Research update: Characterization and mitigation of *Salmonella* and *Listeria* risks

Presented by:
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Objectives

• Preventing cross contamination
  • Pathogens: *Salmonella* and *Listeria monocytogenes*
  • Citrus packinghouse systems:
    • Imazalil, soda ash, sodium bicarbonate
    • Tanks, spray, drench systems
    • Recirculated/reused
  • Compatible sanitizers:
    • Chlorine and peracetic acid

• Evaluating survival of inoculated *Salmonella, L. monocytogenes* on citrus fruit as a function of inoculum carrier
Imazalil with PAA
Stability of PAA in water and Imazalil (300 ppm) at 23ºC (73ºF)

Water

IMZ

Stability of PAA in water and Imazalil (300 ppm) at 23ºC (73ºF)
Effect of PAA concentration (10 to 20 ppm) and exposure time (0.5 to 5 min) on the inactivation of *Salmonella* in 300 ppm imazalil at 16\(^\circ\)C (60\(^\circ\)F)

![Graph](image)

\[\text{Salmonella (n=6)}\]

- LOD = 1.3
- ≥5 log reduction of *Salmonella* was observed in ≥2 min at ≥15 ppm PAA

# positive enrichments (48 h at 37\(^\circ\)C)
Effect of PAA concentration (10 to 20 ppm) and exposure time (0.5 to 5 min) on the inactivation of *Salmonella* and *Listeria* in 300 ppm imazalil at 16°C (60°F)

**Salmonella (n=6)**

<table>
<thead>
<tr>
<th>Log CFU/ml</th>
<th>Control</th>
<th>10 ppm</th>
<th>15 ppm</th>
<th>20 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 min</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>1 min</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>2 min</td>
<td>6 6 6</td>
<td>6 6 6</td>
<td>6 6 6</td>
<td>6 6 6</td>
</tr>
<tr>
<td>3 min</td>
<td>3 min</td>
<td>2 min</td>
<td>2 min</td>
<td>2 min</td>
</tr>
<tr>
<td>5 min</td>
<td>4 2 min</td>
<td>4 2 min</td>
<td>4 2 min</td>
<td>4 2 min</td>
</tr>
</tbody>
</table>

**Listeria (n=9)**

<table>
<thead>
<tr>
<th>Log CFU/ml</th>
<th>Control</th>
<th>10 ppm</th>
<th>15 ppm</th>
<th>20 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 min</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>1 min</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>2 min</td>
<td>9 9 9</td>
<td>9 9 9</td>
<td>9 9 9</td>
<td>9 9 9</td>
</tr>
<tr>
<td>3 min</td>
<td>8 8 8</td>
<td>8 8 8</td>
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<td>8 8 8</td>
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<tr>
<td>5 min</td>
<td>9 9 9</td>
<td>9 9 9</td>
<td>9 9 9</td>
<td>9 9 9</td>
</tr>
</tbody>
</table>

≥5 log reduction of *Salmonella* in ≥2 min at ≥15 ppm PAA

≥5 log reduction of *Listeria* in ≥3 min at ≥20 ppm PAA
Effect of PAA concentration (20 to 60 ppm) and exposure time (0 to 5 min) on the inactivation of *Listeria* in 300 ppm imazalil at 16°C (60°F) (n=3)

A ≥5 log reduction of *Listeria* in IMZ was observed in ≥3 min at 20 and 25 ppm PAA or ≥2 min at 30 and 45 ppm or 1 min at 60 ppm.
Effect of temperature 16° C vs 40° C (60 vs 104° F) on the inactivation of *Salmonella* with or without imazalil (300 pp) or PAA (20 ppm)

Greater survival of *Salmonella* was observed at 16° C compared to 40° C. At 40° C ≥5 log reductions of *Salmonella* was observed within 0.5 min exposure of IMZ with 20 ppm PAA.
Greater survival of *Listeria* was observed at 16°C compared to 40°C.
At 40°C ≥5 log reductions of *Listeria* was observed within 1 min exposure of IMZ with 20 ppm PAA.

Effect of temperature 16 vs 40°C (60 vs 104°F) on the inactivation of *Listeria* with or without imazalil (300 pp) or PAA (20 ppm)

Log CFU/ml (n=6)
Log reduction of *Salmonella* as a function of minimum effective dose of PAA at 16 and 40°C (60 and 104°F)

For 16°C:
- 5 Log reduction @ 30 ppm 1 min
- 60 ppm 0.5 min
- 15 ppm 2 min

For 40°C:
- 5 Log reduction @ 10 ppm 1 min
- 20 ppm 0.5 min

CT = Antimicrobial concentration (ppm) × Contact time (min)
Log reduction of *Listeria* as a function of minimum effective dose of PAA at 16 and 40°C (60 and 104°F)

16°C

- 5 Log reduction @ 60 ppm 1 min
- [30 ppm 2 min 20 ppm 3 min]

40°C

- 5 Log reduction @ 20 ppm 1 min
- [10 ppm 2 min]

[Graph showing log reduction vs. CT (mg.min⁻¹) at 16°C and 40°C, with water and IMZ (300 ppm) curves, and specific log reduction points marked by red circles.]
Minimum effective dose of PAA to achieve $\geq 5$ log reduction of *Salmonella* and *Listeria* in water and imazalil (300 ppm) at 16°C and 40°C in the absence of organic matter.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Temperature (°C)</th>
<th>CT value $\geq 5$ log reduction (mg.min$^{-1}$)</th>
<th>Salmonella</th>
<th>Listeria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>16</td>
<td>40</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>20</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Imazalil (300 ppm)</td>
<td>16</td>
<td>30</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>10</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>
Soda Ash
Survival of *Salmonella* and *Listeria* in water and 3% soda ash at 16 and 40°C (60 and 104°F)

3% soda ash was not effective in reduction of *Listeria* population at 16°C and 40°C.

3% soda ash was not effective in reduction of *Salmonella* population at 16°C, while at 40°C a ≥5 log reduction of *Salmonella* was observed in ≥3 min.
SBC with chlorine
Salmonella were reduced 6 log CFU/ml after 20 seconds of exposure to ≥18 ppm free chlorine in 3% SBC at pH 8.3 and 9.6.

*Salmonella* were reduced 6 log CFU/ml after 20 seconds of exposure to ≥18 ppm free chlorine in 3% SBC at pH 8.3 and 9.6.
Effect of free chlorine and exposure time on the inactivation of *Listeria* in 3% SBC at 16ºC (60ºF) (in the absence of organic matter)

Consistent dose of chlorine; different free chlorine
Survival of *Listeria* after 20 sec exposure of chlorine at 16°C (n=3)

![Graph showing survival of Listeria after 20 sec exposure of chlorine at different pH levels.](image)

- **Water (pH=7.8)**
- **SBC (pH=8.10)**
- **SBC (pH=9.5)**

Log CFU/ml after 20 sec:
- **Water**: 0, 0, 0, 0
- **SBC (pH=8.10)**: 0, 0, 0, 0
- **SBC (pH=9.5)**: 0, 9, 11, 15

Free chlorine concentration (ppm):
- **Water**: LOD=1.3
- **SBC (pH=8.10)**: 15
- **SBC (pH=9.5)**: 15

*Note: LOD = Limit of Detection*
Summary (no organic matter)

**IMZ+ PAA**

- *Listeria* was more resistant than *Salmonella*
- ≥5 log reduction of *Salmonella*: ≥2 min at ≥15 ppm PAA at 16°C; CT=30 (mg.min⁻¹)
- ≥5 log reduction of *Listeria*: ≥3 min at ≥20 ppm PAA 16°C; CT= 60 (mg.min⁻¹)
- At 40°C, minimum effective dose of PAA to achieve ≥5 log reduction of *Listeria and Salmonella* reduced to the CT of 10 and 20 mg.min⁻¹, respectively.

**3% Soda ash**

- No reduction of *Listeria* at 16°C or 40°C after 5 min
- No reduction of *Salmonella* at 16°C after 5 min
- ≥5 log reduction of *Salmonella* in ≥3 min at 40°C

**3% SBC+ Chlorine**

- ≥5 log reduction of *Listeria and Salmonella*: 18 ppm free chlorine in 20 sec at pH 8 and 9.5