Efficacy of a Washing System and Commercial Produce Washes to Reduce Escherichia coli Surrogates on Green Leaf Lettuce Surface

Keyla Lopez¹, Donka Milke¹, Nicholas Bloedow², and Kelly J.K. Getty¹
¹Food Science Institute and Department of Animal Sciences and Industry; and ²Department of Statistics, Kansas State University, Manhattan, KS, USA

Introduction
Lettuce has repeatedly been associated with foodborne outbreaks connected to Escherichia coli O157:H7. Leafy greens are highly perishable food commodities that are generally consumed raw. Leafy greens are washed before consumption, primarily to remove soil, pesticide residues, and spoilage-causing and pathogenic microorganisms. The objective of this study was to test the efficacy of a continuous water motion washing system for foodservice reducing rifampicin-resistant E. coli surrogates on the surface of green leaf lettuce.

Materials and Methods

Samples
• Green leaf lettuce was purchased at a local retail store in Manhattan, Kan.
• Lettuce leaves (1,000 g per each container) were placed in 6 separate plastic containers with lids.

Inoculation (Figure 1)
• Lettuce leaves in each plastic container were inoculated with a fine mist of a five-strain cocktail of rifampicin-resistant E. coli surrogates inoculum (ca. 10 mL a total of ten full sprays).
• Then the plastic containers were covered with lids and manually shaken to assist inoculum distribution.
• Inoculated lettuce was allowed to dry for 1 h at 25±2°C in a biosafety cabinet to allow attachment of cells.

Experimental Treatments
• Inoculated lettuce samples (1,000 g) were wash separately with tap water (TW), a commercial antimicrobial for fruit and vegetable wash [CAFVT; lactic acid (1,061 – 1,391 ppm), sodium hydrogensulfate, dodecyl[b]sulfonic acid (76 -11 ppm; sodium salt)], or a 5% vinegar solution containing 0.24% acetic acid [VS; The Kroger Co., Cincinnati, OH, with 5% acetic acid].

Washing Process (Figure 2)
• Treatments were applied for 120 s either by using a continuous water motion washing system or by hand resulting in six treatment combinations.
• Lettuce samples washed by hand were submerged in and out of the washing solution (ca. 120 L) for 2 min by gloved-covered hands. After washing, lettuce was removed from the wash tank by using a stainless steel basket, shaken, and allowed to air dry for 5 min.

Sampling and Enumeration
• Following washing procedures, lettuce samples (250±0.3 g) from each treatment combination (n= 2 per replication) were separated for enumerations.
• Remaining lettuce leaves were stored at 4±1°C for further sampling at days 1, 4, and 6.
• Leaves were blended and aliquots from the resulting homogenate were surface-plated onto tryptic soy agar (TSA) supplemented with 100 μg of rifampicin per mL.

Statistical Analysis
• The experiment was replicated three times and followed a randomized complete block design with a factorial arrangement of treatments.
• Data were analyzed using PROC GLIMMIX procedures of SAS.
• Appropriate main and interaction effects were tested at P<0.05.
• Mean log_{10} reductions were estimated from contrasts of the treatment combination minus the inoculated control treatment for each trial.

Results

On Day 0 After Washing
• Mean log reductions of E. coli populations were not affected by the interaction of wash solution x wash action (P>0.05).
• Therefore, main effects were compared across wash solutions and then across wash actions to determine statistical differences.

Table 1. Mean log reductions of E. coli surrogate populations on green leaf lettuce after application of washing treatments on day 0

<table>
<thead>
<tr>
<th>Effect</th>
<th>Treatment</th>
<th>Log Reduction (CFU/g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash solution</td>
<td>Tap water</td>
<td>1.34ab</td>
</tr>
<tr>
<td></td>
<td>CAFVT</td>
<td>2.25b</td>
</tr>
<tr>
<td></td>
<td>VS</td>
<td>2.09ab</td>
</tr>
<tr>
<td>Washing action</td>
<td>Hand</td>
<td>1.53b</td>
</tr>
<tr>
<td></td>
<td>Agitation</td>
<td>2.26bc</td>
</tr>
</tbody>
</table>

Changes of E. coli surrogate populations over storage time

Implications
• Incorporation of wash solutions and/or agitation in the washing process compared to water alone reduced greater E. coli surrogate populations on green leaf lettuce surface.
• Storage of green leaf lettuce at refrigeration temperatures (4±1°C) after washing reduced the risk of potential proliferation of E. coli surrogates.
• The vinegar solution (5%) represents a good alternative at foodservice to decrease potential microbial contamination.

Acknowledgments
• This research was conducted by Kansas State University and was partially funded by the U.S. Department of Agriculture.