Considerations for Limiting and Preventing Microbial Contamination in Whole Grain Cereal Based Products

Dr. Andreia Bianchini
The Food Processing Center
Food Science and Technology Department
University of Nebraska - Lincoln
Why Worry About Safety?

✓ Foodborne diseases/outbreaks
  - Worldwide over 1 billion cases occur per year
  - Over 5 million children die each year from foodborne diseases
  - In the US 9.4 million cases are estimated to occur annually

✓ Economic losses associated with contaminated product:
  - Production Losses
  - Product Recall
  - Product Liability
  - Loss of Business

✓ It is the right thing to do!
Wheat Flour: A Food Safety Risk Factor?

Historically: Flour and grain based products have always been considered a safe product, regarding pathogens!

More recently, however...

✓ Foodborne outbreaks associated with flour:
  
  ❖ 2005 - United States (*Salmonella*)
  
  ❖ 2008 - South Island of New Zealand (*Salmonella*)
  
  ❖ 2009 - United States (*Escherichia coli O157:H7*)
### Mycotoxins of Greatest Concern in Grains

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Molds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td><em>Aspergillus flavus</em>, <em>A. parasiticus</em>, <em>A. nomius</em></td>
</tr>
<tr>
<td>Ochratoxin</td>
<td><em>Aspergillus ochraceus</em>, <em>A. niger</em></td>
</tr>
<tr>
<td><em>Penicillium verrucosum</em></td>
<td></td>
</tr>
<tr>
<td>Fumonisins</td>
<td><em>Fusarium verticillioides (moniliforme)</em></td>
</tr>
<tr>
<td></td>
<td><em>F. proliferatum</em>, <em>F. subglutinans</em></td>
</tr>
<tr>
<td>Deoxynivalenol (DON, Vomitoxin)</td>
<td><em>Fusarium graminearum</em>, <em>F. culmorum</em></td>
</tr>
<tr>
<td></td>
<td><em>F. pseudograminearum</em></td>
</tr>
<tr>
<td>Zearalenone</td>
<td><em>Fusarium graminearum</em>, <em>F. culmorum</em></td>
</tr>
<tr>
<td></td>
<td><em>F. crookwellense</em></td>
</tr>
</tbody>
</table>
Challenges for the Grain Industry

- Microbiological quality of wheat grains
- Microbiological quality of milling-end products
- Trends in grain based foods
- Trends in grain processing
- Consumer eating habits
The Food Production Chain

Production

Distribution

Processing

Retail

Home Preparation

Restaurant Preparation

Home Consumers

Restaurant Consumer

Restaurant

Consumers
Microbiological Quality and Safety of Grains

Raw agricultural commodities:
✓ Subject to contamination from the environment
✓ Broad diversity of microbial contaminants

<table>
<thead>
<tr>
<th>Microbial Test</th>
<th>Standard*</th>
<th>Flour**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic plate count</td>
<td>&lt;10⁵</td>
<td>1.1×10⁵</td>
</tr>
<tr>
<td>Enterobacteriaceae/Coliforms</td>
<td>&lt;100</td>
<td>150</td>
</tr>
<tr>
<td>E. coli (total)</td>
<td>&lt;100</td>
<td>12.8% positive</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Not detected in 25g</td>
<td>1.3% positive</td>
</tr>
</tbody>
</table>

*Ready-to-eat standards for pasta, pizza, meals, tarts, pies, cakes & pastries without dairy cream at the point of sale in UK
**Data from 4,000 wheat flour samples from the US in 1993
Microbiological Quality and Safety of Grains

Fluctuation in microbial populations

- Production practices, meteorological conditions and method of harvesting

![Map of Nebraska showing precipitation and temperature by districts]

2011-2012 growing season precipitation for Nebraska
Source: USDA NASS Nebraska Field Office

<table>
<thead>
<tr>
<th>Weather Related Data</th>
<th>Panhandle</th>
<th>South Central</th>
<th>Southeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation*</td>
<td>8.96”</td>
<td>15.90”</td>
<td>18.96”</td>
</tr>
<tr>
<td>Temperature*</td>
<td>70.2 °F</td>
<td>70.5 °F</td>
<td>65.7 °F</td>
</tr>
</tbody>
</table>

*Average precipitation and temperature during the growing season
Microbiological Quality and Safety of Grains

Fluctuation in microbial populations

✓ Production practices, **meteorological conditions** and method of harvesting

Microbiological Profile*

![Bar chart showing microbial populations in different regions.]

- Panhandle
- South Central
- Southeast

**Log CFU / g**

- TPC
- Yeast
- Mold
- Enterobactereaceae
The majority of the microorganisms are concentrated towards the surface of the kernel

- Some species can occupy the inner part of the kernel

Laca et al., 2006 – Distribution of microbial contamination within cereal grains.
Microbiological Quality and Safety of Grains

Control Measures

- Trend/Surveillance data
- Analysis of incoming grain or “Supplier Approval Program”
- Awareness of the current weather issues *locally* and *globally*

Informed decision about where to source grain in a global market.
The Food Production Chain

Production

Processing

Distribution

Retail

Home Preparation

Home Consumers

Restaurant

Restaurant Preparation

Restaurant Consumer
Cleaning
**Cleaning**

APCs (log CFU/g) in durum wheat samples (2001 crop season):

<table>
<thead>
<tr>
<th>District</th>
<th>Dirty(^a)</th>
<th>Clean(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montana</td>
<td>7.2</td>
<td>6.1</td>
</tr>
<tr>
<td>NW North Dakota</td>
<td>8.4</td>
<td>8.6</td>
</tr>
<tr>
<td>NC North Dakota</td>
<td>8.5</td>
<td>8.2</td>
</tr>
<tr>
<td>NE North Dakota</td>
<td>8.4</td>
<td>8.3</td>
</tr>
<tr>
<td>SW North Dakota</td>
<td>8.2</td>
<td>5.3</td>
</tr>
<tr>
<td>SE North Dakota</td>
<td>8.4</td>
<td>6.9</td>
</tr>
<tr>
<td><strong>Region(^c)</strong></td>
<td><strong>8.2 ± 1.3</strong></td>
<td><strong>7.2 ± 0.5</strong></td>
</tr>
</tbody>
</table>

\(^a\) Sample as collected.

\(^b\) Seived grain.

\(^c\) Average of 6 district values ± standard deviation.

Manthey et al., 2004.
Cleaning

Effect on mycotoxins:

✓ Remove moldy kernels, broken kernels, and fine materials
✓ Remove scab infested wheat and barley: ↓ DON 5.5 – 19%
✓ In corn: ↓ Fumonisin by 26-69%
✓ Moldy kernel, seed or nut:
  ↓ Aflatoxin (40-80%)
Cleaning

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>COMMODITY</th>
<th>INITIAL DON CONCENTRATION (mg/kg)</th>
<th>REDUCTION AFTER CLEANING (%)</th>
<th>CLEANING</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITALY</td>
<td>Durum wheat</td>
<td>0.4 - 13.1</td>
<td>1 - 31%</td>
<td>Rational Kernel Service Mod. M220V sifter (equipped with an aspiration system and two sieves)</td>
<td>Visconti et al 2004 (138)</td>
</tr>
<tr>
<td>USA</td>
<td>Soft red winter wheat</td>
<td>0.6 - 20</td>
<td>average 51%</td>
<td>Carter Day dockage tester</td>
<td>Delwiche et al 2005 (139)</td>
</tr>
<tr>
<td>CZECH REPUBLIC</td>
<td>Wheat</td>
<td>0.09 - 3.0</td>
<td>45 - 59%</td>
<td>Sieving, scouring and polishing (the laboratory aspirator of dust particles Labofix Brabender)</td>
<td>Lancova et al 2008 (140)</td>
</tr>
<tr>
<td>UNITED KINGDOM</td>
<td>Wheat</td>
<td>0.01 - 0.32</td>
<td>- 8* - 78% (average 48%)</td>
<td>Separator/classifier (sieving) with aspiration</td>
<td>Scudamore and Patel 2008 (141)</td>
</tr>
</tbody>
</table>

* Represents an increase

### Washing

Microbial load in durum wheat after different washing treatments:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bacteria (log CFU/g)</th>
<th>Fungi (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Grain (Control)</td>
<td>4.3a</td>
<td>4.9a</td>
</tr>
<tr>
<td>Distilled Water Wash</td>
<td>4.2a</td>
<td>4.9a</td>
</tr>
<tr>
<td>Ozonated Water Wash (16.5 mg ozone/L)</td>
<td>3.8b</td>
<td>4.7a</td>
</tr>
<tr>
<td>Chlorinated Water Wash (700 mg/L)</td>
<td>2.4c</td>
<td>4.5a</td>
</tr>
<tr>
<td>Acetic acid (1%) Wash</td>
<td>0.5d</td>
<td>1.9b</td>
</tr>
<tr>
<td>Acetic Acid (1%) and Ozonated Water (20.5 mg/L) Wash</td>
<td>0.2e</td>
<td>1.7b</td>
</tr>
</tbody>
</table>

Dhillon, et al., 2009.
Tempering
**Tempering**

**Effect of ozone during wheat tempering:**

<table>
<thead>
<tr>
<th></th>
<th>Flour obtained from soft wheat (Adiyaman Beyaz)</th>
<th>Flour obtained from hard wheat (Arjantin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteria (log CFU/g)</td>
<td>Fungi (log CFU/g)</td>
</tr>
<tr>
<td>No Treatment</td>
<td>4.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Water</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Ozonated Water 1.5 mg ozone/L</td>
<td>3.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Ozonated Water 11.5 mg ozone/L</td>
<td>2.5</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Tempering

Effect of organic acids and salt solutions during hard wheat tempering:

Aerobic Plate Counts

Log_{10} (CFU/g) reduction

Organic Acids

Acetic Acid  Citric Acid  Lactic Acid  Propionic Acid  Salt  LA 2.5% + Salt  LA 5% + Salt  AA 2.5% + Salt

0.0%  1.0%  2.5%  5.0%  2.0%  2.5% + 1%  2.5% + 2%  5% + 1%  5% + 2%
Tempering

Effect of organic acids and salt solutions during hard wheat tempering:

*Enterobacteriaceae*

![Bar graph showing the effect of organic acids and salt solutions on the reduction of Enterobacteriaceae.](image-url)
Milling
Dry Milling

Microbial distribution during milling of hard wheat:

The chart illustrates the Log10 (CFU/g) reduction of microbial distribution during milling. The y-axis represents the log reduction in CFU/g, while the x-axis shows the milling fractions: Bran, Shorts, First Break, and Fine Reduction. The chart indicates the following:

- **APC** (in blue) shows a significant reduction in all milling fractions.
- **Yeast** (in red) exhibits moderate to high reductions, particularly in Fine Reduction.
- **Mold** (in green) displays minimal reduction across all fractions.
- **Eb** (in purple) shows the highest reduction, especially in Fine Reduction.

The Food Processing Center
FB$_1$ content in corn and milled fractions. Each bar represents a sample of corn and various fractions from consecutive weeks (Katta et al., 1997)
Dry Milling

Wheat, barley, rice and other cereals:

DON
Zearalenone
Aflatoxin
Ochratoxin A

Bran fraction
Germ
**Wet Milling**

Distribution of Fumonisins in Different Fractions of Wet Milled Yellow Corn

<table>
<thead>
<tr>
<th>Fraction</th>
<th>$FB_1$ (ppm)</th>
<th>$FB_2$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber</td>
<td>4.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Germ</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Gluten</td>
<td>5.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Starch</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Steep Water</td>
<td>1.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Process Water</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Modified from Bennett *et al.* 1996
Wet Milling

Aflatoxins
Zearalenone
Fumonisins

Steep water
Gluten fiber
Germ

Starch: Relatively free of mycotoxins
After Milling
Reduction of microbial load in wheat flour by heating:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Standard Plate Count (CFU/gm)</th>
<th>Coliform Count (CFU/gm)</th>
<th>Yeast Count (CFU/gm)</th>
<th>Mold Count (CFU/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Control flour</td>
<td>2700</td>
<td>30</td>
<td>200</td>
<td>150</td>
</tr>
<tr>
<td>2  Heat-treated flour</td>
<td>120</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Crust (% moisture)</th>
<th>Crumb (% moisture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread made from treated flour</td>
<td>18.8%</td>
<td>42.6%</td>
</tr>
<tr>
<td>Bread made from untreated flour</td>
<td>16.5%</td>
<td>39.7%</td>
</tr>
</tbody>
</table>

After Milling

Reduction of *Salmonella* in corn meal by heating:

VanCauwenberge et al., 1981.
After Milling

Effect of irradiation from a $^{60}$Co source on the microbial load of meals:

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Wheat Meal</th>
<th>Corn Meal</th>
<th>Oat Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation levels (Kgy)</td>
<td>0 1 10 25</td>
<td>0 1 10 25</td>
<td>0 1 10 25</td>
</tr>
<tr>
<td>Escherichia</td>
<td>--- --- --- ---</td>
<td>+++ +++ --- ---</td>
<td>+++ +++ --- ---</td>
</tr>
<tr>
<td>Bacillus</td>
<td>+++ +++ --- ---</td>
<td>+++ +++ --- ---</td>
<td>+++ +++ --- ---</td>
</tr>
<tr>
<td>Serratia</td>
<td>+++ ++- --- ---</td>
<td>+++ +---- --- ---</td>
<td>+++ ++- --- ---</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>+++ +++ --- ---</td>
<td>++- +---- ++- ---</td>
<td>--- --- --- ---</td>
</tr>
<tr>
<td>Clostridium</td>
<td>+++ +++ --- ---</td>
<td>+++ +++ +++ +++ ---</td>
<td>+++ +++ --- ---</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>--- --- --- ---</td>
<td>--- --- --- ---</td>
<td>++- --- --- ---</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>--- ++- --- ---</td>
<td>--- --- --- ---</td>
<td>--- --- --- ---</td>
</tr>
<tr>
<td>Molds</td>
<td>+++ +++ --- ---</td>
<td>+++ +++ --- ---</td>
<td>+++ +++ --- ---</td>
</tr>
</tbody>
</table>

Hanis et al., 1988.
Thermal Processing
Effect of moisture and temperature on the inactivation by extrusion of *Enterococcus faecium* as a surrogate for *Salmonella*:

Minimum survival: 81.1°C and 28.1% moisture

Bianchini et al., 2012. Validation of extrusion as a killing step for *Enterococcus faecium* in a balanced carbohydrate-protein meal by using a response surface design.
Thermal Processing

Corn Flakes Processing

Corn Grits

Cooking
(Steam Pressure Cookers)

Hot-Air Drying

Flaking

Roasting

Reductions of 85% in fumonisins may be achieved.
Thermal Processing

Corn flakes processing:

✓ Aflatoxin:
  - Cooking and flaking with and without sugar
    64-67% ↓ Aflatoxin
  - After toasting with and without sugar
    78-85% ↓ Aflatoxin
Thermal Processing

- Corn Muffin (5 mg/Kg FB$_1$) (Jackson et al., 1997)
  - 175°C: 16% Reduction
  - 200°C: 28% Reduction
  - Higher Loss at the Surface

- Corn Muffin (5 mg/Kg FB$_1$)
  - 204°C: No Reduction
  - 232°C: 48% Reduction (Castelo et al., 1998)
Thermal Processing

Fried Tortilla Chips

Corn

Nixtamalization (Cooking/Steeping)

Masa (Rinsed and Ground)

Sheeting/Cutting/Baking

Frying

Reductions vary from 37-78%
(Voss et al., 2001)
Thermal Processing

Fried Tortilla Chips:

- **Aflatoxin**
  - Tortilla: ↓ 52%
  - Tortilla chips: ↓ 84%
  - Corn chips: ↓ 79%

- **Zearalenone**
  - Tortilla: ↓ 59-100%

- **Deoxynivalenol**
  - Tortilla: ↓ 72-82%
Thermal Processing

Extrusion process:

- **Aflatoxin**
  - 140-185°C → 33-38% Reduction
  - >185°C → 66% Reduction
- **Zearalenone (120-160°C)** → 70% Reduction
- **Deoxynivalenol (120°C)** → 11-27% Reduction
- **Fumonisin (160-200°C)** → 46-76% Reduction
- **Fumonisin (160°C, Glucose)** → 93% Reduction

*Temperature and screw type are important!*
Consumer Behavior

✓ In the 2009 raw cookie dough outbreak in the US:
  - 94% of case patients consumed raw cookie dough
  - 11% of control consumers reported eating raw cookie dough
  - Several patients bought the dough with the intention of eating it unbaked

✓ A national survey in the US in 2011 revealed that consumers eat raw products occasionally:
  - 58% - Cookie dough
  - 24% - Biscuit
  - 22% - Pie crust
  - 11% - Pizza crust
Microorganisms and mycotoxins are naturally associated with grains

- Keeping them out from the food chain is the best approach

Several factors influence the level of contamination and/or mycotoxin in the final grain based product

- Ingredient storage
- Processing choices and parameters
  - Functionally of the flour?
  - Mycotoxins: True reduction or chemical conversion?

Consumer education could reduce pathogen risks associated with certain products