FORMULATING FOODS TO CONTROL BACTERIAL PATHOGENS

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Agenda

• Background
  • Food safety risks

• Critical factors controlling microbial growth
  • Available moisture (water activity)
  • Acidity (pH)
  • Ingredients with antimicrobial activity

• Validating formulation as a preventive control in food safety plan
  • Examples of controlling bacterial pathogens
Microbes Cause Foodborne Illness

• 48 Million episodes of foodborne illness per year
  • Viruses, bacteria, parasites
• 128,000 hospitalizations
  • *Salmonella*, *Campylobacter*, Norovirus
• 3,000 deaths
  • *Salmonella*, *Listeria monocytogenes*
  • *Toxoplasma gondii*
• Economic impact
  • Direct medical costs & lost wages
  • Recall costs and litigation/liability
    • Peanut butter – *Salmonella* 2008-2009, Recall cost $50-60 million; Peanut Corp. Am. bankrupt

Scallon et al., 2011; University of Florida Emerging Pathogens Institute, 2011
Factors that contribute to foodborne illness (typically more than one)

| Contamination of raw commodities | Flour, *E. coli* O121 and O26  
Soynut butter, *E. coli* O157:H7  
Raw milk gouda, *E. coli* O157:H7  
Sprouts, *Salmonella* |
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Slow acid development</td>
<td><em>Staphylococcus aureus</em> cheese, yogurt, fermented sausage</td>
</tr>
</tbody>
</table>
| Improper hot-holding temperature | *Clostridium perfringens*, buffets, catering  
*Clostridium botulinum*, nacho cheese sauce                    |
| Recontamination of products      | *Listeria monocytogenes*, ice cream                              |
| Ability of microbes to grow at refrigeration temperatures | *Listeria monocytogenes*, packaged salads                         |
| Lack of growth inhibitors        | *Listeria monocytogenes*, soft, high pH cheeses                  |
| Temperature abuse                | *Clostridium botulinum*, carrot juice                            |
| Susceptible Consumers            | *Listeria monocytogenes*, cantaloupe                            |
Bacterial Pathogens: General Concepts

- Pathogens generally found at low levels
- Pathogens do not always cause spoilage
- Can survive or grow in adverse conditions
  - Survive cooking; grow under refrigeration temperatures
- Infectious dose varies
- Toxin formation requires growth
  - *No growth = no toxin = no illness*
  - *Examples: S. aureus, B. cereus, C. botulinum*
  - Growth can occur in hours, days or weeks
Formulation Strategies Focus on Gram Positive Bacteria

- Tend to be more resistant to thermal inactivation (especially spores)
- Typically require growth to cause illness

- Vegetative pathogens
  - *Listeria monocytogenes*
  - *Staphylococcus aureus*

- Sporeforming bacteria
  - *Bacillus cereus*
  - *Clostridium perfringens*
  - *Clostridium botulinum*
Do not rely on temperature alone

- Cooking/pasteurization is not perfect
  - Spores survive heating
  - Post-pasteurization contamination
- Temperature abuse is common
  - During distribution, transportation, consumer homes, power-outages
- Growth of psychrotrophic pathogens
  - *Listeria monocytogenes*
  - Some *Clostridium botulinum* strains
  - Some *Bacillus cereus* strains
Formulating Foods for Safety

- Goal: Maintain safety through the point of consumption
  - Delay pathogen growth until gross spoilage
- Requires understanding of microbial physiology and ecology in foods
  - Adequacy of control depends on target microbe
- Consider whole food, individual components, and interfaces of components
Critical Factors for Formulation

- Available moisture
  - Water activity $a_w$
  - Function of moisture, salt, other ingredients
- pH
  - Acid type, titratable acidity
- Addition of growth inhibitors
  - Preservatives (synthetic or clean label/”natural”)
  - Additive or synergistic interaction means that lower levels of each factor can be used
    - Less effect on sensory attributes
- Use in combination with temperature control
**pH and a$_w$ combinations that inhibit growth of vegetative cells and spores**

<table>
<thead>
<tr>
<th>Critical a$_w$ values</th>
<th>Critical pH values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;4.2</td>
</tr>
<tr>
<td>&lt;0.88</td>
<td>No growth</td>
</tr>
<tr>
<td>0.88 – 0.90</td>
<td>No growth</td>
</tr>
<tr>
<td>&gt;0.90 – 0.92</td>
<td>No growth</td>
</tr>
<tr>
<td>&gt;0.92</td>
<td>No growth</td>
</tr>
</tbody>
</table>

? = Requires time/temperature control unless product testing demonstrates otherwise

Antibacterial ingredients

- Secondary barrier during temperature abuse or mishandling
- Typically bacteriostatic (delays growth, does not kill)

<table>
<thead>
<tr>
<th>Synthetic</th>
<th>Clean Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate*, propionate*</td>
<td>Cultured sugar, cultured milk</td>
</tr>
<tr>
<td>Diacetate*, acetic acid*</td>
<td>Dry vinegar, buffered vinegar</td>
</tr>
<tr>
<td>Nitrite*</td>
<td>Cultured celery</td>
</tr>
<tr>
<td>Erythorbate, ascorbate*</td>
<td>Acerola cherry powder</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>None (derived from rowanberries)</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>Cranberries, prunes, plums, cinnamon</td>
</tr>
<tr>
<td>Phenolics, flavonoids</td>
<td>Fruit / spice extracts</td>
</tr>
</tbody>
</table>

* Clean label substitute with documented efficacy
Limitations of Preservatives

- **NOT** a substitute for good manufacturing practices
  - Component of food safety plan: preventive controls
- Consumer acceptance, effect on sensory, functional attributes, cost
- Efficacy affected by food components
  - Fat level (solubility), moisture, temperature, pH, nitrite, smoke,
- Competitive microflora may also be inhibited
Validating Formulation Safety

- Validation establishes the scientific basis for process preventive controls in the Food Safety Plan
  - Ex. Validate critical limit values for process controls
- May include:
  - Using scientific principles and data
  - Use of expert opinion (including predictive models)
  - Challenging the process at the limits of its operating controls
- Performed or overseen by a preventive controls qualified individual (PCQI)
Assessing Risks

- Specific for a given food/characteristics
- Requires understanding of:
  - Likelihood of contamination
  - Processing and handling in facility
  - Distribution
  - Shelf-life
    - Quality based on microbial changes vs. organoleptic changes
    - Temperature of storage
  - Potential use/abuse at retail, consumer level
VALIDATING FORMULATION SAFETY EXAMPLES

Effect of formulation on processed meat
   *Listeria monocytogenes* (extended refrigerated storage)
   *Clostridium perfringens* (cooling)

Effect of formulation on natural cheese
   *Listeria monocytogenes*

Effect of formulation on process cheese
   *Staphylococcus aureus*
   *Clostridium botulinum*

Effect of formulation on refrigerated meals
   *Clostridium botulinum*
High moisture RTE Meats
*L. monocytogenes*, 41°F (5°C), no growth inhibitors

Critical factors
- Competitive microflora
- pH, moisture
- Nitrite

Glass and Doyle, AEM, 1989
Buffered vinegar, moisture, pH
Uncured RTE Meats: L. monocytogenes

Turkey: ~73% moisture, pH 6.15, 1.1% NaCl, no nitrite
Beef: ~66% moisture, pH 5.75, 0.6% NaCl, no nitrite

Change Populations L. monocytogenes (Log CFU/g)

- Turkey Control No Antimicrobials
- Beef Control No Antimicrobials
- Turkey - 2.0% buffered vinegar
- Beef - 2.0% buffered vinegar

Adapted from JFP 76:1366, 2013
Similar results with 1.5% lemon/cherry/vinegar blend; 3.0% cultured sugar-vinegar blend
Effect of nitrite source + cure accelerator, *Clostridium perfringens*, 15 h biphasic cooling, cured meats, Appendix B

- Deli turkey model system
  - 74% moisture, 1.3% NaCl, pH 6.3
- No effect of conventional versus alternative (natural) ingredients
- 100 ppm nitrite alone not sufficient
  - Requires ascorbate for inhibition

King, et al., J. Food Prot. 78: 1527-1535.
ComBase Perfringens Predictor
Effect of pH and NaCl, cured meat (NaNO₂)
No erythorbate in model, *Clostridium perfringens*

Similar trends in ham with 156 ppm nitrite plus 547 erythorbate cooled over 25 h
Effect of cultured dairy solids (clean label antimicrobial) on *L. monocytogenes* on Reduced Salt Mozzarella

Same trends, but less differences (more growth) at pH 6.0
Effect of acid type, pH, and moisture on *L. monocytogenes*

**Lactic Acid**

**Acetic Acid**
Process cheese slices, 85°F

Results: no growth
L. monocytogenes
E. coli O157:H7
Salmonella
B. cereus

Variable growth Staph
Growth at 1-7 days; pH>5.6, no sorbate

Population at which staphylococcal enterotoxin is detected

Upper SD of growth for S. aureus

Average S. aureus

Minimum detection limit

40% moisture for Cheddar based
20 formulations tested; Staph greatest variation for growth

FRI, Unpublished data, 2001
Sponsored by DMI
S. aureus on process cheese and cheese food
Effect of pH and water activity/moisture

All formulations contain 0.2% potassium sorbate

- Pepper Jack (45% moisture, pH 5.5, 1.7% NaCl, aw 0.945)
- American (43% moisture, pH 5.3, 1.8% NaCl, aw 0.955)
- Cheddar2 (42% moisture, pH 5.3, 2.0% NaCl, aw 0.955)
- Cheddar1 (42% moisture, pH 5.6, 1.8% NaCl, aw 0.945)
- Cheddar3 (40% moisture, pH 5.3, 2.5% NaCl, aw 0.935)

FRI, unpublished data, 2004; sponsored by industry
Formulation Safety for Process Cheese Spread
FRI Model (aka Tanaka Model)

Safety factors for control of Clostridium botulinum in shelf-stable process cheeses

- Moisture
- pH
- Sodium chloride
- Sodium phosphate
- Not modeled
  - Aw
  - lactate

Tanaka et al, 1986 JFP
Formulation safety for non-standard process cheese: sorbic acid and *Clostridium botulinum*

<table>
<thead>
<tr>
<th>moisture</th>
<th>pH</th>
<th>NaCl</th>
<th>DSP</th>
<th>total salts</th>
<th>fat</th>
<th>sorbic acid</th>
<th>Predicted time to toxicity (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.0</td>
<td>5.8</td>
<td>2.0</td>
<td>1.7</td>
<td>3.70</td>
<td>22</td>
<td>0</td>
<td>FRI 2017: 7</td>
</tr>
<tr>
<td>54.0</td>
<td>5.8</td>
<td>2.0</td>
<td>1.7</td>
<td>3.70</td>
<td>22</td>
<td>0.1</td>
<td>FRI 1986: 7</td>
</tr>
<tr>
<td>54.0</td>
<td>5.8</td>
<td>2.0</td>
<td>1.7</td>
<td>3.70</td>
<td>22</td>
<td>0.15</td>
<td>FRI 2017: 80</td>
</tr>
<tr>
<td>54.0</td>
<td>5.8</td>
<td>2.0</td>
<td>1.7</td>
<td>3.70</td>
<td>22</td>
<td>0.2</td>
<td>FRI 1986: 7</td>
</tr>
</tbody>
</table>

Note: 2017 model validation in progress
Probability of failure for 2017 model set to 0.001
Use for guidance only for product developers
Cultured celery, pH, product matrix  
Refrigerated foods:  
Proteolytic *C. botulinum*

<table>
<thead>
<tr>
<th>Matrix</th>
<th>pH</th>
<th>Treatment</th>
<th>Storage Temp °F</th>
<th>Toxicity (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dijon Pork</td>
<td>6.0</td>
<td>Control</td>
<td>59</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cultured Celery (80 ppm NO₂)</td>
<td>59</td>
<td>&gt;8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td>Cauliflower-potatoes</td>
<td>5.5</td>
<td>Control</td>
<td>59</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cultured Celery (60 ppm NO₂)</td>
<td>59</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>

Golden et al., 2017, JFP, in press

Received “non-prot botulinum cook” 90°C, 10 min  
Tested for toxin weekly  
No toxicity for pH ≤6.0 during storage for 8 weeks at 50°F
Dry vinegar, Fruit-Spice Blend, pH
Uncured Chicken: *C. botulinum* Time to toxin production

<table>
<thead>
<tr>
<th>Temperature °F</th>
<th>pH 6.15</th>
<th>pH 6.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5% Dry Vinegar + 0.6% Fruit-Spice-Vinegar blend</td>
<td>Control + 0.5% Dry Vinegar + 0.6% Fruit-Spice-Vinegar blend</td>
</tr>
<tr>
<td>77</td>
<td>2 d</td>
<td>2 d</td>
</tr>
<tr>
<td>55</td>
<td>1 mo</td>
<td>1 mo</td>
</tr>
<tr>
<td>45</td>
<td>&gt;6 mo</td>
<td>1 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 mo</td>
</tr>
</tbody>
</table>
Summary

• Formulation alone will not guarantee safety
  • Heat or alternative pasteurization
  • Proper sanitation
  • Part of the well designed food safety system
• Factors to consider
  • Storage temperature distribution, at retail, by consumers
  • Water activity, pH/total acidity
  • Synthetic and clean label antimicrobials
• Requires that manufacturing specifications are met
• Success also depends on education and cooperation of consumer for safe food handling
Acknowledgements

- FRI Applied Food Safety Lab
- Meat and Muscle Biology Lab
- WI Center for Dairy Research

Funding
- University of Wisconsin Foundation
- North American Meat Institute Foundation
- USDA
- Dairy Management Inc.
- FRI Summer Scholar Program
- International Dairy Foods Association
- Wisconsin Association of Meat Processors
- Industry support
FSPCA PCQI Training (Human Foods)

- UW-Madison August 22-24, 2017
  - 2.5 day standard curriculum
- Blended Course
  - Part 1 online
  - Part 2 instructor-led
- Other courses held around US, several times per month
  - See https://fspca.force.com for registration links
  - Upcoming classes in WI
    - Cherney Microbiology
    - Covance
    - NSF
    - Safe Food Resources