Staphylococcus aureus and Whey
A Collaboration between Industry, Regulatory and Academia

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Wisconsin Center for Dairy Research

Center for Dairy Research “Solution Based Research Backed by Experience, Passion and Tradition”
Some History

- Whey handling in Wisconsin covered by ATCP 65
  - Focus is safety
  - Cooled to <45°F or heated to/maintained at >140°F
  - Within 4 hours of draw from vat
  - Legal pasteurization

- Cooling to <45°F
  - Cost, cost, cost
  - Most plants would have to add cooling capacity

- Heating to >140°F
  - Damage to whey proteins
  - Undesired color changes

- Concern is *Staphylococcus aureus*
Why do We Care About *Staphylococcus aureus*?

- Also known as *S. aureus*
- Gram-positive, aerobic cocci
- Optimal growth conditions
  - pH 6.5
  - 86-99°F
- Ubiquitous (air, dust, clothing, floors, water, sewage and insects)
  - Principal source is human nose (6 – 50% of population)
  - Also found on hands, infected wounds, burns, etc.
- Poor competitor
- **Produces heat stable toxin** (not all strains)
  - Requires $10^{5-6}$ vegetative cells to produce toxin
  - Withstands 250°F for up to 10 minutes
Why do We Care About *Staphylococcus aureus*? (continued)

- Low populations can recontaminate whey
  - Personnel, improperly cleaned equipment, etc.
- Uncooled whey has optimum temperature for growth
  - *S. aureus* optimum 86-99°F
- If toxin produced then remains in the product throughout processing. Not inactivated by heat.
- *S. aureus* is in the Top 5 of food borne pathogens in U.S.
Some History (continued)

- Plants have trouble complying with temperature requirement
  - Cost of equipment
  - Operating cost for cooling
- CDR and DATCP worked together to provide options
- Development of variance process
  - Establish parameters for time/temperature for holding whey
  - Demonstrate product safe under those conditions
  - Monitoring process
  - Process for handling product outside of allowed parameters
  - Conditional use of 100 ppm hydrogen peroxide
Current Options

- Within 4 hours of start of whey draw
  - Meet heating/cooling requirement OR
  - Pasteurize the whey (time and seal) OR
  - Allow pH of whey to drop below 4.6
- Option to use 100 ppm hydrogen peroxide to control pH
- Obtain a variance
  - A central processing facility needs a variance for each whey supplier
  - Monitoring time/temperature/coliforms
  - Option to use 100 ppm hydrogen peroxide
  - Product disposed of if conditions not met
  - Applies only to whey from pasteurized cheese milk
Variances and Whey Handling

- Focus is food safety
- Must demonstrate the product is safe despite being held outside allowed Ag80 temperatures
- *Staphylococcus aureus* concern
- Coliforms used as an indicator of possible problems
- Temporary allowance to use hydrogen peroxide
The Basic Problem

- **Food safety**
  - If no cooling then lower pH is desired
  - If pH decreases then no *S. aureus* growth/toxin

- **Product quality**
  - pH decrease (acid production) makes sticky whey

- **Use of hydrogen peroxide to control microorganisms but does it stop both *S. aureus* and cheese cultures?**

- **Quality versus food safety issue**
  - Low pH keeps *S. aureus* from growing BUT
  - Low pH is an unacceptable product for whey processor
A Balancing Act

Product safety and quality

Product at a reasonable cost

Balance
Partnering to Solve the Problem

- Initially DATCP and CDR working together
  - Ensuring safety for whey not meeting cooling requirements
  - Variance program
  - Limited use of hydrogen peroxide
- Quickly apparent information lacking on *S. aureus* and whey
- Spring 2016 a group met
  - Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP)
  - Wisconsin Cheese Makers Association (WCMA)
  - Food Research Institute (FRI)
  - Center for Dairy Research (CDR)
Regulatory, Industry and Science groups agreed on information needed.

Group developed a plan for finding answers to questions:
- How does starter bacteria and acid production affect *S. aureus*?
- What is the effect of temperature on *S. aureus*?
- What is the effect of hydrogen peroxide on *S. aureus* growth?
- What are the Z- and D- values for *S. aureus* in whey?

FRI would do the research.

WCMA would fund the study.

DATCP and CDR hopefully would be able to use the data to develop alternatives to current processes.
Approaches for Inactivating *S. aureus*

- **Addition of hydrogen peroxide**
  - Hydrogen peroxide (H$_2$O$_2$) has very reactive oxygen atoms
  - Able to punch holes in bacterial cell wall thereby killing the bacteria

- **Thermal inactivation**
  - Heat denatures enzymes of the bacteria causing death
  - Damages cell envelope causing fluid inside to burst out
Study Design

- **Whey from cheese making at CDR**
  - No starter, mesophilic or thermophilic
  - Average starting pH 6.3 – 6.6
- **S. aureus added to whey**
  - 3 strain mixture known to cause food poisoning
  - 3-log CFU/ml
- **Hydrogen peroxidized added**
  - 0, 10 or 100 ppm
- **Incubated at 70 or 90°F**
- **Sampled at 0, 4, 8, 12 and 24 hours**
Acid Production by Cheese Starter Cultures

- Starter cultures will continue to produce acid in whey unless:
  - Temperature is too high
  - Temperature is too low
  - Inactivated by hydrogen peroxide
  - Inactivated by heating

- Acid negatively affects whey quality

- How cool does whey have to be to stop acid production?
- How does pasteurization affect acid production?
How Bacteria Grow

Bacterial Cell Division

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Generation time in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41°F</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Coliforms</td>
<td>8</td>
</tr>
</tbody>
</table>
pH Development in Raw and Pasteurized Cheddar Whey

- **40 F**
- **100 F**
- **80 F**
- **70 F**

**Black dots** — Raw
**Colored dots** — Pasteurized
# Effect of Heat Treatment and Storage Temperature on pH Development

Time for raw or pasteurized whey to drop to pH 6.0

<table>
<thead>
<tr>
<th>Temperature (°F)</th>
<th>Raw (h)</th>
<th>Pasteurized (h)</th>
<th>Difference (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>80</td>
<td>3</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>70</td>
<td>4</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>40</td>
<td>&gt; 48</td>
<td>&gt; 48</td>
<td>-</td>
</tr>
</tbody>
</table>
How Bacteria Die

- Bacteria not all killed instantly
- Population typically dies exponentially
- Decimal reduction time (D-value)
  - Time to kill 90% of the bacteria
  - Time in minutes to achieve a 10-fold reduction at a given temperature
- D-value is for a specific set of conditions
  - Temperature
  - Composition of media - pH, total solids, etc.
  - Bacteria present
## Microbial Exponential Death Rate (10-Fold Reduction)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Deaths per Minute</th>
<th>Number of Survivors</th>
<th>Log</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1,000,000</td>
<td>$10^6$</td>
</tr>
<tr>
<td>1</td>
<td>900,000</td>
<td>100,000</td>
<td>$10^5$</td>
</tr>
<tr>
<td>2</td>
<td>90,000</td>
<td>10,000</td>
<td>$10^4$</td>
</tr>
<tr>
<td>3</td>
<td>9,000</td>
<td>1,000</td>
<td>$10^3$</td>
</tr>
<tr>
<td>4</td>
<td>900</td>
<td>100</td>
<td>$10^2$</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>10</td>
<td>$10^1$</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>1</td>
<td>$10^0$</td>
</tr>
</tbody>
</table>
For this example:

D-value = 1 min

To kill $10^6$ bacteria need to heat at D-Value temperature for 7 min

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Number of Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,000,000</td>
</tr>
<tr>
<td>1</td>
<td>100,000</td>
</tr>
<tr>
<td>2</td>
<td>10,000</td>
</tr>
<tr>
<td>3</td>
<td>1,000</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>
Why the pH Decline After Pasteurization?

- Very high population of starter culture in the whey
  - Do not have enough log cycles to kill all of the starter bacteria
  - Starter resumes growing and producing acid if held at favorable temperatures

![Graph showing the affect of starting population on pH decline](image)
What happens to *S. aureus* with:
- Storage temperature
- Acid production
- Hydrogen peroxide addition
- Competition from starter cultures
S. aureus Growth in Whey at 90ºF with and without Peroxide and Starter Culture

Δlog CFU/ml

Hours

0 ppm, No Starter
0 ppm, Starter
10 ppm, No Starter
10 ppm, Starter
100 ppm, No Starter
100 ppm, Starter

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S. aureus Growth in Whey at 70°F with and without Peroxide and Starter Culture
**S. aureus and Hydrogen Peroxide**

- *S. aureus* produces catalase that inactivates hydrogen peroxide

\[ \text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} \text{O}_2 + \text{H}_2\text{O} \]

- Low concentration of hydrogen peroxide

- High concentration of hydrogen peroxide
# Growth of *S. aureus*

(Initial population of $10^3$ *S. aureus/ml whey)*

## Whey without Cheese Starter

<table>
<thead>
<tr>
<th>Peroxide (ppm)</th>
<th>Storage Temperature 70°F</th>
<th>Storage Temperature 90°F</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Growth &gt;1 log</td>
<td>Growth &gt;2 log</td>
</tr>
<tr>
<td>10</td>
<td>No Growth</td>
<td>Growth &gt;2 log</td>
</tr>
<tr>
<td>100</td>
<td>Decrease 2 log</td>
<td>Decrease &gt;2 log</td>
</tr>
</tbody>
</table>

## Whey with Cheese Starter

<table>
<thead>
<tr>
<th>Peroxide (ppm)</th>
<th>Storage Temperature 70°F</th>
<th>Storage Temperature 90°F</th>
<th>Final pH 70°F</th>
<th>Final pH 90°F</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Growth</td>
<td>Decrease 2 log</td>
<td>4.3</td>
<td>3.8</td>
</tr>
<tr>
<td>10</td>
<td>No Growth</td>
<td>Decrease 2 log</td>
<td>4.3</td>
<td>3.7</td>
</tr>
<tr>
<td>100</td>
<td>Decrease &gt;1 log</td>
<td>Decrease 2 log</td>
<td>6.6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

UW-Food Research Institute, November 2016
Growth of *S. aureus* in Whey

- **Whey with Starter Culture and *S. aureus***
  - pH decreases to <5.0 without H$_2$O$_2$ addition
  - Starter culture competitively inhibits the growth of *S. aureus* at 70°F and 90°F for up to 24 hours
  - Use of 100 ppm H$_2$O$_2$ inhibits both acid production and *S. aureus*
Growth of *S. aureus* in Whey
(continued)

- Whey with No Starter Culture and *S. aureus*
  - Require time-temperature control and/or the addition of hydrogen peroxide
    - *S. aureus* grows > 1 log increase
      - 4 – 8 h at 90°F
      - ~12 h at 70°F
  - Conditions that limit *S. aureus* growth
    - 10 ppm H$_2$O$_2$ - Safe up to 24 hours at 70°F
    - 10 ppm hydrogen peroxide if stored at 90°F for < 8 hours
    - 100 ppm hydrogen peroxide if stored at 90°F for > 8 hours

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Determining Thermal Inactivation of *S. aureus*

**D-value** – Time for 1 log kill

**Z-value** – measure of resistance to temperature change
D- and Z-values for *S. aureus* in Whey

- Time/Temperature for a 3 or 5-log kill of *S. aureus*
  - No difference for whey with or without starter culture
- Increased heat = Faster kill

<table>
<thead>
<tr>
<th>Temperature (°F)</th>
<th>D-value (min)</th>
<th>Time to 5 log reduction (min)</th>
<th>Time to 5 log reduction (sec)</th>
<th>Time to 3 log reduction (min)</th>
<th>Time to 3 log reduction (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>1.32</td>
<td>6.60</td>
<td>396</td>
<td>3.96</td>
<td>238</td>
</tr>
<tr>
<td>145</td>
<td>0.38</td>
<td>1.90</td>
<td>114</td>
<td>1.14</td>
<td>69</td>
</tr>
<tr>
<td>150</td>
<td>0.12</td>
<td>0.60</td>
<td>36</td>
<td>0.36</td>
<td>22</td>
</tr>
<tr>
<td>155</td>
<td>0.07</td>
<td>0.35</td>
<td>21</td>
<td>0.21</td>
<td>13</td>
</tr>
</tbody>
</table>
What Does This Mean for Industry?

- Remember - Need both product safety and product quality
  - Safety Issue – Growth of *S. aureus* and possible toxin production
  - Quality Issue – Production of lactic acid by starter cultures
  - Regulatory Issue – Use of hydrogen peroxide to control pH

- Customer requirements will continue to dictate what options are possible
  - Hydrogen peroxide use not allowed for some customers
  - Limits on total plate counts for final powder
What Does This Mean for Industry? (continued)

- **S. aureus** can grow in sweet whey
  - Much slower growth at 70 versus 90°F

- Peroxide effects **S. aureus** growth in sweet whey
  - 10 ppm hydrogen peroxide slows growth of **S. aureus**
  - 100 ppm hydrogen peroxide kills **S. aureus** (starting population of $10^3$)

- Pasteurization significantly slows acid production especially if held at 70°F post pasteurization

- CFR permits use of 10 ppm hydrogen peroxide
  - 21CFR 173.356 Food Additives
What Does This Mean for Industry? (continued)

- Are there other options that limit acid production and *S. aureus* growth besides cooling to 45°F or addition of peroxide?
  - Is thermalizing whey a viable option?
  - How cold is cold enough?
  - How many log cycles of kill should be built into the process?
  - Currently being determined at CDR

- Peer reviewed publications
  - Answers questions asked 30 years ago
  - Gives credibility to research
  - Able to incorporate results into regulations (PMO)
Conclusions About the Study

- Only made possible because industry, regulatory and scientific groups worked together
- Group decided at the start what worked for everyone
  - Put down on paper what everyone needed from the work
  - Everyone agreed to the needs of others in the group
- Group then decided
  - Variables to be studied
  - Exact conditions (temperatures, concentrations, etc.)
  - Cost
  - Meet and review as study progressed
Conclusions About the Study (continued)

- After initial work was completed
  - Group met again
  - Discussed what it all meant
  - Decided what else needed to be done

- After remainder of work completed
  - Met again to discuss
  - Agreed to additional funding for extra work to explore promising options

- Finally
  - Met to agree on what it means for each group
  - Next steps
  - Celebrate
What Everyone Came Away With

Science
- Papers for peer reviewed publication
- Expanded knowledge on product (whey) safety

Regulatory
- Options for safe handling of whey
- Peer reviewed data that supports changes in whey handling

Industry
- Additional options for safe whey handling that preserve quality and safety while reducing costs
Special Thanks

- **Food Research Institute**
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