Current Food Safety Research in Dairy and Non-Dairy Products

Kristin Schill, Research Assistant Professor, Food Research Institute
University of Wisconsin-Madison
Contact: Kristin.Schill@wisc.edu; 608-264-1368
Overview

• Introduction to the Food Research Institute
• Applied Food Safety Research Highlights at the FRI
  – Cheese Milk Thermization App
  – Inactivation of *Listeria monocytogenes* and *Salmonella* in plant-based process cheese
  – Inhibition of *L. monocytogenes* in plant-based cream cheese using clean label antimicrobials
  – Expanding the 2017 Glass Model for botulinal safety of process cheese
• Focus Topic - Sodium Reduction in Dairy
• Efficacy of Hydrogen Peroxide on Pathogen Inhibition
  – Cheese Brines and Queso fresco
Food Research Institute

- Address select food safety issues
- Cooperation with regulatory agencies
  - FDA, USDA
- Interdepartmental collaboration
- Source of future well-trained scientists
- Outreach and communications
  - Seminars/webinars
  - Newsletter
  - Literature reviews
  - Conferences/workshops

*Demonstrating food safety leadership through research, training, and outreach for more than 70 years*
Interdepartmental, Integrative and Multi-Interdisciplinary
HPAI in US Dairy Cattle – Inquiry from Dairy Industry

Keith Poulsen
Stacey Schultz-Cherry
St. Jude Faculty
Yoshihiro Kawaoka

School of Medicine and Public Health
Bacteriology
Food Science
Veterinary Medicine
Kathy Glass
Pathobiological Sciences
Food Research Institute
John A. Lucey
Alex O’Brien

Veterinary Diagnostics Lab
Center for Dairy Research

Wendy Bedale
Animal & Dairy Sciences
Medical Microbiology and Immunology
Meat Science and Animal Biologics Discovery

Keith Poulsen
Stacey Schultz-Cherry
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Dean Sommer

Kathy Glass

Veterinary Medicine

Animals & Dairy Sciences

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Bacteriology

Food Research Institute

 Veterinarian Diagnostics Lab

Center for Dairy Research
Applied Food Safety Lab

- **Food challenge studies**
  - Dairy, meat, bakery, refrigerated foods
  - Effect of cooling rate and storage temperature on microbiological safety of meat and dairy products
  - Microbiological safety of pasteurized process cheese products and processed meats
  - Traditional and “natural” antimicrobials
  - Reduced-sodium applications
  - Thermal inactivation

- **Impact**
  - Food regulations (changes or support)
    - Pasteurized Milk Ordinance, antimicrobials approved for use in meats
  - Validation of process/formulation efficacy
  - Identify novel strategies/formulations to improve safety
Dairy Research at FRI

- Cheese Milk Thermization App
  - *Listeria monocytogenes*
  - Shiga-toxin producing *E. coli*
  - Coming soon...*Lactobacillus parabuchneri*
- User-Friendly!
- IAFP MARKETPLACE presentation
  - Tuesday July 16, 2024
  - Long Beach, California

Developed by: Sarah Engstrom (Grande Custom Ingredients Group) & Kathy Glass (FRI)
Thermal Inactivation of *Listeria monocytogenes* and *Salmonella* spp. in a Plant-Based Process Cheese

- USDA requirements for process cheese pasteurization – >74°C/ 165°F for >30 seconds
- *L. monocytogenes* is more heat resistant than *Salmonella* in PC
- D-values are affected by water activity and pH
  - Casein substituted with plant proteins (pea)
  - Whey substituted with starches

### Table 2. D-values (minutes; time for 1-log reduction) for *Listeria monocytogenes* and *Salmonella* spp. in plant-based dairy analog

<table>
<thead>
<tr>
<th>°F</th>
<th>°C</th>
<th>a&lt;sub&gt;90&lt;/sub&gt; pH-4.8</th>
<th>a&lt;sub&gt;90&lt;/sub&gt; pH-5.8</th>
<th>a&lt;sub&gt;95&lt;/sub&gt; pH-4.8</th>
<th>a&lt;sub&gt;95&lt;/sub&gt; pH-5.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td>54.4</td>
<td>13.45</td>
<td>20.73</td>
<td>15.45</td>
<td>69.71</td>
</tr>
<tr>
<td>135</td>
<td>57.2</td>
<td>5.71/9.91</td>
<td>11.81/14.29</td>
<td>10.23/13.18</td>
<td>25.52*/34.06</td>
</tr>
<tr>
<td>140</td>
<td>60.0</td>
<td>2.92/1.02</td>
<td>5.85/2.21</td>
<td>4.37/2.34</td>
<td>12.38/13.82</td>
</tr>
<tr>
<td>145</td>
<td>62.8</td>
<td>0.83/0.38</td>
<td>1.43/0.40</td>
<td>2.10/1.34</td>
<td>4.90/2.88</td>
</tr>
<tr>
<td>150</td>
<td>65.6</td>
<td>0.45/0.07</td>
<td>0.65/0.1</td>
<td>0.96/0.42</td>
<td>1.77/0.82</td>
</tr>
<tr>
<td>155</td>
<td>68.3</td>
<td>0.26</td>
<td>0.38</td>
<td>0.54</td>
<td>1.10</td>
</tr>
</tbody>
</table>

*aTime for samples to reach the target temperature ranged from 8-10 seconds and were included in the calculations.
*bD-value data for all four formulations is an average of triplicate trials.
*cReplicate 2 values exceeded remaining three trials (e.g. D<sub>95</sub> = 40.98)
Inhibition of *Listeria monocytogenes* in Cold-Filled Plant-based Cream Cheese at 27°C

- 0.7% Rowan Berry Extract
  - Equivalent to ~0.2% potassium sorbate
- Effective at controlling *L. monocytogenes* in plant-based cream cheese
- Need to demonstrate efficacy of *Staphylococcus aureus* control

![Graph showing inhibition of *Listeria monocytogenes* with different antimicrobial agents.](image)

Preliminary results indicate that 0.7% Rowan Berry Extract is effective in controlling *Listeria monocytogenes* in plant-based cream cheese. The graph shows a log reduction in CFU/g over time for different antimicrobial treatments: 0% Antimicrobial, 0.1% Potassium Sorbate, 0.35% Rowan Berry Extract, and 0.7% Rowan Berry Extract. The conditions for these tests are pH 5.2, 60% moisture, aw=0.98, and 0.4% salt.
Shelf stable pasteurized process cheese spreads: *Clostridium botulinum*

- **Major safety factors**
  - Moisture
  - Sodium Chloride (NaCl)
  - Disodium phosphate (DSP)
  - pH
  - Decrease 0.2 units ≈ 0.75% total salts ↓

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*Safety line*

- Unsafe
- Safe

- ~400 mg/28 g
- ~320 mg/28 g

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*Tanaka et al, 1986 JFP*
Expanding the FRI 2017 Glass Model for Botulinal Safety Evaluation of Non-Standard-of-Identity Process Cheese

• 2017 Glass Model = Expanded Tanaka model (for standard-of-identity process cheese spreads)

| ranges for | 50 | 5.4 | 1.7 | 0.8 | 10 | 0 | 0 |
| 2017 model | 60 | 6.2 | 2.4 | 1.6 | 30 | 50 | 0.2 |

Revised Aug 10, 2015

<table>
<thead>
<tr>
<th>total salts</th>
<th>moisture</th>
<th>pH</th>
<th>NaCl</th>
<th>DSP solids</th>
<th>fat</th>
<th>%K replacemnt</th>
<th>% sorbic acid</th>
<th>failure probability</th>
<th>EXCEL Predicted Fail time in Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.00</td>
<td>54.0</td>
<td>5.5</td>
<td>2.4</td>
<td>1.60</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>1E-12</td>
<td>6</td>
</tr>
<tr>
<td>4.00</td>
<td>54.0</td>
<td>5.5</td>
<td>2.4</td>
<td>1.60</td>
<td>20</td>
<td>0</td>
<td>0.1</td>
<td>1.00E-12</td>
<td>66</td>
</tr>
<tr>
<td>4.00</td>
<td>54.0</td>
<td>5.5</td>
<td>2.4</td>
<td>1.60</td>
<td>20</td>
<td>0</td>
<td>0.2</td>
<td>1.00E-12</td>
<td>781</td>
</tr>
</tbody>
</table>

• 202X FRI Model = Expanded 2017 Glass Model
  – Lactic acid (0.25-1.75%)
  – Anhydrous disodium phosphate (1.6-2.8%)
Reducing sodium in dairy products

- “Reduced sodium” claim must decrease by at least 25%
- Butter: salt main preservative during short term non-refrigerated storage
  - Salted butter: $a_w$ 0.83-0.92
  - Unsalted butter: $a_w$ 0.96-0.98 requires strict refrigeration or acid addition/cultured cream
- No salt standards for specific cheeses
  - Cheddar; range 0.8 to 2.0%
  - High-salt cheeses (2.5-3.5% salt) Blue, Romano, Parmesan, Feta select for desirable microbes; inhibits undesirable microbes
  - Lower salt cheeses (0.9-1.2%) Swiss (Propionibacterium gas formation), fresh Mozzarella
- “Salt” defined as NaCl
- Current standards of identity (SOI) for most cheeses do not allow use of salt substitutes
  - 2023 FDA proposal to amend 80 SOI that specify “salt” to allow salt substitutes
Effect of NaCl, pH, water activity in Cheddar

[Listeria, Cheddar, 4C]

Shrestha et al., 2011 J. Dairy Science

[Salmonella, Cheddar, 4C]

Shrestha et al., 2011 J. Food Science
2017 Glass Model - focusing on 50% sodium reduction in shelf-stable pasteurized process cheese

### Reduce sodium by reducing salts

<table>
<thead>
<tr>
<th>total salts (NaCl+DSP)</th>
<th>Est. sodium mg/28 g</th>
<th>Predicted Fail time in Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.00</td>
<td>460</td>
<td>42</td>
</tr>
<tr>
<td>3.25</td>
<td>290</td>
<td>5</td>
</tr>
</tbody>
</table>

### Reduce sodium by replacing with K-based salts

<table>
<thead>
<tr>
<th>total salts (including emulsifier)</th>
<th>Est. sodium mg/28 g</th>
<th>Predicted Fail time in Weeks</th>
<th>%K molar replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.00</td>
<td>460</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>4.00</td>
<td>345</td>
<td>49</td>
<td>25</td>
</tr>
<tr>
<td>4.00</td>
<td>230</td>
<td>57</td>
<td>50</td>
</tr>
</tbody>
</table>

1. Predictive model developed to ≤50% replacement, molar basis
2. K replacement on molar basis
   a. 1.28% KCl to replace 1.0% NaCl
   b. On a % basis, NaCl is more effective than KCl
3. Previous work (mid-1990s) with K-replacement up to 75% suggest less activity than at 25 or 50% replacement levels

Ex. Non-standard-of-identity product, 15% cheese, low lactate levels
50% moisture, pH 5.4, 10% fat; no sorbate; probability of failure set to 0.001
Pathogen control in cheese brines using hydrogen peroxide
Background

• Brining/salting important step during cheese manufacturing
  – Pure salt solutions: correlation between salt and water activity
• Brine can serve as a reservoir of salt tolerant pathogens
  – *Listeria monocytogenes*
    • “zero tolerance” pathogen
    • Growth at 13% salt; survive in up to 30% salt; lower limit for growth $a_w$ 0.92
  – *Staphylococcus aureus*
    • Requires growth in high levels to develop enterotoxin
    • Poor competitor
    • Salt growth limits affected by pH
    • Tolerance 20% salt; lower limit for growth $a_w$ 0.86

<table>
<thead>
<tr>
<th>% NaCl</th>
<th>Aw</th>
</tr>
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<tbody>
<tr>
<td>0.9</td>
<td>0.995</td>
</tr>
<tr>
<td>1.7</td>
<td>0.99</td>
</tr>
<tr>
<td>3.5</td>
<td>0.98</td>
</tr>
<tr>
<td>7.0</td>
<td>0.96</td>
</tr>
<tr>
<td>10.0</td>
<td>0.94</td>
</tr>
<tr>
<td>13.0</td>
<td>0.92</td>
</tr>
<tr>
<td>16.0</td>
<td>0.90</td>
</tr>
<tr>
<td>22.0</td>
<td>0.86</td>
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</table>
Acidification of model cheese brines (pH 2.0)

Lactic acid

Acetic acid

Hydrochloric acid

Citric acid

Chemical treatments that have reduced the survival of *L. monocytogenes* in commercial cheese brines

10-100 ppm Sodium hypochlorite

0.005-0.02% H$_2$O$_2$

1% Potassium sorbate

1% Sodium benzoate

Hydrogen Peroxide

- Hydrogen peroxide (H₂O₂): reactive oxygen induces cellular damage
- Decomposes to water and oxygen
- 3% concentration used as antiseptic, skin care, oral hygiene, cleaning
- Potential use
  - In combination with PAA to inactivate *Listeria* on apples
  - As tool to mitigate contamination by *Listeria monocytogenes* and *Staphylococcus aureus* in brine, cheese, and whey
- Relatively inexpensive and easy to administer/test/verify by small manufacturers
- Need to monitor activity and effects on product quality and equipment
Phase 1: Inactivation of *L. monocytogenes* in Fresh Model Brines

- **Model brines (filtered)**
  - 10% salt pH 4.6
  - 10% salt pH 5.4
  - 20% salt pH 4.6
  - 20% salt pH 5.4

- **Temperatures**
  - 10°C (50°F)
  - 15.6°C (60°F)

- **Hydrogen peroxide**
  - 0 ppm
  - 50 ppm
  - 100 ppm

- **Inoculated with 5.5-log CFU/mL of 5 – strain cocktail of acid adapted *L. monocytogenes***
  - LM301 (Cheddar Isolate, 1/2a)
  - LM108M (low moisture, low pH salami isolate, 1/2b)
  - LM310 (Feta cheese isolate, 4b)
  - FSL-R2-500 (Hispanic style soft cheese isolate, 4b)
  - FSL J1-110 (Jalisco cheese isolate, serotype 4b)

- **Assayed at t=0, 24 h and 7 days (1 week)**
Average log change in populations of *L. monocytogenes* (log CFU/ml)

**Model Brine, 10°C, 0 ppm H2O2**

- 10% salt, pH 4.6
- 10% salt, pH 5.4
- 20% salt, pH 4.6
- 20% salt, pH 5.4

**Model Brine, 10°C, 50 ppm H2O2**

**Model Brine, 10°C, 100ppm H2O2**

**Model Brine, 15.6°C, 0 ppm H2O2**

**Model Brine, 15.6°C, 50 ppm H2O2**

**Model Brine, 15.6°C, 100ppm H2O2**

1 hour 24 hour 1 week

1 hour 24 hour 1 week

1 hour 24 hour 1 week

1 hour 24 hour 1 week
**Phase 2: Commercial Cheese Brines**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Cheese type</th>
<th>Use temperature</th>
<th>pH range</th>
<th>Salt range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Parmesan</td>
<td>50-53 F</td>
<td>5.00-5.10</td>
<td>21-22%</td>
</tr>
<tr>
<td>B</td>
<td>Brick</td>
<td>71-73 F</td>
<td>5.05-5.25</td>
<td>27-29%</td>
</tr>
<tr>
<td>C</td>
<td>Gorgonzola</td>
<td>50-60 F</td>
<td>4.55-4.75</td>
<td>20-23%</td>
</tr>
<tr>
<td>D</td>
<td>Mozzarella</td>
<td>30-32 F</td>
<td>5.30-5.40</td>
<td>25-28%</td>
</tr>
<tr>
<td>E</td>
<td>Feta</td>
<td>55 F</td>
<td>4.50-4.60</td>
<td>15-18%</td>
</tr>
<tr>
<td>H</td>
<td>Parmesan</td>
<td>53-55 F</td>
<td>5.05-5.20</td>
<td>27-29%</td>
</tr>
<tr>
<td>J</td>
<td>Brick</td>
<td>No data</td>
<td>5.40-5.55</td>
<td>10-19%</td>
</tr>
</tbody>
</table>
Experimental Design

• 7 brines (see previous slide)
  – Each trial used brines from a different season (Summer/August 2020, Autumn/October 2020, Winter/January 2021)
• *L. monocytogenes* – acid adapted, 5-strain cocktail – 5.5-log CFU/mL
• *S. aureus* – acid adapted, 3-strain cocktail (FRI196E, whipped butter isolate, SEA; FRI S6, shrimp isolate, SEA&B; FRI 952, ham isolate, SEA&D) – 4.5-log CFU/mL
• H$_2$O$_2$ levels: 0 ppm, 50 ppm, 100 ppm
• Temperatures: 12.8°C, 7.2°C, 0°C (only 12.8°C for *S. aureus*)
• Testing at: Time 0, 1 day, 1 week
L. monocytogenes inactivation in 7 brines over 1 week with differing levels of H$_2$O$_2$ addition at 12.8°C (55°F)

![Graph showing log reduction of L. monocytogenes in different brines with varying pH and NaCl levels. The graph indicates rapid inactivation of H$_2$O$_2$ due to high yeast counts.]

A- Parmesan pH 5.04; 21.92% NaCl
B- Brick pH 5.16; 27.88% NaCl
C- Blue/Gorg pH 4.60; 21.48% NaCl
D- Mozzarella pH 5.38; 26.50% NaCl
E- Feta pH 4.54; 16.53% NaCl

Rapid inactivation of H$_2$O$_2$ due to high yeast counts.
L. monocytogenes inactivation in 7 brines over 1 week with differing levels of H$_2$O$_2$ addition at 7.2°C (45°F)

Rapid inactivation of H$_2$O$_2$ due to high yeast counts
*L. monocytogenes* inactivation in 7 brines over 1 week with differing levels of H$_2$O$_2$ addition at 0°C (32°F)
*Staphylococcus aureus* inactivation in 7 brines over 1 week with differing levels of H$_2$O$_2$ addition at 12.8°C (55°F)
Conclusions

• Hydrogen peroxide effective in killing *Listeria* in brine
  – Most consistent kill with 100 ppm H$_2$O$_2$ across all temperatures and brine types
  – 24 hours insufficient to generate consistent >2 log kill; 7 days
  – Lethality similar with salt-tolerant *Staphylococcus aureus*

• Factors that accelerate inactivation
  – Lower pH
  – Higher salt
  – Warmer brine temperatures

• Certain indigenous microbes can inactivate H$_2$O$_2$
  – Must be validated in specific brines to ensure activity

• H$_2$O$_2$ levels must be monitored for effective levels

• Watch out for unintended consequences: flavor issues, pitting of equipment
Effect of hydrogen peroxide to control *Listeria* on surface of queso fresco

- Queso fresco: high moisture, high pH, frequently associated with outbreaks of listeriosis
- Recontamination during cheese make process or finished cheese during packaging
- U-Conn Research: ~53% moisture; ~pH 6.3, ~4% salt, no starter culture
  - Cubed pressed curd inoculated with *L. monocytogenes* (4-log CFU/g), dried
    - 1. 1-min dip in water, 5% hydrogen peroxide, 10% SC, 25% acidified calcium sulfate with lactic acid, 10% ε-polylysine, 2 and 5% lauric arginate ethyl ester (and combinations)
  - Drain/dry 30 min, vacuum sealed, stored at 45°F
  - Enumerate at day 1, 7, 14, 21, 28, 35

*Kozak, Bobak and D’Amico, JFP. 2018*
Efficacy of antimicrobial dips on surface *Listeria*

- >2+ log kill within 24 h
- Sustained inhibition for 35 days
Efficacy of Hydrogen Peroxide dips on surface *Listeria*

- 2+ log kill within 30 min
- Sustained inhibition even though residual hydrogen peroxide diminished 10x by day 1 and was undetectable at 21 d

Robinson and D’Amico, 2021, Int Dairy J.
Summary

- Hydrogen peroxide is an effective lethal treatment against *Listeria monocytogenes* and *Staphylococcus aureus* in brines, cheese surfaces and whey
- Levels and exposure time needed for lethality will depend on application, salt/pH, temperature
- Low concentrations require longer time for inactivation but can be used as an ongoing pathogen mitigation program/brine management program
- Use as an adjunct to pasteurization, GMPs and EMPs
Acknowledgements

- Funding – National Dairy Council, Dairy Farmers of Wisconsin, Wisconsin Cheesemakers Association, Wisconsin Center for Dairy Research
- Technical expertise of the Applied Food Safety Laboratory – Jieyin Lim, Quinn Huibregtse, Ashley Chung, Brandon Wanless – Max Golden, Harneel Kaur, Calvin Slaughter, Christopher Sailing
- Consultants/helpful discussions – Larry Bell, Jim Mueller for securing brine – Bob Wills – Tim Stubbs, Chad Galer – Dennis Seman – Wendy Bedale
- Sarah Engstrom