8072-41420-020: Development of Alternative Intervention Technologies for Fresh or Minimally Processed Foods

Brendan A. Niemira, PhD
Research Leader
Food Safety and Intervention Technologies Research Unit

USDA - Agricultural Research Service
Eastern Regional Research Ctr.
600 E. Mermaid Ln, Wyndmoor, PA, USA
http://www.tinyurl.com/FSIT-RU
Food Safety and Intervention Technologies Research Unit

“Developing and validating novel processes that improve food safety and quality”

- Determine incidence and prevalence of pathogenic microorganisms in foods.
- Develop and validate non-thermal and advanced thermal intervention technologies, individually and in combination, to improve the safety of meat, poultry, shell eggs, seafood, produce, and other foods.
- Transfer these novel processes
- And procedures to our customers and stakeholders in industry and regulatory agencies in the US and internationally.
Development of Alternative Intervention Technologies for Fresh or Minimally Processed Foods

- Novel antagonists - e.g. *Bacteriovorax*, *Bdellovibrio* and non-pectolytic *Pseudomonas* - for biological-based intervention strategies [M. Olanya]
- Chemical decontamination interventions - e.g. novel sanitizer formulations and advanced gas-phase antimicrobial treatments [B. Annous]
- Nonthermal technologies - e.g. cold plasma, high-intensity monochromatic light, and irradiation - as effective, waterless physical treatments [B. Niemira]
- Engineering prototype development and systems integration support [J. Sites]
- Scientific support: G. Boyd, A. Burke, D. Buckley
Modesto Olanya: Biological controls
Presentation Outline – Biocontrol aspect of CRIS 020

• Sophorolipids & antimicrobial compounds for pathogen control (12 Month)

• Predatory bacteria effects on pathogen inactivation (24 Month).

• Combined effects of biocontrol strains /microbes, sanitizer, radiation on pathogen reductions (36 Month).

• Evaluate biocontrol strains, gas-phase antimicrobials, biosurfactant effects on pathogen control (48 Month)

• Highlight some research accomplishments

• Briefly mention considerations for future research directions

• Outline research collaborators.
1. *Salmonella enterica*:
Gram(-), Facultative anaerobe, rod-shaped
More recent outbreaks (CDC): Shell eggs, dried coconut, chicken salad, raw sprouts, etc.

2. *Escherichia coli* O157:H7:
G(-), Facultative anaerobe, rod-shaped
More recent outbreaks (CDC): Romaine lettuce, leafy greens, peanut butter, etc.

3. *Listeria monocytogenes*:
G(+), Facultative anaerobe, rod-shaped
More recent outbreaks (CDC): cheese, frozen vegetable, raw milk, packaged salads, etc.
12 month milestone

Sophorolipids and antimicrobial compounds for pathogen inactivation on post-harvest produce
Effects of storage time and sophorolipid (C16) concentration on reduction of *E. coli* and *Salmonella* strains in-vitro.
### SL-p and sanitizer combinations on inhibition of *Listeria monocytogenes* inoculated on grape tomatoes (25 C)

<table>
<thead>
<tr>
<th>Pathogen strains</th>
<th>Treatments</th>
<th>Bacterial populations (Log CFU/ml)</th>
<th>Pathogen reductions (Log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lm 2625</td>
<td>Control (untreated)</td>
<td>5.67 ±0.20 a</td>
<td>-</td>
</tr>
<tr>
<td>Lm 2625</td>
<td>SL-p (0.12%)</td>
<td>4.36 ±0.22 b</td>
<td>1.31 c</td>
</tr>
<tr>
<td>Lm 2625</td>
<td>SL-p (5%)</td>
<td>2.01 ±0.92 d</td>
<td>3.66 b</td>
</tr>
<tr>
<td>Lm 2625</td>
<td>Sanitizer</td>
<td>&lt;1.00 (ND) e</td>
<td>5.67 a</td>
</tr>
<tr>
<td>Lm 2625</td>
<td>SL-p (0.12%) + sanitizer</td>
<td>&lt; 1.00 (ND) e</td>
<td>5.67 a</td>
</tr>
<tr>
<td>Lm 2625</td>
<td>SL-p (5%) + sanitizer</td>
<td>2.31 ±1.04 c</td>
<td>3.36 b</td>
</tr>
<tr>
<td>Lm 2625</td>
<td>EtOH (3%)</td>
<td>5.03 ±0.23 a</td>
<td>0.34 c</td>
</tr>
</tbody>
</table>

Grape tomato inoculated with 50 µl of pathogen, air dried (5 mins). SL-p applied at 200 µl, sanitizer applied at 200 µl; and combinations of two treatments applied at 200 µl each. Samples stored for 24h, 25 C and processed.

Data are means and associated standard errors. Means with same letters within columns are not significantly different (*P* > 0.05). ND = non-detectable populations.

*Chlorine dioxide was applied at 0.4 mg/L, 90% R.H., and 13 °C.*
### Control of *Salmonella* & *E. coli* on tomato by SL-p and Sanitizer

<table>
<thead>
<tr>
<th>Pathogen strains</th>
<th>Treatments</th>
<th>Bacterial populations (Log CFU/ml)</th>
<th>Pathogen reductions (Log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM G46390</td>
<td>Control</td>
<td>5.22 ±0.07 a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SL-p (0.12%)</td>
<td>5.08 ±0.08 a</td>
<td>0.41 c</td>
</tr>
<tr>
<td></td>
<td>Sanitizer</td>
<td>&lt;1.00 ND d</td>
<td>5.22 a</td>
</tr>
<tr>
<td></td>
<td>SL-p + Sanitizer</td>
<td>&lt;1.00 ND d</td>
<td>5.22 a</td>
</tr>
<tr>
<td></td>
<td>EtOH</td>
<td>4.86 ±1.22 a</td>
<td>0.36 c</td>
</tr>
<tr>
<td>Ec 35150</td>
<td>Control</td>
<td>5.59 ±0.10 a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SL-p (0.12%)</td>
<td>5.20 ±0.28 ab</td>
<td>0.39 e</td>
</tr>
<tr>
<td></td>
<td>Sanitizer</td>
<td>3.86 ±0.24 a</td>
<td>1.64 b</td>
</tr>
<tr>
<td></td>
<td>SL-p + Sanitizer</td>
<td>3.12 ±0.68 c</td>
<td>2.47 b</td>
</tr>
<tr>
<td></td>
<td>EtOH</td>
<td>6.36 ±0.17 a</td>
<td>0</td>
</tr>
</tbody>
</table>

Means with the same letters within columns are not significantly different (\(P>0.05\)).
Inactivation of foodborne pathogens on tomatoes by SL-\(p\) & sanitizer treatments

- Temp & storage time of SL-\(p\) affected (\(P<0.05\)) pathogen populations. Reductions were > at 25C than 5C.
- Some strains of Lm were more sensitive (008 & 2625) to Sl-p than others.
- At 2-5% SL-p, reductions of Salmonella (1.91-3.86 logs) & E. coli (0.87-4.09 logs).
- Sanitizer & sanitizer + Sl-p reduced bacterial populations by 5.29-5.76 logs and 0.71-3.66 logs.
- Combinations of sanitizer + SL-p were synergistic on E. coli, but not Salmonella & Listeria.
- SL-P exhibited antimicrobial activity
24 month milestone

Predatory bacteria (*Bdellovibrio & Halobacteriovorax* sp.) effects on pathogen reductions on post-harvest produce
Predatory bacteria effects on pathogenic bacteria
### Bacteria reduction by *Bdellovibrio bacateriovorus* 109J in HM buffer

<table>
<thead>
<tr>
<th>Storage time (h)</th>
<th>Pathogen combinations</th>
<th>Bacterial populations (Log CFU/ml)</th>
<th>Pathogen reductions (Log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td><em>E. coli</em> O157:H7 (35150)</td>
<td>7.26 ±0.16 a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> O157:H7 +Bb109J</td>
<td>3.31 ± 0.06 d</td>
<td>3.85 c</td>
</tr>
<tr>
<td>48</td>
<td><em>E. coli</em> O157:H7 (43894)</td>
<td>7.35 ±0.00 a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> O157:H7 +Bb109J</td>
<td>5.55 ±0.53 c</td>
<td>1.80 f</td>
</tr>
<tr>
<td>48</td>
<td><em>E. coli</em> O157:H7 (43895)</td>
<td>8.40 ±0.12a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> O157:H7 +Bb109J</td>
<td>6.95 ±0.18 b</td>
<td>1.44 f</td>
</tr>
<tr>
<td>48</td>
<td><em>S. enterica</em> (2380)</td>
<td>7.55 ±0.35 a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>S. enterica</em> +Bb109J</td>
<td>6.55 ±0.40 b</td>
<td>1.00 f</td>
</tr>
</tbody>
</table>

Means with the same letters within columns are not significantly different (*P*>0.05).
Reduction of nalidixic acid resistant bacterial strains by *Bdellovibrio Bacateriovorus* 109J

<table>
<thead>
<tr>
<th>Storage time (h)</th>
<th>Pathogen &amp; predator combinations</th>
<th>Bacterial populations (Log CFU/ml)</th>
<th>Pathogen reductions (Log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td><em>E. coli</em> O157:H7 (43895)</td>
<td>7.53 ±0.23 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> 43895 +Bb109J</td>
<td>6.41±0.12 c</td>
<td>1.12 h</td>
</tr>
<tr>
<td>24</td>
<td><em>E. coli</em> 11775</td>
<td>7.79 ±0.06 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> 11775 +Bb109J</td>
<td>3.80±0.50 e</td>
<td>3.99 f</td>
</tr>
<tr>
<td>24</td>
<td><em>Salmonella</em> Newport (H1275)</td>
<td>8.86±0.12 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. Newport</em> +Bb109J</td>
<td>8.60±0.26 a</td>
<td>0.26 ns</td>
</tr>
<tr>
<td>24</td>
<td><em>Salmonella</em> Montevideo (G4639)</td>
<td>7.26±0.39 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. Montevideo</em> +Bb109J</td>
<td>5.22±0.16 d</td>
<td>2.04 g</td>
</tr>
</tbody>
</table>

Means with the same letters within columns are not significantly different (*P* > 0.05).
Reduction of *E. coli* O157:H7 by *B. bacteriovorus* 109J on lettuce

<table>
<thead>
<tr>
<th>Produce / predator</th>
<th>Temp (°C)</th>
<th>Treatment</th>
<th>Pathogen reduction (Log CFU/g)</th>
<th>Bb 109J Plaques (PFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td><em>E. coli</em> 35150 + <em>Bb</em> 109J</td>
<td>0.86 a</td>
<td>2.29 ×10⁵ - 1.8×10⁶</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td><em>E. coli</em> 35150 + <em>Bb</em> 109J</td>
<td>1.84 b</td>
<td>4.0×10⁴ - 3.1×10⁵</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td><em>E. coli</em> 35150 + <em>Bb</em> 109J</td>
<td>0.32 a</td>
<td>5.0×10⁴ - 4.2×10⁵</td>
</tr>
</tbody>
</table>
Predatory bacteria effects on pathogen reductions

- Bacterial reductions by Bb 109J was documented on E. coli & Salmonella strains; & ranged from 1-3.5 Log CFU/ml (HM buffer)
- On antibiotic resistant strains, pathogen reductions differed among strains. Non-significant on S. Newport (H1275), but > 3 logs on Ec 11775 (HM buffer).
- Lower pathogen reductions were achieved on produce assays (<2 logs). Variation in strains sensitivity & impediments to motility of attack cells (Bb 109J) on produce may lead to reduced predation efficacy.
- Temp optima for host (pathogen) growth relative to attack prey cells & ratio of predator to prey may influence predation.
- Plans for assessing synergy with other controls. Some limitations e.g. whether Bb 109J has effect on gut microbiota. Delivery systems which can enhance contact of predator/prey would be beneficial.
36 month milestone

Combined effects of biocontrol strains/microbes, radiation and sanitizer on pathogen reductions (36 Month).
Biocontrol, radiation and sanitizer effects on *Salmonella* on produce

![Image of carrots and tomatoes](image1.png)

**Graph 1:**
- **Salmonella enterica populations (Log CFU/g)** vs **Radiation Dosage (kGy)**

**Graph 2:**
- **Injury of *Salmonella enterica* (%) inoculated on carrots** vs **Radiation Dosage (kGy)**
Biocontrol, sanitizer, radiation effects on *Listeria* on produce

**Survival of *Salmonella enterica* on post-harvest produce (Log CFU/g)**

- **Untreated**
- **Biocontrol**
- **Radiation**
- **Sanitizer**
- **R+B**
- **R+S**
- **S+B**
- **S+R**

**Survival of *Listeria monocytogenes* on post-harvest produce (Log CFU/g)**

- **Untreated**
- **Biocontrol**
- **Radiation**
- **Sanitizer**
- **R+B**
- **R+S**
- **S+B**
- **S+R**

*Note: Data shows survival levels for Carrot at 5°C and Tomato at 5°C and 25°C, with letters indicating significant differences.*
Biocontrol, radiation and sanitizer effects on pathogens (*Salmonella, E. coli & Listeria*) on produce

- Treatment combinations significantly (P<0.05) reduced pathogens on produce.

- On carrot, 2.5-5.1 log reductions (5 C), & 1.3-4.7 log reductions (25 C). On tomato, log reductions ranged from 3.1-5.0 (5 C) & 2.2-5.0) at 25 C.

- Relative efficacy of treatments were: Sanitizer > radiation > biocontrol were observed regardless of produce & temp.

- Synergistic effects of 2 & 3-way treatment combinations were recorded, indicating pathogen inactivation may be optimized by treatment combinations.

- Several pubs are under way / student thesis documentation.
48 month milestone

Biocontrol strains, biosurfactants, gas-phase antimicrobials, effects on pathogen control
Relative efficacy of *Pseudomonas chlororaphis* and gaseous chlorine dioxide for suppression of *Salmonella* populations on tomatoes and soybean sprouts

<table>
<thead>
<tr>
<th>Biocontrol + pathogen</th>
<th>Populations (Log CFU/g)</th>
<th>ClO$_2$ 0.4 mg/L (Log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Montevideo</td>
<td>5.26 ± 0.26 ab</td>
<td>0.83 ±0.00 a</td>
</tr>
<tr>
<td>S. Montevideo + <em>P. chlororaphis</em>)</td>
<td>4.79 ± 0.41 a</td>
<td>&lt; 0.30 a</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>5.62 ± 0.14 b</td>
<td>&lt; 0.30 a</td>
</tr>
<tr>
<td>S. Typhimurium + <em>P. chlororaphis</em>)</td>
<td>5.12 ± 0.16 ab</td>
<td>2.59 ± 1.46</td>
</tr>
<tr>
<td>Means</td>
<td>5.19</td>
<td>-</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.81</td>
<td></td>
</tr>
</tbody>
</table>

*Int. J. Food Sci. & Technol. 2015, 50, pg 1102-1108; Food Sci. & Biotech, 2017, 26, pg 513-520;*
Cost comparison of competitive exclusion microbe (CEM) production and application to competing technologies on tomato

<table>
<thead>
<tr>
<th>Pathogenic microbe control</th>
<th>Production scale</th>
<th>Cost (US$/ kg of tomato)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competing technologies (mean)</td>
<td>Large</td>
<td>$0.001</td>
</tr>
<tr>
<td>Chlorine wash</td>
<td>Large</td>
<td>$0.00046</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>Small</td>
<td>$0.21</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>Large</td>
<td>$0.02</td>
</tr>
<tr>
<td>CEM</td>
<td>Small</td>
<td>$0.073</td>
</tr>
<tr>
<td>CEM</td>
<td>Large</td>
<td>$0.0058</td>
</tr>
<tr>
<td>PEF (orange juice)</td>
<td>Commercial process.</td>
<td>$3.70/L</td>
</tr>
<tr>
<td>Thermal pasteurisation</td>
<td>Commercial process.</td>
<td>$0.107/L</td>
</tr>
</tbody>
</table>

Production scale – general scale of pathogenic microbe control  
Mean total costs for application of technology / kg of tomato using conventional methods of biocontrol application

*Biocon. Sci & Technol. 2016, 26, pg 651-664*
Research Accomplishments

• SL-p & antimicrobial compounds, predatory bacteria and effects on pathogen inactivation quantified & documented. Several pubs. & presentations made.

• Combined treatment effects (biocontrol, sanitizer, radiation & gas-phase antimicrobials – significant pathogen reductions & synergistic effects demonstrated. Collaborations e.g. agreement with University of Puerto Rico & numerous presentations & publications & others in progress.

• Quantified price tags for biocontrol / competitive exclusion microbes (CEM). Documented CEM costs relative to competing technol e.g. thermal pasteurization or HPP. Research highlighted in ARS Annual Report (2016).

• Numerous disseminations of published data & presentations of reports. Exploratory interest by Ebiotech for potential collaborations.

• Collaboration with Durban University of Technology (S. Africa), Dept of Health Kwazulu (S. Africa), University of Maine, e.t.c. led to cost quantification of listeriosis impacts in S. Africa & demonstrated food safety implications (published).
Future research considerations

• Completion of 48 month milestones, & continuation of 60 month milestone research (asses the effects monochromatic blue light + predatory bacteria + biocontrol strains on leafy greens.

• Inactivation of foodborne pathogens by predatory bacteria and combinations of biocontrol strains (competitive exclusion). Potential collaborations with biocontrol industry e.g. Ebiotech.

• Potential utility of antimicrobial peptides for pathogen control.

• The most synergistic aspects of combinations of biocontrol, physical and chemical measures for pathogen reductions on post-harvest produce.

• Pathogen inactivation by sanitizer, natural anti-pathogen derived products (protein isolates).
Research collaborations

Dr. Aaron Hoshide, University of Maine
Dr. Oluwatosin Ijabadeniyi, Durban University of Technology
Dr. Olusankanmi Ayeni, Dept of Health, South Africa
Professor Lynette Orellana, University of Puerto Rico, Mayaguez
Dr. Richard Nyankanga, University of Nairobi, Kenya
Dr. Peter Ojiambo, North Carolina State University
Dr. Daniel Kadouri, Newark University, Dental School, NJ
Dr. Susan Koval, Western Ontario University, Canada
FSIT CRIS 20 Research Scientists
FSIT Research Scientists / collaborators, BOAC & RCPM Scientists
Dr. Zhongqi He, USDA-ARS, SRRC, New Orleans, LA
Dr. Bob Larkin, USDA-ARS, NEPSWL, Orono, ME
Thanks for your attention

Modesto.Olanya@ars.usda.gov
Bassam Annous: Chemical and thermal treatments
Efficacy of hot water treatments (90 s) in reducing *Salmonella* on mung bean seeds

<table>
<thead>
<tr>
<th>Treatment Temperature (°C)</th>
<th>Log CFU/gm seed</th>
<th>Log CFU/ gm sprout</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>5.44 ± 0.12</td>
<td>8.24 ± 0.10</td>
<td>97</td>
</tr>
<tr>
<td>70</td>
<td>0.30 ± 0.00</td>
<td>7.32 ± 0.14</td>
<td>98</td>
</tr>
<tr>
<td>75</td>
<td>No Growth</td>
<td>7.11 ± 0.55</td>
<td>96</td>
</tr>
<tr>
<td>80</td>
<td>No Growth</td>
<td>No Growth</td>
<td>95</td>
</tr>
<tr>
<td>90</td>
<td>No Growth</td>
<td>No Growth</td>
<td>25</td>
</tr>
</tbody>
</table>

(Dr. Bassam Annous)
Cantaloupes

Control

Hot water (70°C) Treated
Fresh-cut prepared from treated cantaloupes and stored at 4°C for Two Months
Biobased Insert (Patent pending)

On-demand release of ClO$_2$ into a closed package
Post-packaging control of pathogens on fresh and fresh-cut fruits and vegetables

Figure 2. Inter-layer binding of pectin and gelatin layers. Application of the positively charged gelatin glue solution on the surface of slightly negatively charged pectin followed by pressing onto the negatively charged gelatin layer to promote binding and achieve contact between the layers.
Brendan A. Niemira: Nonthermal processes
ERRC gliding arc cold plasma emitter

- e^-
- Free radicals
- Elemental Oxygen
- NO_x
- Ozone
- Nanoparticles
- UV light
- Oxygen
- Injected volatiles
- Nitrogen
- Carbon dioxide
Flexible, broadly effective
Plasma jet: rapid treatments

- Almonds
  - 20s at 6cm reduced *E. coli O157:H7 C9490* by 1.34 log

- Sliced tomatoes
  - 20s at 2cm reduced *S. Stanley* by 0.71 log

- Time x Distance: complex & isolate-dependent.

- Nitrogen was less effective than air

- Optimized trade off of amount of kill vs. speed (Niemira 2012 J Food Sci)
Cold plasma: blueberries

- Blueberries as model system for small, fragile fruits
- Inoculated with Norovirus surrogates:
  - Murine Norovirus (MNV)
  - Tulane virus (TV)
- Native microflora: TAPC, Y&M
- Sensory impact:
  - compression firmness
  - surface color
  - total anthocyanins
- Measurements of heat buildup, with and without active cooling measures:
  - Determine non-thermal kill level
    
    (Lacombe et al., Food Micro 2015)
Cold plasma: viral inactivation

- Both surrogates effectively inactivated by cold plasma.
  - Significant (P<0.05) effects after 45s (TV) or 15s (MNV).
- Antimicrobial effects not related to heat.
- Engineering controls allowed for minimizing heat buildup on berries.
Cold plasma: Salmonella on oranges

- Dyne-A-Mite HP AC plasma jet
- 47kHz, 549 W
- Feed gas: dried air, 60 psi
- Distances: 0 or 7.5 cm
- Inoculated oranges set on a rotating platform
  - Simulates “tumbling” of a commercial setting
- Treatment times: 0 (non-treated control), 1, 3, or 5 minutes, at room temperature
Antimicrobial efficacy

Survival of *Salmonella Anatum* on Valencia oranges after treatment with cold plasma jet

<table>
<thead>
<tr>
<th></th>
<th>Stem Scar 0 cm</th>
<th>Stem Scar 7.5 cm</th>
<th>Blossom Scar 0 cm</th>
<th>Blossom Scar 7.5 cm</th>
<th>Peel 0 cm</th>
<th>Peel 7.5 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.52</td>
<td>6.52</td>
<td>6.13</td>
<td>6.13</td>
<td>6.11</td>
<td>6.11</td>
</tr>
<tr>
<td>1 min</td>
<td>5.84</td>
<td>6.10</td>
<td>5.63</td>
<td>5.78</td>
<td>3.88</td>
<td>6.00</td>
</tr>
<tr>
<td>3 min</td>
<td>4.97</td>
<td>6.33</td>
<td>3.08</td>
<td>5.78</td>
<td>3.61</td>
<td>5.54</td>
</tr>
<tr>
<td>5 min</td>
<td>4.29</td>
<td>5.27</td>
<td>2.64</td>
<td>4.68</td>
<td>2.51</td>
<td>4.38</td>
</tr>
</tbody>
</table>

Legend:

- Control
- 1 min
- 3 min
- 5 min
Antimicrobial efficacy (2)

- All treatments reduced *Salmonella* vs. control
- Log reductions dependent on distance
  - 0 cm: 0.94 – 2.09 (stem scar), 1.57 – 3.56 (blossom end), and 2.4 – 4.09 (peel)
  - 7.5 cm: 0.15 – 1.57 (stem scar), 1.01 – 1.80 (blossom end), and 0.37 -1.22 (peel).
- Longer time, greater reductions
  - For 0 cm, 3 and 5 min NSD
- Effective against all inoculation sites
  - In contrast to chemical sanitizer treatments
  - Stem scar, blossom end typically more difficult
Cold plasma: in-package treatment

- Romaine lettuce, inoculated with E. coli O157:H7
- 5’ treatment, with plasma generated inside the commercial clamshell package
- 1.0 – 1.5 log reduction, persistent through 7d refrigerated storage
- Suggests new applications for post-packaging antimicrobial treatment

*(Min et al. 2016. JFP; Min et al. 2017 Food Micro)*
Cold plasma: in-package treatment of bulk Romaine lettuce

Schematic diagram of the cold plasma reactor holding a clamshell package treatment sample containing seven layers of lettuce leaves in a three-column configuration.
Cold plasma: in-package treatment

- The *L. monocytogenes* population declined by an additional 0.6 log CFU/g after being stored at 4 °C for 24 h
  - Cold storage can enhance the treatment effect against *L. monocytogenes*
- For bulk packaged lettuce, inhibition rates of *E. coli* O157:H7 were consistent in all locations for 1-, 3-, 5-, and shaken 7-leaf stacks.
- Both the rigid and flexible types of conventional and commercial plastic packages can be directly used for packaging lettuce that will be decontaminated by DACP
  - Current produce packaging lines can be utilized with DACP processing, with further optimization
Inactivation of *Escherichia coli* on Blueberries using cold plasma with chemical augmentation inside a partial vacuum

**Inactivation of *Escherichia coli* on Blueberries Using Cold Plasma with Chemical Augmentation inside a Partial Vacuum**

Sarah M. Hertrich, Glenn Boyd, Joseph Sites and Brendan A. Niemira
Food Safety and Intervention Technologies Unit, Eastern Regional Research Center, USDA-ARS, Wyndmoor PA 19038

**RESULTS**

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>0.00</th>
<th>0.50</th>
<th>1.00</th>
<th>1.50</th>
<th>2.00</th>
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<tbody>
<tr>
<td>Vacuum Only</td>
<td>0.32</td>
<td>1.84</td>
<td>0.41</td>
<td>0.71</td>
<td>0.28</td>
</tr>
<tr>
<td>Vacuum + H2O</td>
<td>0.19</td>
<td>1.61</td>
<td>0.56</td>
<td>0.51</td>
<td>0.35</td>
</tr>
<tr>
<td>Vacuum + 25% EtOH</td>
<td></td>
<td></td>
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<tr>
<td>Vacuum + 50% EtOH</td>
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<tr>
<td>Vacuum + CO2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuum + CO2 + H2O</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Legend**

- **10 min**
- **5 min**
- **1 min**
Cold plasma Inactivation of Biofilms

- *Salmonella* and *E. coli* O157:H7, adherent biofilms grown for 24, 48 or 72h on a test surface
- Cold plasma applied to a moving conveyor belt. Very short treatments reduced the most durable biofilms
  - *E. coli* O157:H7: 1.25 log (5s), 2.46 log (10s), 3.03 log (15s)
  - *Salmonella*: 0.92 log (5s), 1.59 log (10s), 2.12 log (15s)
- Rapidly and effectively inactivated persistent contamination on food contact surfaces associated with fruits and vegetables processing. (Niemira et al. 2014 J Food Sci)
Cold plasma Inactivation of Biofilms (2)

- Rapid reductions of adherent biofilms: 99.96% reduction in 15s without sanitizers or scrubbing
- Infra-red imaging confirms a nonthermal process (Niemira et al. 2018 Fr Sust Food Systems)
Cold plasma Inactivation of Biofilms (3)

- *Salmonella* biofilms grown on glass slides 24h, placed inside food containers
- Corona discharge cold plasma (gentler than plasma jet) for 3 minutes
- Biofilm EPS structure degraded and dispersed
- Image analysis of Live/Dead confocal microscopy to quantify biofilm dispersal (*Hertrich et al, in preparation*)
Vortex tube-coupled cold plasma

- In closed containers, heat can accumulate from plasma treatment
- Air jet cooling works, but advanced vortex tube cooling is better
- Replacing air jets with vortex tubes reduces temperatures by up to 20°C on conveyor belts, and by up to 62°C in closed containers
- Simple, robust, non-electrical cooling will expand applications for cold plasma treatment
Conclusions

- New sanitizers seeing commercial application
- Biological controls = new opportunities
- Precision thermal application to produce
- Cold plasma is an effective, waterless, chemical-free, contact-free antimicrobial process

Key areas for future research
- Effective combinations with liquid chemical sanitizers
- Integration of new technologies with existing equipment
- Energy costs, overall process efficiencies
- Adaptation to the particular needs of individual commodities
Joseph Sites: Engineering support and systems integration
Parametric Modeling

Software: Solidworks

Benefits:

- Virtually design and test prototypes
- Design changes of components are carried through assemblies, eliminating rework
  - Structural, Thermal and Dynamic testing available using the same models
- Direct import for 3D printers
- Direct import for Injection Modeling
- Direct import for CNC Milling and Turning
Additive Manufacturing Capabilities
(3D Printing)

**Stratasys Eden 260V**
- **Build Volume:** 255 x 252 x 200 mm (10 x 9.9 x 7.9 in)
- **Process:** Poly Jet (Liquid Resin / UV Cure)
- **Layer Resolution:** 16 microns (0.006 in)
- **Best uses:** Close tolerance prototype models, limited use molds for vacuum forming and injection molding.
- **Material:** Soft rubber to polycarbonate like material

**MakerBot (Stratasys) Replicator +**
- **Build Volume:** 285 x 153 x 155 mm (11.2 x 6.0 x 6.1 in)
- **Process:** Fused Deposit Modeling (Thermoplastic extrusion)
- **Layer Resolution:** 100 micron (0.0039 in)
- **Best uses:** Inexpensive rapid prototype – proof of concepts.
- **Material:** PLA (polylactic acid)
Rapid Prototype Advantages
(3D Printing)

-No hard tooling required
-Low Cost: No significant penalty for redesigns
-Reduced Design-Build-Test cycle by 75% - 80%
-Faster turn around time: All work done in-house
-Allows for more complex designs
-Allows for organic designs

Example: RF Antenna Zero Torsion Brackets – Project Evolution