Validation and Verification of Thermal and Non-Thermal Processes

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Objectives

• Validation of thermal and non-thermal processes
  – Key concepts
  – Examples – Blanching, High Pressure Processing

• Verification
Considerations When Establishing a Processes

Considerations when establishing the efficacy and equivalency of processes include:

- the identification of risk, hazards and most resistant pathogen(s) of concern in the food (be realistic)
- the efficacy of the process to reduce the risk/hazard and performance criteria
- what are possible control measures and their acceptance
- the food matrix characteristics
- normal conditions of distribution and storage
- the intended use of the food
- Monitoring, review and failure process

(NACMCF, 2004)
Defining the Microbial Target

Spoilage Microorganisms
eg Yeast, Molds
*Lactobacilli*

Toxigenic pathogens
eg *Staphylococcus aureus*
*Clostridium botulinum*

Infectious pathogens
eg *Listeria, Salmonella*
*E.coli O157:H7*

- Delay Growth
- Prevent Growth
- Inactivate

- Prevent Growth
- Inactivate

- Inactivate
Resources

• NACMCF, IFTPS documents
• Food Safety Preventive Controls Alliance
• Regulatory agencies – FDA, USDA, EFSA, FSANZ etc…
• Scientific literature – PubMed, scientific journals
• Trade associations – California Almond Board, American Frozen Foods Institute
• University outreach and extension
Performance Standard Examples
Thermal Log Reduction

- 5 log reduction for fruit and vegetable juices
- 5 log reduction for in-shell egg pasteurization
- 5 log reduction for pasteurized almonds
- 6 log reduction for pasteurization of milk (71.1°C, 3 s)
- 8.75 log reduction for liquid egg pasteurization
- 6 log non-proteolytic *C. botulinum* cook – 90°C, 10 min
- 12 log reduction for sterilization of LACF/ proteolytic *C. botulinum* cook 121.1°C, 3 min
Validation Considerations
Steps in Validation

• Objectives of the validation
• Description and defining the process
• Products covered in the validation
• Validation methodology
• Validation report
Validating Processes

• Process optimization
  – Design and delivery of parameters e.g. water, air, drains, vents, sensors

• Heat and mass transfer models
  – Identify slowest to heat locations and repeatability
  – Locations of heat penetration containers or packs

• Time and Temperature Indicators (TTIs)
  – Alternative to thermocouples
  – Microbiological, enzymatic, chemical or physical

• Heat penetration and uniformity of temperatures in pack
  – Formulation, solids, weight, packaging material
Validation Studies

• When conducting validation tests, conditions for critical factors should reflect the “worst case” expected operating conditions.

• It is useful to “test to failure.”
  – Understand the boundaries between inactivation and survival
  – Establish critical limits for the process
  – Provide information for deviation evaluation
Common Causes: day to day variation in a process that fall inside control limits

Special Cause: a special event or deviation in a process that fall outside the control limits

Source: W.E. Deming
Temperature Mapping of the Blancher at Multiple Locations
18 Data Trace Probes Recording at 2-Minute Increments
The decimal reduction time is commonly referred to either as just $D_T$, or as the D-value.
Temperature Coefficient, z-value

z-value is defined as the temperature coefficient of microbial destruction or the number of degrees of temperature to cause 10-fold or 1-log change in the D-value.

z-value is obtained for an organism in a specific medium by generating survivor curves at different temperatures, obtaining the D-values at these temperatures, then plotting $D_T$ vs. temperature.

\[ z = \frac{(T_2 - T_1)}{\log D_1 - \log D_2} \]
# Heat Resistance of Selected Spore Formers

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium</th>
<th>Temperature (°C)</th>
<th>D value (min)</th>
<th>z (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium sporogenes</em> (PA 3679)</td>
<td>Several substrates including pea purée</td>
<td>104.4–143.3</td>
<td>0.75–2.03</td>
<td>9.0–14.7</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer pH 7</td>
<td>112.8–148.9</td>
<td>1.06</td>
<td>9.3</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>Several substrates</td>
<td>104.0–132.2</td>
<td>0.051–0.58</td>
<td>8.2–10.4</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer pH 7</td>
<td>120.0–140.0</td>
<td>0.13</td>
<td>11.0</td>
</tr>
<tr>
<td>Other <em>Clostridium</em> sp.</td>
<td>Several substrates</td>
<td>85.0–121.0</td>
<td>0.2–195</td>
<td>6.9–11</td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>Several substrates including milk</td>
<td>100.0–121.0</td>
<td>0.3–16.0</td>
<td>4.1–7.7</td>
</tr>
<tr>
<td><em>Desulfotomaculum nigrificans</em></td>
<td></td>
<td>121</td>
<td>55.0</td>
<td>9.5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Nutrient broth</td>
<td>70</td>
<td>0.006</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>70</td>
<td>0.04</td>
<td>6.5</td>
</tr>
<tr>
<td><em>Lactobacillus</em> sp.</td>
<td>Tomato juice</td>
<td>70</td>
<td>4.0–11.0</td>
<td>11.5–12.5</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Beef, chicken, and carrot homogenates</td>
<td>70</td>
<td>0.14–0.27</td>
<td>5.98–7.39</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Aqueous sucrose/glucose (a_w 0.995)</td>
<td>70</td>
<td>0.03–816</td>
<td>6.8–19.0</td>
</tr>
<tr>
<td></td>
<td>Milk chocolate (51% milk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Milk</td>
<td>70</td>
<td>0.30</td>
<td>5.1</td>
</tr>
<tr>
<td><em>Streptococcus</em> sp.</td>
<td>Broth and ham</td>
<td>70</td>
<td>0.015–2.84</td>
<td>3.5–17.0</td>
</tr>
<tr>
<td><em>Microbacterium lacticum</em></td>
<td>Skim milk</td>
<td>70</td>
<td>4.0</td>
<td></td>
</tr>
</tbody>
</table>

Sterilization Value, $F$ (also, F value)

The number of minutes required at any given temperature to inactivate the above spore load of *C. botulinum* is designated the $F$ value for that particular temperature and for the z-value of the organism. Specifically,

$$F_{T_{ref}}^{Z} = \int_{0}^{t} 10^{(T - T_{ref})/z} \, dt$$

Other $F$ values:

$F_{232 \, ^{\circ}F}^{18 \, ^{\circ}F} = 24.5\, \text{min}$  
$F_{214 \, ^{\circ}F}^{18 \, ^{\circ}F} = 245\, \text{min}$  
$F_{250 \, ^{\circ}F}^{18 \, ^{\circ}F} = 3.00\, \text{min}$

$111.1\, ^{\circ}C$  
$101.1\, ^{\circ}C$  
$121.1\, ^{\circ}C$

Industry uses as the “minimum botulinum cook” and designates it as $F_{o}$. Commercial sterility, $F_{o}$ of 5 or 6
Thermal-Death-Time (TDT) Curve

- Logarithmic nature of Time
- Linear with Temperature
- Total kill end point is a two point destruction line

Correlation of Surrogates
Challenge Studies

Inoculate food

- Levels of inoculation
- Type of microorganism
- Inoculum preparation method
- Method of inoculation

Process

- Times
- Temperatures
- Other processing conditions

Determine number of survivors

- Gas formation
- Recovery of inoculum
- Incubation time
- Injured cells recovery

Shelf life

- Microbiological and/or chemical assessment
- Quality changes
- Abuse conditions
Acid Stress

Adaptive Acidification Tolerance Response of *Salmonella typhimurium*

JOHN W. FOSTER* AND HOLLY K. HALL

Department of Microbiology and Immunology, College of Medicine, University of South Alabama, Mobile, Alabama 36688

Received 14 August 1989/Accepted 1 November 1989

- Tolerance response to mild acid treatments
- Classic example by Foster *et al.* in 1990s on *Salmonella* ATR
- Adaptation of Salmonella to mild acid by gradual exposure to lowering pH
- Subsequent generations survived and grow in low pH

**FIG. 1.** ATR. Cells grown to $10^8$ cells per ml in pH 7.6 minimal glucose medium were adapted by adjusting the medium pH to 5.8 (○). After one doubling, the cells were challenged by readjusting the pH to 3.3 (Δ). Unadapted cultures (●) remained at pH 7.6 until achieving a cell density of $2 \times 10^8$ cells per ml and then were directly challenged at pH 3.3 (△). Viable counts were determined at timed intervals. The results are expressed in terms of log percent survival.
Acid adapted using 1% glucose

Type of acid and amount used in acidification are important

Could be important for ingredients that were low acid e.g. watermelon, carrots and vegetables

Breidt et al., 2013 JFP 76:1245-1249
Protein and Fat along with water activity can affect heat sensitivity

<table>
<thead>
<tr>
<th>Actual $a_w$</th>
<th>High-Protein</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>High-Fat</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(avg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>35.34 (0.87)</td>
<td>9.78 (0.13)</td>
<td>0.41</td>
<td>0.50</td>
<td>37.7 (1.56)</td>
<td>11.46 (0.32)</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.63</td>
<td>15.02 (0.44)</td>
<td>9.46 (0.17)</td>
<td>0.57</td>
<td>0.63</td>
<td>16.16 (0.39)</td>
<td>8.72 (0.14)</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.73</td>
<td>6.09 (0.09)</td>
<td>10.4 (0.20)</td>
<td>0.65</td>
<td>0.73</td>
<td>4.77 (0.06)</td>
<td>7.8 (0.09)</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.81</td>
<td>2.41 (0.03)</td>
<td>9.85 (0.12)</td>
<td>0.57</td>
<td>0.81</td>
<td>1.47 (0.03)</td>
<td>7.17 (0.08)</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.90</td>
<td>0.57 (0.02)</td>
<td>7.42 (0.12)</td>
<td>0.34</td>
<td>0.91</td>
<td>0.24 (0.01)</td>
<td>7.01 (0.08)</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.98</td>
<td>0.001 (0.0002)</td>
<td>5.08 (0.09)</td>
<td>0.76</td>
<td>0.004 (0.0005)</td>
<td>6.03 (0.10)</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: $D_{75}^i$ (min), $z_T$-value$^i$ (°C), RMSE (log CFU/g)

Standard error is reported in parenthesis

Jin et al. (2018) J. Food Protection (accepted)
Blanching Validation of Almonds

Objectives

- Verify how long almond kernels are immersed from point A to B under certain operating parameters in a blancher
- Verify the temperatures at the coldest point in the hot water immersion process

Blanching Line Description

- Flow chart of line configuration
- Scalding and drying mechanism
- Scalder speed setting and calibration
- Maximum throughput
- Raw input and blanched product segregation
- Line sanitation procedures
Blanching Validation of Almonds

Blanching Process Flow Chart

Scalding Illustration

Almond Board of California
Blanching Validation of Almonds

Products Covered by Validation
• List all products made through line
• List all products to be validated
• Maximum throughput for each product
• Worst case scenario parameters for each product
Blanching Validation of Almonds

Validation Methodology

- Temperature measurement of hot water
- Duration measurement of hot water immersion
- Replication of validation runs
- Minimum input almond temperature
- Lethality validation for Salmonella

<table>
<thead>
<tr>
<th>Hot Water Temperature (°F)</th>
<th>Time to Meet Required Lethality (min)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-Log</td>
</tr>
<tr>
<td>180</td>
<td>2.47</td>
</tr>
<tr>
<td>181</td>
<td>2.37</td>
</tr>
<tr>
<td>182</td>
<td>2.27</td>
</tr>
<tr>
<td>183</td>
<td>2.17</td>
</tr>
<tr>
<td>184</td>
<td>2.08</td>
</tr>
<tr>
<td>185</td>
<td>1.99</td>
</tr>
<tr>
<td>186</td>
<td>1.90</td>
</tr>
<tr>
<td>187</td>
<td>1.82</td>
</tr>
<tr>
<td>188</td>
<td>1.75</td>
</tr>
<tr>
<td>189</td>
<td>1.67</td>
</tr>
<tr>
<td>190</td>
<td>1.60</td>
</tr>
</tbody>
</table>

** z = 53 F°
Blanching Validation of Almonds

Validation Report submitted by Process Authority
• Handler or manufacturer information
• Production line validated
• Product validated
• Validation methodology
• Results summary
• Handling procedure for the product produced during process deviation
• Dates of the validation conducted
• Products not validated or did not meet 4-log reduction
• Conclusions and recommendations
• Process authority information
**HPP Processing Conditions**

- Need to understand the capacity and limits
- Thermal conditions are well defined
  - E.g. 71.1°C, 3 sec for juice pasteurization same as milk pasteurization
- Non-thermal technologies are not as well defined
- Research and pilot equipment may not necessarily be the same as commercial equipment
  - E.g. HPP research units 600MPa max some may be up to 890 MPa – Commercial units may have 590 MPa max
- Does variation in pressure come up times affect inactivation/survival?
- Pressure variation between pilot and commercial
HPP Juice Validation

Inoculate juice
- Type of microorganism
- Levels of inoculation
- Inoculum preparation method
- Method of inoculation

Process
- HPP processing containment
- HPP pressure
- Time at pressure
- Temperatures

Determine number of survivors
- Recovery of inoculum
- Incubation temp & time
- Injured cells recovery

Shelf life
- Microbiological and/or chemical assessment
- Quality changes
- Abuse conditions

- Effect of containment
- Stability of inoculum
Bacterial Strain Selection

**Salmonella species (7)**
- S. Anatum S102 - sprout
- S. Cubana S120 – alfalfa sprout
- S. Montevideo S240 - tomato
- S. Muenchen S250 - sprout
- S. Newport J1894 - tomato
- S. Thompson RM1987 - cilantro
- S. Typhimurium S337 – orange juice

**E. coli O157:H7 (8)**
- Ec F4546 – alfalfa sprout
- Ec M-11-0450i-1 – hazelnut
- Ec Sakai E417 – radish sprout
- Ec SEA13B88 – apple juice
- Ec TW14359 – spinach
- Ec 960212 - sprouts
- Ec 960218 - sprouts
- Ec F4637 - sprouts

**L. monocytogenes (8)**
- Lm 7-16-3 – dairy
- Lm 573-035 – caramel apple
- Lm 1838 – cabbage
- Lm CDC L316 – coleslaw
- Lm F2365 – clinical human
- Lm MAD328 - environmental
- Lm Scott A – clinical human
- Lm V37 - milk
# HPP (586 MPa) *E. coli* O157:H7 in Orange Juice

<table>
<thead>
<tr>
<th>Day</th>
<th>No HPP</th>
<th>CUT</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>24</th>
<th>38</th>
<th>52</th>
<th>66</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>log cfu/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30s</td>
<td>7.96</td>
<td>7.17</td>
<td>5.60</td>
<td>3.85</td>
<td>2.56</td>
<td>1.13</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
</tr>
<tr>
<td>90s</td>
<td>7.96</td>
<td>7.17</td>
<td>3.20</td>
<td>0.39</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
</tr>
<tr>
<td>120s</td>
<td>7.89</td>
<td>6.97</td>
<td>2.11</td>
<td>0.35</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
</tr>
<tr>
<td>180s</td>
<td>7.96</td>
<td>7.17</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enrichment</th>
<th>30s</th>
<th>90s</th>
<th>120s</th>
<th>180s</th>
</tr>
</thead>
<tbody>
<tr>
<td>pos</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
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<tr>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
</tbody>
</table>

*Note: N/A indicates not applicable or not tested.*
Storage Conditions

• Packaging of product
  – Different sizes/volumes
  – Packaging materials

• Storage and Shipping
  – Abuse during storage
  – Abuse during shipping
Sampling Consideration

- Sampling schemes
  - Usually a minimum of two but typically three
- Replicates or trials
- Sampling method and size
- Sample analysis
  - Usually 1:10 dilution in appropriate buffer
  - Plating – use of nonselective media or with selective overlay media
  - Use of standard methods
- Controls
Duration of Study and Sampling Intervals

• At least to the intended shelf life of the product
  – Typically 1.5 times of intended shelf life (as recommended by NACMCF)
• Sampling intervals based on prior knowledge
  – Depends on intent – growth vs inactivation
• Incorporation of abuse conditions
Interpreting of Results

• Check calculations
• Data mining
• Statistical analysis
• Report results in validation report
Expert Advice and Laboratory

- Relevant FDA and USDA guidance documents, trade association guidance documents
- Use of expert food microbiologist or consultant familiar with HPP
- Consult statistician on study design
  - Ensure consistency and reproducibility
- Review/consultation by regulatory body or the intended recipient of the work
- Choose a laboratory that is credible
- Ask questions
Verification Considerations
Equipment Calibration

• Essential to assure that the data generated are correct
• Performed on equipment and instruments used to monitor or verify parameters in the Food Safety Plan
• Performed at a frequency that ensures equipment will provide an accurate measurement

Institute for Thermal Processing Specialists
http://www.iftps.org

# Calibration and Accuracy Check Examples

<table>
<thead>
<tr>
<th>Calibration (Periodic)</th>
<th>Accuracy check (Routine)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thermometer</strong></td>
<td></td>
</tr>
<tr>
<td>A dial thermometer is checked against an NIST* standardized thermometer for two or more temperatures</td>
<td>Thermometer used to monitor cold temperatures measures the correct temperature of an ice slurry (32°F (0°C))</td>
</tr>
<tr>
<td><strong>pH Meter</strong></td>
<td></td>
</tr>
<tr>
<td>Meter is adjusted to read between two pH points of buffer standards</td>
<td>pH of a single standard near that of the product is measured correctly under plant conditions</td>
</tr>
<tr>
<td><strong>Metal Detector</strong></td>
<td></td>
</tr>
<tr>
<td>Detector is adjusted by manufacturer to detect standardized metal slugs</td>
<td>Detector rejects product with metal standards</td>
</tr>
</tbody>
</table>

*NIST = National Institute of Standards and Technology*
Accuracy Check and Calibration Frequency

• Considerations:
  – Design of the monitoring device
  – Reliability and sensitivity of the device
  – Environment or conditions in which it is used

• Records must:
  – Document results of accuracy checks and calibration procedures
  – Be reviewed or review overseen by a preventive controls qualified individual

• Records should:
  – Provide a traceability to a reference device e.g. National institute of Standards and Technology (NIST)
Intelligent Indicator and Film Technology
High Pressure Pasteurization Indicators

-Time/pressure indicator confirms 87,000psi for 3 minutes

- Visual cues for fast reference, improved administrative compliance
- Verifies accurate machine parameters (tunable)
- Flags inadequate processing - quick identification allows fast re-run
- Supports achieving standards
- Low cost
- Non-reversible
BlindSpotz™
HIGH-PRESSURE PASTEURIZATION INDICATORS

ABUNDANCIA MEXICANA
GUACAMOLE
COLD PRESSURE INDICATOR:
Only accept if the cheesemask is darker than the outer ring.

DO NOT ACCEPT
Under 87,000 PSI

ACCEPT
Over 87,000 PSI

ABUNDANCIA MEXICANA
GUACAMOLE
COLD PRESSURE INDICATOR:
Only accept if the cheesemask is darker than the outer ring.

FARMERS SELECT
New!
Ultra Thin
Cracked Black Pepper Turkey Meat

BLACK BERRY

ctiinks.com
Summary

• Understand of the process helps define validation parameters
• Understanding inactivation kinetics of your target can narrow scope
• Testing for failure is helpful in defining critical limits and variations
• Verification methods require reliable, accurate and calibrated equipment or an independent calibrated and quantifiable parameter
• Careful inclusion of data in validation reports including verification procedures
Thank You and Questions

My Definition of Validation

**VERIFICATION**
- 2 sleeves?
- Is it size L?
- Is it blue?
- Are any buttons missing?

**VALIDATION**
- Does it fit?
- Is it comfortable to drive in?
- Does the colour match my eyes?
- Can I afford it?
- Is it good quality?
- Will my date like it?