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

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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 application combined with chemical wash solutions or tap water alone in 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.

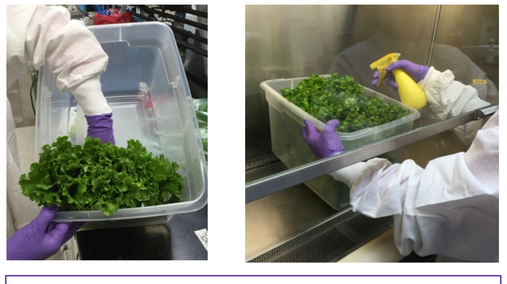


Figure 1. Inoculation procedure for lettuce samples

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, dodecylbezesulfonic 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 glove-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 (25±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.

a) b) c) d)

Figure 2. Washing process: a) Continuous water motion washing system, b) two bay wash tank, c) lettuce samples to be washed d) stainless steel basket used to remove lettuce from the wash tank

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 × wash action ($P>0.05$).
- Therefore, main effects were compared across wash solutions and then across wash actions to determine statistical differences.

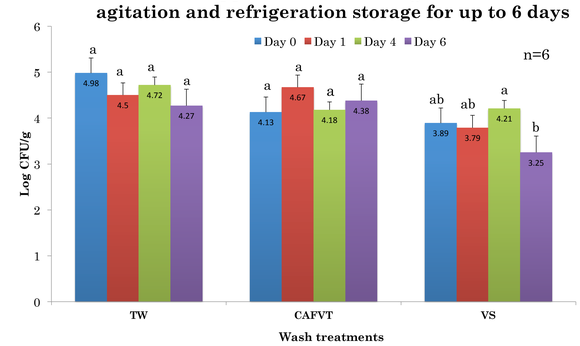
Table 1. Mean log reductions of *E. coli* surrogate populations on green leaf lettuce after application of washing treatments on day 0

Effect	Treatment	Log Reduction (CFU/g)[c]
[1]Wash solution	Tap water	1.34[b]
	CAFVT	2.25[a]
	VS	2.09[ab]
[2]Washing action	Hand	1.53[y]
	Agitation	2.26[x]

CAFVT= Commercial Antimicrobial Fruit and Vegetable Treatment; VS= Vinegar Solution in Water
[1]Data pooled across washing action (n = 12); Standard error (SE) = 0.19.
[2]Data pooled across wash solution (n =12); SE = 0.15.
[ab]Means or [xy]Means with different superscripts within a column section are different ($P < 0.05$).
[c]The initial mean population of *E. coli* on unwashed inoculated samples was ~ **6.57 $\log_{10}$ CFU/g**.

Changes of *E. coli* surrogate populations over storage time

***Figure 3. E. coli* surrogate populations on lettuce leaves after application of washing treatments with agitation and refrigeration storage for up to 6 days**



[ab]Bars with different superscripts within a group are different ($P < 0.05$).

***Figure 4. E. coli* surrogate populations on lettuce leaves after application of washing treatments by hand and refrigeration storage for up to 6 days**

[ab]Bars with different superscripts within a group are different ($P<0.05$).

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 foodservices to decrease potential microbial contamination.

Acknowledgments

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