Validation of Washing Treatments to Reduce Pathogens in Green Leaf Lettuce and Tomatoes

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Introduction

Fresh tomatoes and lettuce have repeatedly been associated with outbreaks caused by *Salmonella* and *Escherichia coli* O157:H7. Although, the specific source of contamination have not been identified, tomatoes and lettuce are grown and handled in environments that facilitate contamination. To minimize pathogen contamination of fresh produce, effective food safety interventions are needed from production to consumption. **Therefore, the objective of this study was to determine the efficacy of a commercial wash solution for reducing pathogens on the surface of green leaf lettuce and tomatoes.**

Materials and Methods

Samples

- Green leaf lettuce and un-waxed tomatoes were obtained from K-State Dining Services.
- Produce were stored at 4°C for no more than 2 days and then tempered at room temperature (22 ± 2°C) prior inoculation.

Inoculation

- Lettuce samples were spot inoculated; 1 ml of *E. coli* O157:H7 (five- strain) inoculum suspension (~7.8 log CFU/ml) was distributed in 10 drops/spots on the upper side of leaves.
- Tomatoes were submerged in *Salmonella* spp. (five-strain) inoculum suspension (~9.39 log CFU/ml) for 30 sec.
- After inoculation samples were allow to air dry in the biosafety cabinet (22 ± 2°C) for 1 h to allow for bacteria attachment.

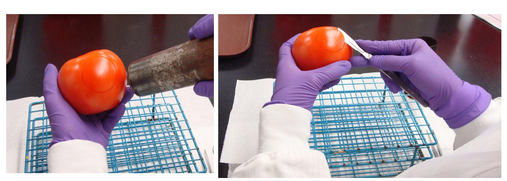

Figure 1. Coring procedures for tomato samples

Experimental Treatments

- Samples were either treated with a commercial fruit and vegetable wash solution (CWS) or regular cold tap water (CTW) for three contact times (30, 60, 120 s) resulting in six treatment combinations.

Washing Process (Figure 2)

- Per treatment combination, two inoculated lettuce samples (25 ± 0.3 g/each) or tomatoes were washed by submerging and gently stirring produce item in the treatment solution.
- A disinfected metallic colander was used to hold produce during washing and rinsing produce with tap water (50 ml per lettuce leaf; 100 ml per tomato).
- After rinsing, produce was allowed to dry for 5 min.

Sampling and Enumeration

- Lettuce (25 ± 0.3g) and tomato (core of 11.34 cm^2) samples (Figure 1) were diluted and stomached for 1 min and plated onto sorbitol MacConkey agar with cefixime tellurite supplement (CT-SMAC) and xylose-lisine deoxycholate (XLD) agar plates for *E. coli* O157:H7 and *Salmonella,* respectively (Figure 1).
- Experiment consisted of three replications and two samples per treatment (n=6).

Statistical Analysis

- Data were analyzed as a split-plot design using PROC MIXED procedures of SAS.
- Least squares means were determined and used to compare the interactions at a significance level of $P<0.05$.
- Mean $\log_{10}$ reductions were estimated from contrasts of the treatment combination minus the inoculated control treatment for each trial.

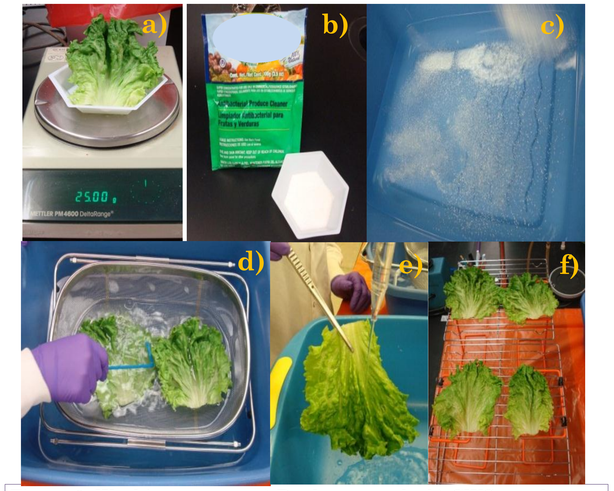

Figure 2. Washing process: a) Produce sample, b) CWS powder, c) preparation of CWS solution, d) CWS solution and washing of produce, e) rinsing step, f) drying time after washing (5 min)

Results

No significant 2- or 3-way interactions of washing treatments with contact time or sample were observed; therefore only the main effect of washing treatment was addressed.

Lettuce

- Lettuce samples that were washed with different CTW or CWS treatment combinations showed *E. coli* O157:H7 populations between 4.53 and 5.58 log CFU/g.
- However, overall washing treatment with CWS showed a higher effect in log reductions of *E. coli* O157:H7 than CTW treatment ($P<0.05$; Figure 3) .

Tomatoes

- The level of recovered *Salmonella* population was below detection in several samples. Therefore, samples were enriched to confirm presence of *Salmonella* . The majority of (33 out of 36) samples showed presence of *Salmonella* after 24 hours of enrichment (Table 1).
- Overall, washing with CTW treatment showed 2.50 log reduction, while CWS treatment showed 2.96 log reduction (Figure 4).

Figure 3. Log CFU /g reductions and standard error of *E. coli* O157:H7 populations on inoculated lettuce treated with wash solutions. Superscripts indicate differences ($P < 0.05$).

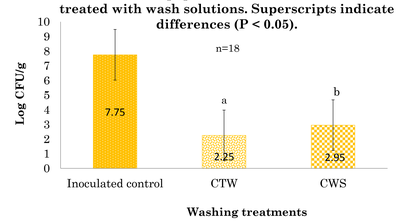

Figure 4. Log CFU/cm² reductions and standard error of *Salmonella* spp. populations on inoculated tomatoes after treatment with wash solutions. Superscripts indicate differences ($P < 0.05$).

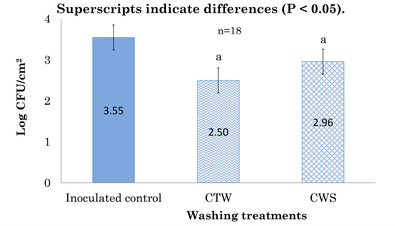

Table 1. Presence (+) or absence (-) of *Salmonella* spp. on tomatoes treated with washing treatments and non-inoculated tomatoes.

Group	Contact time (Seconds)	Repetition 1		Repetition 2		Repetition 3	
		S1*	S2**	S1	S2	S1	S2
Cold tap water	30	+	+	+	+	+	+
	60	+	+	+	+	+	+
	120	+	+	+	+	+	+
Commercial wash solution	30	-	+	+	+	+	+
	60	-	+	+	+	+	+
	120	+	+	-	+	+	+
Non-inoculated samples	n/a	-	-	-	-	-	-

*S1: Sample; ** S2: Sample 2

Implications

- Overall, treatment with commercial wash solution is more effective than cold tap water and should be applied for 120 seconds to reduce the risk of pathogens, such as *E. coli* O157:H7 and *Salmonella* spp.

Acknowledgments

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