27 ppb
  - Median = 0.7 ppb
  - Mean = 6.07 ppb
  - Storage length = 1-11 months
Discussion - Stored Wheat

Crop Year
- Year-to-year variation
- 2011 vs. 2012

<table>
<thead>
<tr>
<th>Wheat</th>
<th>Year</th>
<th># Positive/Total (%)</th>
<th>Storage Time (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Durum</td>
<td>2011</td>
<td>11/37 (29.7%)</td>
<td>6-13</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>2/21 (9.5%)</td>
<td>1-4</td>
</tr>
<tr>
<td>HRS</td>
<td>2011</td>
<td>10/65 (15.4%)</td>
<td>5-16</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>3/79 (3.8%)</td>
<td>&lt;1-5</td>
</tr>
</tbody>
</table>

Temperature
- January-December 2011 Statewide Ranks
- January-December 2012 Statewide Ranks

Precipitation
- January-December 2011 Statewide Ranks
- January-December 2012 Statewide Ranks

Cool/Rainy
- Map showing temperature distribution

Hot/Dry
- Map showing temperature distribution

Source: NCDC_NOAA
Discussion - Stored Wheat

Location
- Durum – all samples from ND
- HRS – 4/5 states had positives

<table>
<thead>
<tr>
<th>State</th>
<th># Positive</th>
<th>Total # of Samples</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>3</td>
<td>5*</td>
<td>60.0</td>
</tr>
<tr>
<td>Montana</td>
<td>2</td>
<td>13</td>
<td>15.4</td>
</tr>
<tr>
<td>North Dakota</td>
<td>7</td>
<td>102</td>
<td>6.9</td>
</tr>
<tr>
<td>South Dakota</td>
<td>1</td>
<td>18</td>
<td>5.6</td>
</tr>
<tr>
<td>Washington</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

*All samples were from same location


**Discussion - Stored Wheat**

**Storage Time**

- Positive samples stored for 4-12 months (durum); 1-11 months (HRS)
  - 20/26 positives (76.9%) were stored ≥7 months
- Effect of temperature and moisture content

![Number of OTA-Positive Samples Related to Storage Time (Months)](chart)

*Source: Nithya et al (2011)*
Discussion

Freshly Harvested Wheat

- One positive durum sample (5.56 ppb)
- Overall – 1/560 (0.2%) positive

  - **Shotwell et al (1976)** - Hard red winter, hard red spring, and soft red winter wheat; 1970-1973; n=848
    - 3 HRW (<15-35 ppb) and 8 HRS (15-115 ppb) were positive
    - $11/848 = 1.3\%$
    - LOD was >15 ppb

  - **Trucksess et al (1999)** – Winter wheat (n=383); 1997; sampled from rail cars and trucks
    - 36 winter wheat (0.03-31.4 ppb) were positive
    - $36/383 = 9.4\%$

“Grain of Salt” – Or Wheat?

- Representative sample – “hot spots”
- No true standard method
- Recovery rates
First study in the U.S. that:
- Examines the incidence of OTA present in freshly harvested and stored *durum* wheat
- Surveys for OTA at several different points of storage under *natural conditions*

Contribute to current knowledge of OTA prevalence in wheat produced in the United States

Confirm points at which prevention strategies would be most useful to limit OTA-infected wheat from entering the food chain
Future Research

- Analyze 2012 survey samples
- Test positive samples for OTA-producing fungi
  - Diversity
- Quantify the number of possible OTA-producing fungi in compositied samples of freshly harvested and stored wheat
  - Relation of biomass to presence of OTA
- Utilize meteorological data and mapping tools to determine the effect of environmental factors on the presence of possible OTA-producing fungi
  - HRS survey samples – 2011/2012
  - Long-term → possible predictive model
Questions