Thermal Process Filings – Lessons Learned

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Acid/Acidified Foods
FDA 1979: 21 CFR Part 114

- Definitions
  - Acid foods naturally have a pH at or below 4.6
    - Citrus fruits, fermented foods(?)
  - Acidified food: Acid of acid food ingredients added to bring pH to 4.6 or lower
    - Fresh packed cucumbers or peppers acidified with acetic acid

- Water activity
  - Above 0.85

- Pathogens of public health significance capable of surviving in acidified foods must be destroyed

- Spoilage organisms prevented from growing in the product

- GMP school required

- Records required

- Process filings

- Refrigerated foods exempt?
- Fermented foods exempt?
Disease outbreaks 1990s–2010

- *Escherichia coli* O157:H7 outbreak in Osaka prefecture, Japan
  - Radish sprouts
  - Sakai City outbreak among school children responsible for more than 5,000 cases with 3 deaths.

- Outbreaks of disease from acid foods
  - Apple cider (*E. coli* O157:H7), Orange Juice (*Salmonella*)
    - pH values similar to acidified vegetable products (pH 3 to 4)

- Salmonellosis from acidified pickles
  - *Salmonella* Newport
    - Refrigerated pickles, Chicago 2010
    - Cross contamination?
A model emerging pathogen: 
*E. coli* O157:H7 and related serotypes

- Healthy cattle, deer, goats, and sheep harbor the organism in their intestines.
- Phage encoded Shiga-like toxins (SLT-I and SLT-II) inhibit protein synthesis.
- Acid resistant compared to K12 strains, can survive exposure to stomach acid.
  - Most acid resistant in acidified foods...
- 3-4 days post infection bloody diarrhea and abdominal cramps occur, typically self limiting.
- The organism may be isolated from stool samples on sorbitol-MacConkey agar.
- Roughly 8% may (children and seniors) develop HUS, which can result in kidney failure and death.
Historical perspective

• Acidified foods regulations, 1979 (21 CFR part 114)
  – From 1996 – 1999 there were outbreaks of *Escherichia coli* O157:H7 and various *Salmonella enterica* serotypes in acid foods in the US

• 2001: Should all new process filings have a heat process?

• 2002 – 2004: Juice HACCP implemented (21 CFR part 120)

• 2003 and 2007: Clarification of the acidified food rules...
  – Thermal processes established for acidified foods
  – pH 3.3 cold fill-hold data

• 2009: Linear models for process filings
  – Electronic process filings must have linear kinetic parameters

• 2010: Draft guidance document released by FDA

• 2010: Outbreak of salmonellosis (5 reported cases) due to contamination of refrigerated pickles in Chicago.
FDA and USDA/ARS

• FDA, industry and USDA/ARS
  – 21 CFR part 114 leaves a lot unsaid

• USDA, Agricultural Research Service, Food Science Research Unit, Raleigh, NC
    • Heat processes and holding times for acidified pickled vegetables
  – Control of Human Pathogens Associated with Acidified Produce Foods (2011-2016)
    • Alternative acids, non-heat processed products
    • Internal physiology of *E. coli* under acid stress
    • Safety of refrigerated and fermented foods
Research questions

• Acidified foods shall be thermally processed to an extent that is sufficient...
  – What is a sufficient thermal process?
  – What are the organisms of concern?
  – What temperatures are needed?
  – What methods needed to assure the appropriate process is applied?
• Prevent growth of spoilage organisms
  – What are the organisms of concern?
  – Should a thermal process be specified for preventing spoilage?
  – Can spoilage microorganisms raise pH?
• How do *E. coli* O157:H7 and related pathogens survive in acid solutions?
  – What happens to the internal physiology?
  – What are specific effects of organic acids vs pH effects?
Applied and Basic Research
Generation of TDT data

\[ \text{Log}_{10} \text{CFU/ml} = \text{Log}_{10}(N_0) - (1/\ln[10])*(\frac{\tau}{B_1})^{B_0} \]

Reduction Time = \( B_1 * (-\ln[(10^{\delta})(1/B_0)]) \)

Parameters: \( \tau = \text{Elapsed time}, \ B_0 \text{ and } B_1 = \text{Weibull parameters}, \ \delta = \text{Log}_{10} \text{Reduction} \)
Generation of TDT data

Methods

• Cocktails of *E. coli* O157:H7, or *Salmonella enterica*, or *Listeria monocytogenes* strains
• Multiple lots of cucumbers (no antimicrobial compounds)
• Induce acid resistance
• Plate on non-selective media after neutralization of acid
• Use non-linear models

\[ \log S = N_0 - \left[ \frac{1}{\ln 10} \left( \frac{\tau}{\alpha} \right) \right]^{1/\beta} \]

*E. coli* O157:H7 cocktail, 54C, pH 4.1, Pickle Brine
Summary of TDT data

\[ t^* = 4.86e7 \exp(-0.264 \times T) \]

Breidt et al., 2005. J. Food Prot. 63:305-310
Processing Times

- Novel methods for statistics with the Weibull model
- Add five times the standard error incorporated into the estimates for safe holding times

### TABLE 4. Predicted 5-log reduction times for E. coli O157:H7, Salmonella, and L. monocytogenes for temperatures above 60°C in acidified pickle brines

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>$t^{*a}$ (SE)</th>
<th>$U_{95}^{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>4.94 (1.14)</td>
<td>7.64</td>
</tr>
<tr>
<td>62</td>
<td>3.79 (0.97)</td>
<td>6.08</td>
</tr>
<tr>
<td>63</td>
<td>2.91 (0.81)</td>
<td>4.83</td>
</tr>
<tr>
<td>64</td>
<td>2.24 (0.67)</td>
<td>3.83</td>
</tr>
<tr>
<td>65</td>
<td>1.72 (0.56)</td>
<td>3.04</td>
</tr>
<tr>
<td>66</td>
<td>1.32 (0.46)</td>
<td>2.40</td>
</tr>
<tr>
<td>67</td>
<td>1.01 (0.38)</td>
<td>1.90</td>
</tr>
<tr>
<td>68</td>
<td>0.78 (0.31)</td>
<td>1.50</td>
</tr>
<tr>
<td>69</td>
<td>0.60 (0.25)</td>
<td>1.19</td>
</tr>
<tr>
<td>70</td>
<td>0.46 (0.20)</td>
<td>0.94</td>
</tr>
<tr>
<td>71</td>
<td>0.35 (0.16)</td>
<td>0.74</td>
</tr>
<tr>
<td>72</td>
<td>0.27 (0.13)</td>
<td>0.58</td>
</tr>
<tr>
<td>73</td>
<td>0.21 (0.11)</td>
<td>0.46</td>
</tr>
<tr>
<td>74</td>
<td>0.16 (0.09)</td>
<td>0.36</td>
</tr>
<tr>
<td>75</td>
<td>0.12 (0.07)</td>
<td>0.28</td>
</tr>
<tr>
<td>76</td>
<td>0.09 (0.05)</td>
<td>0.22</td>
</tr>
<tr>
<td>77</td>
<td>0.07 (0.04)</td>
<td>0.18</td>
</tr>
<tr>
<td>78</td>
<td>0.05 (0.03)</td>
<td>0.14</td>
</tr>
<tr>
<td>79</td>
<td>0.04 (0.03)</td>
<td>0.11</td>
</tr>
<tr>
<td>80</td>
<td>0.03 (0.02)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*a* Estimate value and standard error for the 5-log reduction time.

Current Industry Practice

Monroe et al., 165°F (74°C) for 15 min
  - Prevent spoilage
  - Inactivate softening enzymes

Most producers use somewhat lower temperatures

Appropriate temperature varies and is product specific
  - Rely on process authority?
Recommended 5 log reduction times

Time (min)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Temperature °F
135
140
145
150
155
160
165
170

Predicted 5 log reduction plus 5 times the standard error

Monroe et al. (quality)

Pickles are here...
Linear models

**NOTE: D values from non-linear data resulted in under-processing!**

Cold fill and hold

A heat process is not needed for some acidified foods

- pH 3.3 or below
- Acetic acid as the primary acidulent
- Holding times and temperatures recorded
  - 6 Days for 10°C
  - 48 hr. for 25°C
- 5 log reduction achieved

Listeria monocytogenes, a cold-and-acid sensitive psychrotroph?

21 CFR part 114

…and those [microorganisms] of non-health significance capable of reproducing…

• Lactic acid bacteria
  – *Lactobacillus brevis, plantarum* etc.
  – *Leuconostoc mesenteroides, Weissella*

• Yeasts and Molds
  – *Pichia manshurica*
  – *Zagosaccharomyces globiformis*

• Sporeforming bacilli (beverages, tomato products)
  – *Bacillus coagulans, licheniformis*
  – *Alicyclobacillus* species
    • Omega alicyclic fatty acids, guaiacol (smoky/phenolic)
Observed spoilage of pickle products

1. Pasteurizer not working properly
   - *Lactobacillus*
   - *No pH increase*

2. Improper seal
   - Molds
   - pH can increase

3. Rate of sugar addition to sweetening tanks
   - Yeasts
   - *No pH increase*

Bell and Etchells, 1952. Food Technol. 6(12): 468-472
Anaerobic sporeforming bacilli?

  - *B. subtilis, licheniformis* require oxygen to grow in tomato juice at pH 4.4
  - No growth of aciduric sporeforming bacilli under anaerobic conditions in fruit juices.
  - *B. coagulans* growth in tomato juice above pH 4.2 but strict anaerobic condition were not used.
  - *B. licheniformis* growth only seen if oxygen is present (simulated “leaker” jars of canned tomatoes) (pH 4.4).
  - Excellent review of *Alicyclobacillus*. Anaerobic growth not determined for some species.
  - *B. subtilis* and *B. licheniformis* capable of mixed acid fermentation requires nitrate and glucose in a modified Spizizen medium (pH 7). Lower pH limit for anaerobic growth not defined.
Current MS project

• 1 MS student, August 2010 – August 2012
• Co-chair with Dr. Fletcher Arritt
• Objectives:
  – Determine pH limits for anaerobic growth of sporeforming bacilli
    • Alicyclobacillus?
    • B. licheniformis and B. coagulans
  – Effect of oxygen on growth at selected pH values (pH 4 and above)
  – Organic acid inhibition of growth
    • Acetate, citrate, malate, fumarate, benzoate, sorbate
Applied and Basic Research
What Factors influence survival of vegetative pathogens in acidified foods?

- Temperature
- pH and brine components
  - Amino acids (Foster)
  - Oils and marinades
  - Sugar
- Dissolved oxygen
- Acid specific effects
  - Organic acid vs. pH
  - L vs D lactic acid
An interesting trend: what about refrigerated pickles and related products?

Sodium gluconate as a buffer...

Acetic (pK’ = 4.7)

Gluconic (pK’ = 3.7)

Note: pH = 3.1
ionic str. = 0.37
D - and L – lactic acid

Protonated acid (mM)

6 hour reduction (log cfu/ml)

Detection limit

Sugar and amino acids

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dp Value (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
</tr>
<tr>
<td>A+CJ</td>
<td>4</td>
</tr>
<tr>
<td>A+GT</td>
<td>16</td>
</tr>
<tr>
<td>A+AR</td>
<td>4</td>
</tr>
<tr>
<td>A+GT+AR</td>
<td>16</td>
</tr>
<tr>
<td>A+GL+FR</td>
<td>2</td>
</tr>
</tbody>
</table>

A = acetic acid (400 mM)
CJ = cucumber juice (50%)
GT = glutamic acid (1 mM)
AR = arginine (1 mM)
GL = glucose (55 mM)
FR = fructose (55 mM)
How do you explain this?
Ph.D. project...
Acid killing of *E. coli* with aerobic and anaerobic conditions at pH 3.2, 25°C

![Graph showing 4 hour log reduction (cfu/ml) for different solutions under aerobic and anaerobic conditions.](image)
Answers and Questions

• Heat processing conditions were defined for acidified foods
  – Time-temperature table
  – Linear model for process filing forms (in F)
    • $Z = 19.5F$, $F = 160F$ $t = 1.2$ min.
• Acidified foods with pH 3.3 or below do not require a heat process
  – 48 hr at 25°C or 6 days at 10°C
• Dissolved oxygen and brine components (amino acids) are important!
• The colder you get the better $E. coli$ O157:H7 survives
  – Refrigerated foods are exempt from 21 CFR part 114
  – Pathogen kill step?
• Fermentation and safety
  – How safe are fermented products?
• Basic research to improve food safety
  – What happens to internal cell conditions with organic acid treatments (pH, metabolomics)?
  – Novel GRAS acids or preservatives?
  – Understanding the oxygen effects...
  – Mechanistic models in internal anion, pH and killing kinetics
Fermentation and acidification?

• LAB produce a variety of antimicrobials

• Sugar is used up
  – Lack of nutrients

• *Pathogens are killed*
  – Breidt and Caldwell, 2011. J. Food Protect. *In press*

• Fermented hamburger dill chips (large market)
  – Institutional containers that *can not be heat treated*

• If filed, how?
  – Cold fill and hold, batch acidification?

• Problem for FDA inspectors: how can you tell the difference between fermented and acidified?
  – Reducing sugar assay?
Pre-acidification of fermentation brines?

- Acetic acid in brine
  - 0.05% acetic acid to remove CO₂
  - *Costilow and Ubersax, 1982, J. Food Sci. 47:1866-1868*

- Recycled brine
  - Reduce pollution
  - *Palnitkar and McFeeters, 1975, J. Food Sci. 40:1311-1315*

- Calcium acetate in brine
  - Reduce competitive flora and maintain firmness
    - Used in anaerobic tank experiments...
      - *Fleming et al., 1988. J. Food Sci. 53:127-133*

- One of these methods is used by almost all large commercial manufacturers
Definition of Fermentation

1. Low acid vegetables subject to the action of acid producing microorganisms that will naturally achieve and maintain a pH of 4.6 or less, *regardless of whether acid is added.*

2. The primary acidulent(s) in the product are acids naturally produced by the action of microorganisms, *even if acid is added.*

3. Pathogens of public health significance are destroyed during the fermentation process.

4. Spoilage organisms capable of raising pH above 4.6 are prevented from growing in the product.

5. Post fermentation processing or ingredients will not raise the pH above 4.6 *in any part* of the product or allow the growth of spoilage microorganisms.
Repacking and reprocessing

• Three ways to achieve a five log reduction:
  – Fermentation
    • Breidt and Caldwell, 2011. J. Food Protect. *In press*
  – Pasteurization
    • Breidt et al., 2005. J Food Prot 68(2):305-310
  – Cold fill hold (pH 3.3 or lower)
    • 48 h for 77F, 6 days at 50F
    • Breidt et al., 2007. J Food Prot 70(11):2638-2641

• Is re-heating necessary?
  – pH remains below 4.6
  – 21 CFR part 110 covers recontamination
  – Cost considerations and energy use
Specific acid effects: *E. coli* O157:H7

- **Aerobic vs. anaerobic killing**
  - Salt effects...
- **Fumaric acid**
  - Food grade
- **Preservative acids**
  - Redefine for use in safety?
- **Acid + heat + oxygen effects?**

<table>
<thead>
<tr>
<th>Acid</th>
<th>Protonated Acid, mM (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malic</td>
<td>547.00 (128.00)</td>
</tr>
<tr>
<td>Acetic</td>
<td>377.00 (21.00)</td>
</tr>
<tr>
<td>D-lactic</td>
<td>140.00 (7.00)</td>
</tr>
<tr>
<td>L-lactic</td>
<td>124.00 (3.00)</td>
</tr>
<tr>
<td>Fumaric</td>
<td>24.11 (3.28)</td>
</tr>
<tr>
<td>Sorbic</td>
<td>6.59 (0.54)</td>
</tr>
<tr>
<td>Benzoic</td>
<td>6.47 (0.38)</td>
</tr>
<tr>
<td>Sulfite</td>
<td>1.27 (0.12)</td>
</tr>
</tbody>
</table>

• Most significant safety concern for the pickled vegetable industry?
• *E. coli* O157:H7 and related serotypes can survive for 1 month or more
• *No kill step*
• Refrigeration is not always the best thing!
• MS student
  – Reformulate brines
  – Fumaric acid and benzoic acid

![Graph: E. coli O157:H7 in refrigerated pickles](image)

**Graph:** E. coli O157:H7 in refrigerated pickles
- **Cell Count (Log 10 cfu/ml)**: Y-axis ranging from 0 to 10
- **Time (days)**: X-axis ranging from 0 to 40
- **Legend**: Solid and dashed lines indicate different conditions or treatments.
Future research

• Blanching...
  – 80C, 15 sec.
  – Location of bacteria on cucumbers

• Hot fill and hold
  – Dr. Paul Palnitkar

• Microwave heating
  – Plastic containers

• Improve acid killing with novel acid formulations
  – Fumaric acid, benzoic, sorbic...
  – Peroxide and organic acids

http://www.needleminds-gifs.com/wizard-flames.jpg
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