INHIBITION OF
STAPHYLOCOCCUS AUREUS
IN SWEET WHEY

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What is Whey?

- Liquid byproduct during cheese manufacturing
  - Whole milk proteins coagulated in curd via acidification
  - Liquid separating from the curds is the *whey*
  - Uses: Protein powder, whey cheese, baby formula, etc.
Storage of Liquid Whey

• Regulations
  • “If holding > 4 hours, then must either be at <45°F or >140°F.”
  • Time starts at the draw from vat

• Food safety issue
  • Growth of pathogens
  • Production of heat stable toxin by *Staphylococcus aureus*
  • Regulations may dictate time/temp

• Quality versus food safety issue
  • Low pH = *S. aureus* inhibition = Unacceptable product
Why is *S. aureus* a safety issue?

- Aerobic, ubiquitous, gram positive pathogen
- Produces heat-stable enterotoxin
  - Requires $10^5$-$6$ vegetative cells to produce toxin
  - Withstands heat at 121°C for up to 10 minutes
  - Cause of an estimated 241,000 cases of intoxication each year
- Symptoms include diarrhea and vomiting that arise 1-6 hours post exposure
And when the wrong stars align...

- *S. aureus* optimal growth conditions
  - pH 6.5
  - Temp 86-99°F (30-37°C)
- Whey pH is often 5.9-6.6 and kept warm for long durations of time during holding, which could result in...
Solutions

Studies conducted tested these solutions:

• **Addition of hydrogen peroxide**
  - Hydrogen peroxide ($\text{H}_2\text{O}_2$) has very reactive oxygen atoms which steal electrons from the bacterial cell wall, which destroys the cell

• **Thermal inactivation**
  - Heat denatures the enzymes of the bacteria, rendering them useless
  - Heat also damages the cell envelope of the bacteria so that the envelope loses its shape/rigidity, allowing the fluid inside the cell to burst out
Hydrogen Peroxide Study Outline

• Whey obtained during cheesemaking
  • No-starter, mesophilic, or thermophilic types
  • Average pH 6.3-6.6

• 3-strain mixture of *Staphylococcus aureus* added to whey
  • Inoculated at 3-log CFU/ml

• Hydrogen peroxide added
  • Each whey type contained either 0ppm, 10ppm, and 100ppm hydrogen peroxide

• Incubation at 70°F or 90°F

• Enumerated in duplicate at 0, 4, 8, 12, and 24 hours on Baird-Parker agar (selective) overlaid with Tryptic Peptone agar (non-selective)

• Whey batches tested:
  • 4 no starter, 2 mesophilic, and 2 thermophilic
Log change of *S. aureus* in whey at 70°F
Log change of *S. aureus* in whey at 90°F
## Summary of *S. aureus* Enumerations

<table>
<thead>
<tr>
<th></th>
<th>NO STARTER WHEY</th>
<th>70°F</th>
<th>90°F</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm Hydrogen Peroxide</td>
<td>Growth &gt;1 log</td>
<td>Growth &gt;2 log</td>
<td></td>
</tr>
<tr>
<td>10 ppm Hydrogen Peroxide</td>
<td>No Growth</td>
<td></td>
<td>Growth &gt;2 log</td>
</tr>
<tr>
<td>100 ppm Hydrogen Peroxide</td>
<td>Decrease 2 log</td>
<td></td>
<td>Decrease &gt;2 log</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>STARTER WHEY</th>
<th>70°F</th>
<th>90°F</th>
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</thead>
<tbody>
<tr>
<td>0 ppm Hydrogen Peroxide</td>
<td>No Growth</td>
<td></td>
<td>Decrease 2 log</td>
</tr>
<tr>
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<td>Decrease &gt;1 log</td>
<td></td>
<td>Decrease 2 log</td>
</tr>
</tbody>
</table>
Conclusions

Whey with Starter Culture

• Starter culture competitively inhibits the growth of *Staphylococcus aureus* at 70°F and 90°F for up to 24 hours.

Whey with No Starter Culture

• Require time-temperature control and/or the addition of hydrogen peroxide.
  • Safe up to 24 hours at 70°F.
  • Use ≥10ppm hydrogen peroxide if stored at 90°F for < 8 hours.
  • Use 100ppm hydrogen peroxide if stored at 90°F for > 8 hours.
**S. aureus** Thermal Inactivation

- Objective of this study was to determine D- and Z-values of *Staphylococcus aureus* in whey

- **D-value** = Time to reduce a microbial population by 1-log (90%) at given temperature

- **Z-value** = Temperature change required for 1-log reduction of the microbial population
Thermal Inactivation Study Outline

- Sweet whey obtained during cheesemaking
  - No-starter, mesophilic, or thermophilic types
  - Average pH 6.3-6.6

- 3-strain mixture of *Staphylococcus aureus* added to whey
  - Inoculated at 7-log CFU/ml

- 1ml aliquots were cooked and tested at the following intervals:
  - 140°F (60°C) = 0, 2.5, 5, 7.5, 10, 12.5, and 15 minutes
  - 145°F (63°C) = 0, 0.5, 1, 1.5, 2, 2.5, and 3 minutes
  - 150°F (66°C) = 0, 15, 30, 45, 60, 75, and 90 seconds
  - 155°F (68°C) = 0, 15, 30, 45, 60, 75, and 90 seconds

- Duplicate samples enumerated at each time interval on Baird-Parker agar (selective) overlaid with Tryptic Peptone agar (non-selective)

- Whey batches tested:
  - 3 no starter, 3 mesophilic, and 3 thermophilic batches
Results

140°F (60°C)

145°F (63°C)

150°F (66°C)

155°F (68°C)
# D-values

<table>
<thead>
<tr>
<th>Temp °C</th>
<th>Temp °F</th>
<th>D-value (min)</th>
<th>Time to 5-log reduction (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>140</td>
<td>1.32</td>
<td>6.60</td>
</tr>
<tr>
<td>63</td>
<td>145</td>
<td>0.38</td>
<td>1.90</td>
</tr>
<tr>
<td>66</td>
<td>150</td>
<td>0.12</td>
<td>0.60</td>
</tr>
<tr>
<td>68</td>
<td>155</td>
<td>0.07</td>
<td>0.35</td>
</tr>
</tbody>
</table>

\[
y = -0.1564x + 9.4449 \\
R^2 = 0.97568
\]

### Z-value
- Z-value: 11.56°F (6.39°C)
- \( T_{ref} = 145°F (63°C) \)
Conclusions

• No difference between D-values of whey with or without starter culture
• All 4 temperatures achieved 5-log kill
• Increased heat = Faster kill
  • 140°F D-value = 1.32 min
  • 145°F D-value = 0.38 min
  • 150°F D-value = 0.12 min
  • 155°F D-value = 0.07 min
• Z-value = 11.56°F when using T_{ref} = 145°F
Next Step...

- A current study is being conducted that couples thermal processing and extended storage
  - Whey inoculated at low levels (< 3 log CFU/ml)
  - Cooked at 145°F for 70 seconds
  - Incubated at 70°F or 90°F for up to 24 hours
  - Enumerated at 0, 4, 8, 12, and 24 hours

Goal = To determine if any survivors from inadequate thermal processing can grow during extended storage
Also in the works...
Inhibition of *Listeria* in soft cheeses

**Objective:** Model behavior of *L. monocytogenes* to predict growth/inhibition boundaries

**Model Cheese System:** Micellar casein, cream, water, salt, lactose and acid

- **Phase 1:** Acid type and pH
  - Acid Types = Acetic, Lactic, Citric
  - pH Values = 5.25, 5.50, 5.75, 6.00
  - Moisture = 50%, 56%

- **Phase 2:** Cultured dairy solids/clean label antimicrobials

- **Phase 3:** Protective cultures
Acknowledgements

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