Current Research on the Safe Processing of Acidified Foods

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NC Food Safety and Defense Task Force
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USDA/ARS
Food Science Research Unit

• Located within the Food, Bioprocessing and Nutrition Sciences Dept., NC State Univ.
  – Four faculty: vegetable acidification, fermentation, as well as sweet potato processing
• Focus on processing technology and safety
• Since 1935. All publications are now available online.
• Since 2005, ARS Natl. Program project on the safety of acidified foods.
Research priorities

• Prevent an outbreak of pathogenic bacteria in acid and acidified vegetable products...
  – What is the greatest threat?
  – What is the likelihood of occurrence?
• Science based regulation
  – Fill in knowledge gaps
    • Industry needs and regulatory questions
  – Novel ways of producing safe products?
• Fundamental knowledge about pathogens in acidified foods.
  – Acid resistance and survival of pathogens in acid and acidified foods
Current projects

• Applied research (*E. coli*, *Salmonella*, and *Listeria*)
  – Thermal processing at pH 4.6
  – Cold-Fill-Hold studies at pH 3.5
  – Alternative acids (citric, phosphoric, preservative acids)
  – Spore-forming bacilli, pH increase?

• Basic research (primarily *E. coli* O157:H7 and related serotypes)
  – Modeling internal pH, charge/ion balance
  – Internal cell metabolites
  – Acid resistance of alternate *E. coli* serotypes (O104:H4)
  – Modeling buffer capacity
Additional funding and support

• National Integrated Food Safety Initiative: Bridging the Gap: Integrated Research and Extension in Support of Small Processors of Acidified Canned Foods, 3Yr
  – Some funds for research to fill the knowledge gaps: cold fill hold, thermal processing, and bacillus spoilage (pH rise)
  – Project Investigators (PI’s): Dr. Barbara H. Ingham (Lead) & Dr. Fletcher Arritt

• Collaborator with Dr. David Green: Assisting the Integrated Food Safety System’s National Food Training Program, 3 Yr.
  – FDA Training (curriculum committee)

• Direct industry support
Three big questions

1. What conditions are needed for thermal processing acidified foods at 4.6?
   - Vegetative pathogen kill at pH 4.6
   - Thermal processing for spores with organic acids

2. Can bacillus spores germinate and raise pH under anaerobic conditions in a variety of acidified vegetables?
   - What is the mechanism of pH increase?
   - Role of oxygen
   - Buffering

3. Can ‘reasonable’ cold fill hold conditions at pH 3.5 and 10°C (50°F) be identified?
   - Different organic acids and concentrations
1. Thermal processing at pH 4.6

• Current published data is for pH 4.1
  – Acidified pickles
  – Cucumbers juice as a “generic” vegetable medium

• Vegetative cells vs. Spores (questions 1 & 2)
  – Tomato products, bad seals, and FDA concerns?
  – Industry knowledge? (Fred.Breidt@ars.usda.gov)
  – Effects of organic acids on 5D kill of vegetative pathogens
  – pH effect vs. organic acids

• Another important question: *Is a 5-D kill the right target to shoot for?*
  – Risk assessment approach?
  – Not just for thermal processing!
Thermal processing: microbiological methods

- Use a cocktail of acid resistant EHEC strains
  - Most heat/acid resistant in vegetable broth medium
- Induce acid resistance
  - Static growth at 37°C
- Cucumber juice medium
  - Non-inhibitory
  - pH 4.6, 0.6% acetic acid
- Use non-selective media for plating cells
- Independent replication
Modeling approach

1. Generate Log CFU/ml vs. time data
2. Determine 5D reduction value and the standard error (SE) using a version of the Weibull model
   
   Note: Dr. Jason Osborne, NCSU statistics

3. Plot the $\log_{10}(5D + 5xSE)$ vs. Temperature to determine Z value

4. Determine the survival a reference temperature of 160°F ($F_{160}$)
pH 4.1 or lower:

<table>
<thead>
<tr>
<th>Model</th>
<th>Z value (°F)</th>
<th>$F_{160}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. Decay (5SE)</td>
<td>19.50</td>
<td>1.20</td>
</tr>
<tr>
<td>Exp. Decay</td>
<td>15.70</td>
<td>0.34</td>
</tr>
<tr>
<td>Five D Model</td>
<td>11.98</td>
<td>0.08</td>
</tr>
<tr>
<td>One D Model</td>
<td>11.98</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Models as described in the text: Exp. Decay (5SE), exponential decay model with five times the standard error added; Exp. Decay, exponential decay model; Five D Model, linear model based on a five log reduction; One D Model, linear model based on a one log reduction.

$F_{160}$: Time in minutes (F value) needed to achieve the predicted reduction in cell numbers at a reference temperature of 160°F.

TDT data 64°C (147°F)

10^S = N_0 - 5(τ/T^*)^β

<table>
<thead>
<tr>
<th>No</th>
<th>8.30</th>
<th>CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.872</td>
<td></td>
</tr>
<tr>
<td>5D</td>
<td>24.07</td>
<td>min</td>
</tr>
<tr>
<td>Log 5D</td>
<td>1.38</td>
<td></td>
</tr>
</tbody>
</table>
pH 4.6 Data: Z value determination*

\[ Z = 20.3^\circ F \]

\[ F_{160} = 6.9 \text{ min} \]

<table>
<thead>
<tr>
<th>Temp F</th>
<th>pH 4.6</th>
<th>pH 4.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>37.9</td>
<td>7.1</td>
</tr>
<tr>
<td>150</td>
<td>21.5</td>
<td>3.9</td>
</tr>
<tr>
<td>155</td>
<td>12.2</td>
<td>2.2</td>
</tr>
<tr>
<td>160</td>
<td>6.9</td>
<td>1.2</td>
</tr>
<tr>
<td>165</td>
<td>3.9</td>
<td>0.7</td>
</tr>
<tr>
<td>170</td>
<td>2.2</td>
<td>0.4</td>
</tr>
<tr>
<td>175</td>
<td>1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>180</td>
<td>0.7</td>
<td>0.1</td>
</tr>
</tbody>
</table>

pH 4.1 data from Breidt et al., 2010. *Food Prot Trends* 30(5):268-272

* With 5*SE added
Next steps...

• pH 4.6 data: times are 5X greater than pH 4.1 data ($F_{160}$ from 1.2 to 6.9 min)
• Data is non-linear.
• TDT data: higher temperatures (160° F or greater) needed for these experiments
• Alternative acids?
• pH alone
On the drawing board

• Alternative acids, pH 4.6
  – Gluconic acid: **acid independent** data
  – Citric, Phosphoric?

• *Listeria* and *Salmonella*
  – pH 4.1 data showed *Listeria* = EHEC and *Salmonella* was significantly more heat sensitive

• Spore cocktail
  – *Bacillus* spp. (*licheniformis, coagulans*)
  – *Alicyclobacillus*
2. *Bacillus* spores

- Targeting acidified products with pH values between 4.1 and 4.6
- What are the limiting pH values for spore germination and growth?
- What is the mechanism of pH rise?
- How much buffer capacity is there to resist pH change in ‘typical’ product formulations?
- How much oxygen required for spore germination?
- TDT data for bacillus spores with conditions typical of acidified foods (not tomato products) at pH 4.6.
pH increase: microbiology and biochemistry

• Microorganisms of interest
  – *Alicyclobacillus* species
  – *Bacillus licheniformis*
  – *Bacillus coagulans*

• Non-inhibitory medium for studies
  – CJ broth

• Survey of pH elevation
  – *B. licheniformis*

• Mechanism: amino acid deamination?
  – HPLC, amino acid analyzer

• Titrations with standardized base to determine buffer capacity
B. licheniformis: Sugar and pH elevation

- Fermentation vs. deamination
- CJ has 2% fermentable sugar
- Amino acids are present!
pH Elevation and Arginine

- added arginine

+ added arginine
pH 5.5 with Arginine
Buffering

- 10% CJ
- pH 4.55
Bacillus results

• Deamination of arginine can result in an initial pH rise
  – Aerobically AND anaerobically, but only if spores germinate and grow (we used vegetative cells)
• Arginine is not the predominant amino acid in CJ but sufficient amount is present
• Other amino acids can be deaminated as well!
• Buffering is important!
Buffer Capacity and pH

• Buffer capacity is the ability of a solution to resist a pH change.
• Cucumber juice has buffering due to acids, bases, and amphoteric compounds.
• Concentration and pKa values
  – Additive
  – Undefined
• Can be determined by titration?
  – Hypothesis: The complex buffering of CJ (and other vegetable based broths) can be modeled as a simple buffer with a single concentration and pK
Buffer Capacity

\[
\beta = \frac{\partial C_b}{\partial \text{pH}} = \frac{\partial C_b}{\partial [H^+]} \frac{d[H^+]}{d\text{pH}} = -2.303[H^+] \frac{dC_b}{d[H^+]} \\
\beta = 2.303 \left( \frac{C K_a [H^+]}{([H^+] + K_a)^2} + \frac{K_w}{[H^+] + [H^+]} \right)
\]

References


Titration of CJ with acetic acid
Acetic Acid

**FIGURE 4.22.** Buffer index of equimolar acetic acid–sodium acetate as a function of pH.

Buffer capacity data and model
Buffer capacity with hypothetical buffer (pK apx. 3.0)

<table>
<thead>
<tr>
<th></th>
<th>M/L</th>
<th>mM lactic</th>
<th>mM acetic</th>
<th>NaCl %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>2.997</td>
<td>0.125</td>
<td>103.3767</td>
<td>29.57</td>
</tr>
<tr>
<td>7day</td>
<td>2.909</td>
<td>0.066</td>
<td>55.72</td>
<td>27.69</td>
</tr>
<tr>
<td>New</td>
<td>2.843</td>
<td>0.036</td>
<td>11.53</td>
<td>0</td>
</tr>
</tbody>
</table>

Concentration is proportional to lactic acid concentration (Rsq = 0.98)

- Fermentation brines can be modeled using a buffer with a single pKa = 3.0
- NEXT: Allows predictions of pH change with bacillus growth?
3. Cold fill hold at pH 3.5

• Current data shows requires pH 3.3 or below
  – CJ as non-inhibitory medium
  – Acetic acid was used as the primary acidulent to get pH at or below pH 3.3
  – At 25°C (77°F): 48 hr.
  – At 10 C (50 F): 6 days

• Alternatives for acidified foods, pH 3.5
  – 2% and 2.5% acetic acid
  – Citric + acetic (1% each?)
  – Phosphoric
  – Preservative acids (benzoate, sorbate, etc.)
  – Fumaric acid
Microbiology

- Cocktail of 5 EHEC strains
  - Growth conditions to induce acid resistance
- Experiments done at 10°C
  - Above refrigeration, low enough to prevent heating
- Inoculate brined cucumbers with indicated acid conditions
  - Sampling through septa using a syringe
  - Oxygen limited (essentially anaerobic)
  - Brined cucumbers are a non-inhibitory vegetable products that can be representative of a variety of products
- Plate on non-selective media
  - Recovery of injured cells
- Independent replication
- Weibull model for 5-log reduction and statistics
Cold-fill-hold pH 3.5, 2.5% Acetic

\[ \log S = N_0 - 5(\tau/t^*)^\beta \]

<table>
<thead>
<tr>
<th>No</th>
<th>7.86</th>
<th>Log CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>(t^*) (5D+5SE)</td>
<td>4.50</td>
<td>Days</td>
</tr>
<tr>
<td>B</td>
<td>0.80</td>
<td></td>
</tr>
</tbody>
</table>
Cold-Fill-Hold pH 3.5, 2% Acetic

\[ \log S = N_0 - 5(\pi/t^*)^\theta \]

<table>
<thead>
<tr>
<th>No</th>
<th>7.83</th>
<th>Log CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>t* (5D=5SE)</td>
<td>11.97</td>
<td>Days</td>
</tr>
<tr>
<td>B</td>
<td>0.99</td>
<td></td>
</tr>
</tbody>
</table>

Survivors (Log CFU/ml)

Survivors (Log CFU/ml) vs Time (Days)

- No 7.83 Log CFU/ml
- t* (5D=5SE) 11.97 Days
- B 0.99
Conclusions

• Thermal processing at pH 4.6
  – With 100 mM acetic acid pH 4.6: $F_{160} = 6.9$ min., $Z = 20.3^\circ F$.

• Cold fill hold pH 3.5
  – Data for 2.5% acetic acid, apx. 5 days for holding!
  – 2%: apx. 12 days

  *NOTE*: pH 3.3 data, 6 days at 50$^\circ F$ or 48 hr. at 77$^\circ F$

• Spore forming bacilli
  – IFT abstract: “pH elevation by Bacillus licheniformis in Acidified Vegetable Broth” by Meng et al.
  – pH 4.2 was lower limit for increase
  – Glucose represses pH rise, oxygen required!
  – Deamination of arginine responsible for early pH rise
More conclusions

- *Salmonella* and *Listeria* have previously been shown to be less acid resistant
  - Selected trials will be done to confirm this...
- pH 3.5 with 2.5% acetic acid
  - Holding 5 days at 10°C or above
  - 2% acetic acid is probably not useful
- Additional acids and conditions will be done...
  - Suggestions? Fred.Breidt@ars.usda.gov
  - Objective is to meet a wide variety of products with least number of experiments
- White paper/publications with new data being prepared!
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