

# Validation of Washing Treatments to Reduce Pathogens in Fresh Produce Summary Report



The Center of Excellence for  
**FOOD SAFETY RESEARCH IN**  
**CHILD NUTRITION PROGRAMS**



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**KANSAS STATE**  

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**UNIVERSITY**



# **Validation of Washing Treatments to Reduce Pathogens in Fresh Produce Summary Report**

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## Table of Contents

Validation of Washing Treatments to Reduce Pathogens in Fresh Produce .....	ii
Summary Report .....	ii
List of Figures .....	5
List of Tables .....	6
Executive Summary .....	7
Acknowledgements .....	9
Background .....	10
Objectives .....	12
Experiment 1 .....	13
Experimental design .....	13
Materials and Methods .....	13
Bacterial strains .....	13
Inoculum preparation .....	14
Procedure of inoculation .....	14
Washing procedures .....	15
Sampling and enumeration procedures .....	17
Statistical analysis .....	18
Results and Discussion .....	18
Conclusions .....	20
Experiment 2 .....	21
Experimental design .....	21
Materials and Methods .....	21
Bacterial strains .....	21
Inoculum preparation .....	22
Procedure of inoculation .....	23
Washing procedures .....	23
Sampling and enumeration procedures .....	24
Statistical analysis .....	25

Results and Discussions..... 26  
Conclusions..... 28  
References..... 29

## List of Figures

Figure 1. Drying of produce after inoculation .....	15
Figure 2. Commercial wash solution preparation and washing process.....	16
Figure 3. Inoculum preparation.....	22
Figure 4. Mist inoculation of cantaloupes performed under a biosafety cabinet.....	23
Figure 5. Washing procedures used for cantaloupes treatments.....	24
Figure 6. Coring sampling of cantaloupes used for enumeration .....	25

## List of Tables

Table 1. Mean $\pm$ standard error <i>E. coli</i> O157:H7 populations ( $\log_{10}$ CFU/g) after application of washing treatments to leaf lettuce (n=6) .....	19
Table 2. Presence (+) or absence (-) of <i>Salmonella</i> spp. on tomato samples treated with washing treatments and non-inoculated samples .....	20
Table 3. Mean $\pm$ standard error <i>Salmonella</i> spp. population ( $\log_{10}$ CFU/cm <sup>2</sup> ) after application of washing treatments on cantaloupes (n=6).....	26
Table 4. Mean $\pm$ standard error of <i>Listeria monocytogenes</i> populations ( $\log_{10}$ CFU/cm <sup>2</sup> ) after application of washing treatments on cantaloupes for 120 s (n=9).....	27

## Executive Summary

Fresh produce, such as tomatoes, lettuce, and cantaloupes, have repeatedly been associated with foodborne outbreaks connected to various *Salmonella* serovars, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. Usually fresh produce is exposed to minimal processing in order to maintain organoleptic characteristics, which increases the potential risk of contamination. The aim of this research was to determine the efficacy of a tap water and a commercial wash solution for reducing pathogens on the surface of green leaf lettuce, tomatoes, and cantaloupes and to develop recommendations for school foodservice personnel on best practices for washing fresh produce. Produce was inoculated and then inoculated samples were either treated with a commercial fruit and vegetable wash solution or regular cold tap water for three different contact times (30, 60, 120 s). Inoculated and non-inoculated produce was sampled for enumeration procedures.

## Conclusions

Based on this study, we obtained the following conclusions:

1. Treatment with commercial wash solution was more effective than cold water on reducing *E. coli* O157:H7 on inoculated green leaf lettuce and *Salmonella* spp. on tomatoes. However, the efficacy of the washing treatments on the produce surface was not affected by contact times of 30, 60, or 120 s.
2. The commercial wash solution treatment was capable of reducing approximately 3.0 logs of *E. coli* O157:H7 populations on green leaf lettuce and *Salmonella* spp. populations by approximately 3.0 logs on surfaces of tomatoes.
3. When inoculated cantaloupes (*Salmonella* spp.) were treated with commercial wash solution, a 1.26 log<sub>10</sub> reduction of *Salmonella* spp. population was achieved.

4. A main effect of contact time was observed on cantaloupes inoculated with *Salmonella* spp., with 120 s contact time showing the lowest *Salmonella* spp. population recovery after either cold tap water or commercial wash solution treatment.
5. When inoculated cantaloupes with *Listeria monocytogenes* were treated with the commercial wash solution for 120 s, a 1.12 log reduction of *Listeria monocytogenes* population was achieved.
6. Overall, treatment with commercial wash solution was more effective than cold tap water on reducing pathogens on produce surfaces.

### **Recommendation**

Based on our results, we recommend use of a commercial wash solution by submerging and gently stirring the produce item for at least 120 s, followed by a rinsing step, to reduce the risk of pathogens, such as *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* on the surface of green leaf lettuce, tomatoes, and cantaloupes.



## **Acknowledgements**

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## Background

Global food consumption has changed over time. In the United States (U.S), the increasing demand for availability of fresh produce year round, emphasis on increasing consumption of fresh produce for a healthier life style, and a changing ethnic composition of the population have contributed to the increased per capita consumption of fresh produce (Cook, 2011; Pollack, 2001).

Concurrent with the increase in consumption, the U.S. Food and Drug Administration (FDA) has responded to several recalls or foodborne outbreaks linked to fresh produce. The increase in reported outbreaks associated with fresh produce is strongly linked to the increased consumption of these commodities and the improved epidemiological systems used to determine the source of foodborne illnesses outbreaks such as PulseNet of the Centers for Disease Control and Prevention (Doyle and Erickson, 2008; Pangloli et al., 2009).

In a review of outbreaks in the U.S. from 1973 to 1997, Sivapalasingam et al. (2004) reported during that time period, there was an eightfold increase in the proportion of illness attributed to produce. Additionally, Sivapalasingam et al. (2004) stated that during that time 190 produce-associated outbreaks caused 16,058 illnesses, 598 hospitalizations, and 8 deaths in 32 states. Recently, Painter et al. (2013) analyzed data from documented outbreaks between 1998 and 2008 and estimated the annual U.S. foodborne illness attributable to each of 17 commodities and their results attributed 46% of illnesses to produce. Among the 17 commodities analyzed, more illnesses were associated with leafy vegetables (22%) than any other commodity.

Fresh produce, such as tomatoes, lettuce, and cantaloupes, have repeatedly been associated with food outbreaks connected to various *Salmonella* serovars, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. During 2005 and 2006, four multistate outbreaks

of *Salmonella* infections linked to the consumption of raw tomatoes in restaurants resulted in 450 confirmed cases in 21 states (CDC, 2007). During 2011, a large multistate outbreak of listeriosis linked to whole cantaloupes sickened 147 individuals and 33 deaths were reported (CDC, 2012a). In 2012, an outbreak of salmonellosis implicating consumption of cantaloupe contaminated with *Salmonella* Typhimurium and *Salmonella* Newport involved 261 infected people in 24 states (CDC, 2012b). Recently in 2012, a multistate outbreak of *E. coli* O157:H7 infections linked to romaine lettuce affected 58 people from nine states.

Contamination of fresh produce can occur at any point in the food chain (production, harvesting, transportation, processing, or preparation in foodservice or home kitchens) (Pangloli et al., 2009). Usually fresh produce is exposed to minimal processing in order to maintain organoleptic characteristics, which increases the potential risk of contamination. Non-thermal processing is an alternative for improving produce safety without compromising quality and desirability of the food product. Washing produce with tap water is recommended for reducing potential microbial contamination on produce surface. However, it cannot be relied on to completely remove pathogenic contamination (Beuchat, 2001). The potential risk of contamination of produce through the food chain, release of the new dietary guidelines, and effort to introduce fruits and vegetables in the child nutrition programs prompt the continual need to study alternative interventions to improve the safety of fresh produce in school foodservice.

## Objectives

The objectives of this study were:

1. To determine the efficacy of a tap water and a commercial wash solution for reducing pathogens on the surface of green leaf lettuce, tomatoes, and cantaloupes.
2. To develop recommendations for school foodservice personnel on best practices for washing fresh produce.

# Experiment 1

## Experimental design

For microbial analysis, two samples of produce were inoculated with a five-strain cocktail of *E. coli* O157:H7 or *Salmonella* spp. Samples were either treated with a commercial fruit and vegetable wash solution or regular cold tap water for three contact times (30, 60, 120 s), at room temperature ( $22 \pm 2^\circ\text{C}$ ), resulting in six treatment combinations: (1) commercial wash solution, 30 s contact time; (2) commercial wash solution, 60 s contact time; (3) commercial wash solution, 120 s contact time; (4) water, 30 s contact time; (5) water, 60 s contact time; (6) water, 120 s contact time. The experiment was replicated three times.

## Materials and Methods

### Bacterial strains

Mixtures of five strains of each pathogen, isolated from different sources were used as inocula. *Escherichia coli* O157:H7 isolates used in this study included RM 6069 and RM 5280 (associated with 2006 spinach outbreak, clinical isolations). Both strains were kindly provided by Dr. Robert Mandrell; USDA ARS, Albany, CA. *Escherichia coli* O157:H7 mixture also included ATCC 35150 (human feces isolation; Manassas, VA), ATCC 43895 (hemorrhagic colitis outbreak from raw hamburger meat; Manassas, VA), and ATCC 43888 (human feces isolation; Manassas, VA). *Salmonella* spp. strains also were provided by Dr. Robert Mandrell, and included RM33363 (serovar Poona), RM 6832 (serovar Newport), RM 2247 (serovar Baildon), RM 6825 (serovar Gaminara); and ATCC 13311 (*Salmonella* Thyphimurum); these strains have been associated with produce outbreaks.

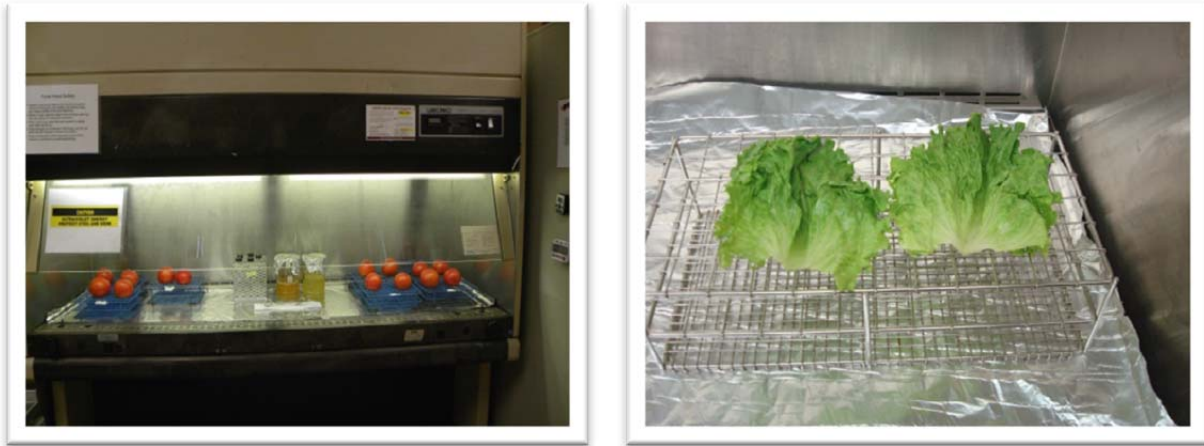
## **Inoculum preparation**

For *E. coli* O157:H7 inocula preparation, one loopful of each culture was individually transferred into 9 ml of tryptic soy broth (TSB; Difco; Flankin Lakes, NJ) and incubated at 37°C for 24 h. The cocktail was prepared by mixing the five cultures in a sterile beaker to deliver a final volume of 50 ml inoculum with a final *E. coli* O157:H7 cell density of 7.86 CFU/ml.

For *Salmonella* spp. inocula preparation, three drops of each culture were individually transferred into 100 ml of tryptic soy broth (TSB; Difco; Flankin Lakes, NJ) and incubated at 37°C for 24 h. For each strain, 20 ml of culture was transferred into a sterile 800 ml beaker containing 400 ml of sterile 0.1% peptone water (Bacto; Flankin Lakes, NJ) for a total inoculum of 500 ml with a final *Salmonella* spp. cell density of 9.39 log CFU/ml. The inoculum was maintained at 22 ± 2°C and applied to produce within 1 h of preparation.

## **Procedure of inoculation**

Green leaf lettuce and un-waxed ripe tomatoes were obtained from the K-State Dining Services (Manhattan, KS). Produce was stored at 4 ± 1°C for no more than 2 days prior to inoculation; before inoculation produce samples were tempered at room temperature (22 ± 2°C). Inoculum suspensions containing *E. coli* O157:H7 and *Salmonella* spp. were used to inoculate green leaf lettuce and tomatoes, respectively. Lettuce samples (25 ± 0.3 g) were spot inoculated; 1 ml of *E. coli* O157:H7 inoculum suspension was distributed in 10 drops/spots on the upper side of leaves and then allowed to dry inside biosafety cabinet for 1 h for attachment of cells (Figure 1). Tomatoes were submerged in *Salmonella* spp. suspension for 30 s and air dried in biosafety cabinet (22 ± 2°C) for 1 h for attachment.

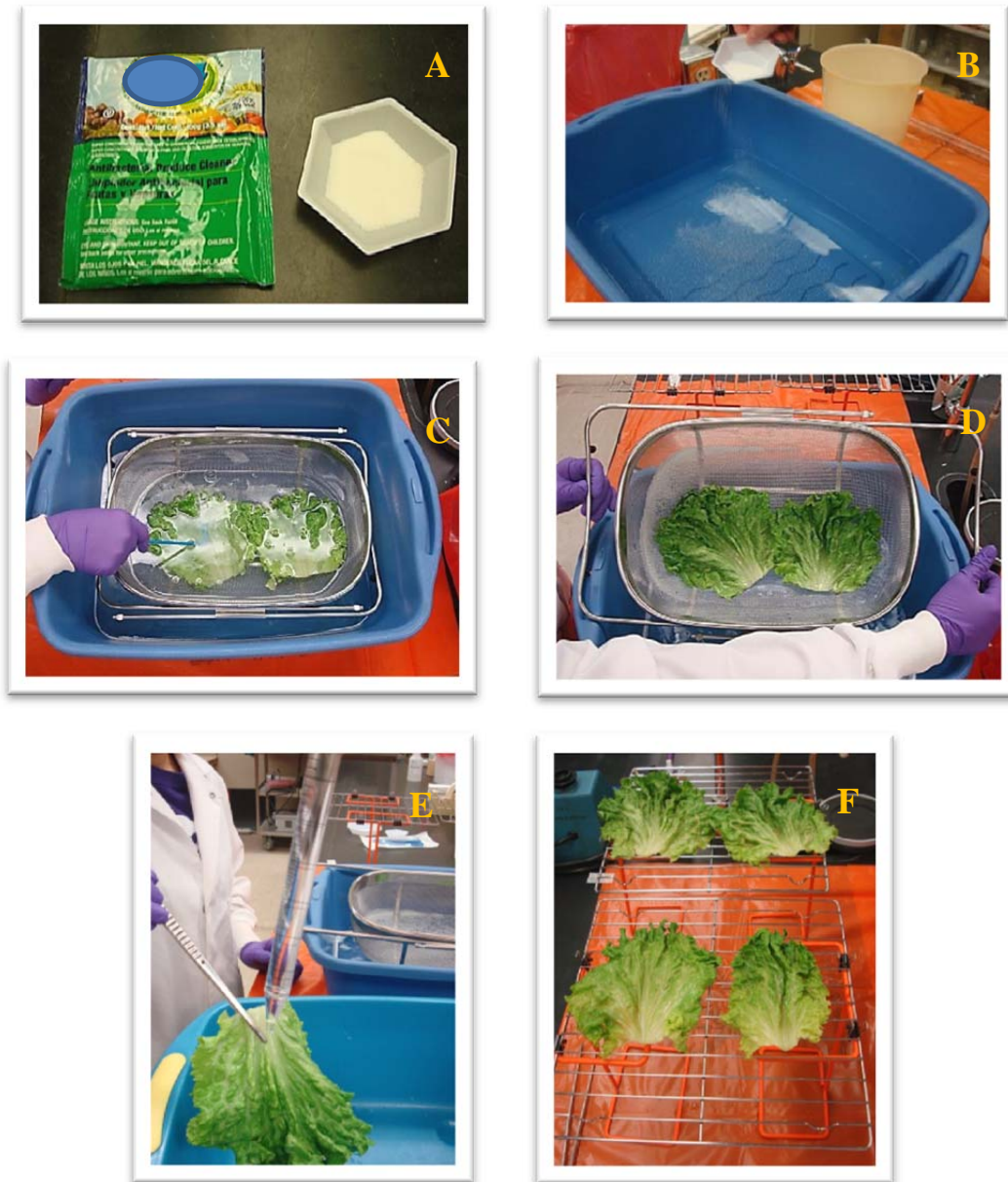


**Figure 1. Drying of produce after inoculation**

### **Washing procedures**

Green leaf lettuce and tomatoes, inoculated with *E. coli* O157:H7 and *Salmonella* spp. as described above, were washed separately with a commercial wash solution (containing citric acid, sodium lauryl sulfate, sodium carbonate, magnesium carbonate, and grapefruit oil extract) or cold tap water for three contact times (30, 60, and 120 s) using a procedure simulating the sequence of steps (washing, rinsing, and drying) followed for preparing produce for consumption in a school foodservice operation (Figure 2). For green leaf lettuce, commercial wash solution was prepared by mixing the antimicrobial powder (14 g) with 4 L of cold tap water according to manufacturer's directions (HealthPro Brands Inc., Cincinnati, OH). For tomatoes, commercial wash solution was prepared by mixing 28 g of antimicrobial powder with 8 L of cold tap water. Per treatment combination, two inoculated lettuce samples ( $25 \pm 0.3$  g/each) or two tomatoes were washed by submerging and gently stirring produce item in commercial wash solution or cold tap water. 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 air dry for 5 min.



**Figure 2. Commercial wash solution preparation and washing process. A) commercial wash solution (CWS) powder, B) preparation of CWS solution, C) CWS solution and washing of produce, D) use of a colander to remove the produce from washing solution plastic tub, E) rinsing step, F) drying time after washing treatment**



## Sampling and enumeration procedures

Populations of *E. coli* O157:H7 and *Salmonella* spp. on untreated and treated produce were determined. Lettuce and tomatoes samples from all treatment combinations were sampled within 5 min after washing procedures. Lettuce samples ( $25 \pm 0.3$  g/each) were transferred to a sterile stomacher bag, 225 ml of sterile 0.1% peptone water (Bacto; Franklin Lakes, NJ) was added to the bag and then stomached on medium speed for 1 min (Seward 400 Stomacher, Seward Limited; Worthing, Great Britain). Samples were serial diluted by using 9 ml of 0.1% peptone water, and dilutions were surface plated (0.1 ml) onto sorbitol MacConkey agar (Difco; Franklin Lakes, NJ) with cefixime tellurite supplement (CTSMAC; Oxoid Limited; Remel In., Lenexa, KS) for *E. coli* O157:H7. Non-inoculated samples had 225 ml of *E. coli* enrichment broth (Difco; Franklin Lakes, NJ) added and were incubated for 18-24 h at 37°C. After enrichment, 0.1 ml was plated onto CTSMAC to verify no *E. coli* O157:H7 was present in the sample.

Cores from two tomatoes from each treatment combination were removed with a sterile scalpel. The procedure consisted of cutting around the core mark ( $11.34 \text{ cm}^2$ ) and excising a circular area of tissue to a depth of  $1 \pm 0.5$  mm. Each core was placed in a sterile stomacher bag, 30 ml sterile 0.1% peptone water (Difco; Franklin Lakes, NJ) was added to the bags and then stomached on medium speed for 1 min. Samples were serial diluted by using 9 ml of 0.1% peptone water, and dilutions were surface plated (0.1 ml) onto xylose-lisine deoxycholate (XLD; Difco; Franklin Lakes, NJ) agar for *Salmonella* spp. enumeration. Also, an additional core from each treated sample and non-inoculated samples had 30 ml of Universal Preenrichment broth (UPB; Difco; Franklin Lakes, NJ) added and were incubated for 24 h at 37°C. After, enrichment, 0.1 ml was plated onto XLD to verify for *Salmonella* spp. presence or absence in the sample.

Additionally, a non-inoculated control sample of each produce item was included for standard aerobic plate counts. Samples were diluted and plated on tryptic soy agar (TSA) before being incubated at 36°C for 24 h to estimate aerobic plate counts.

### **Statistical analysis**

This study followed a split-plot design with replication day as block factor. *Escherichia coli* O157:H7 and *Salmonella* spp. population data were analyzed using PROC MIXED in SAS version 9.2 (SAS institute, Cary, NC). Fixed effects for statistical analysis were treatment, time, treatment × time, sample, treatment × sample, time × sample, treatment×time×sample; while random effects were rep, rep×treatment×time. Least squares means were determined and used to compare the interactions at a significance level of P<0.05. Because there were no 2- or 3- way interactions, the data were pooled for the main effects of treatments and contact time. Mean log<sub>10</sub> reductions and associated standard errors were estimated from contrasts of the treatment combination minus the inoculated control treatment at each trial.

## **Results and Discussion**

Enrichment in non-inoculated samples was performed for detection of *E. coli* O157:H7 and *Salmonella* spp. in the background flora of lettuce and tomatoes, respectively. Following 24 h of enrichment, none of the non-inoculated lettuce and tomato samples had *E. coli* O157:H7 or *Salmonella* spp. populations present.

For the non-inoculated lettuce samples, the average aerobic population (n=6) was 5.3 log<sub>10</sub> CFU/g, while for non-inoculated tomatoes average aerobic population (n=6) was approximately 1.2 log<sub>10</sub> CFU/cm<sup>2</sup>.

For the *E. coli* O157:H7 inoculated lettuce samples, there were no 2- or 3- way interactions for treatment × time × sample, time × sample, treatment × sample, or treatment × time. No differences ( $P > 0.05$ ) existed for contact times used for application of washing treatment solutions (CWS or tap water). However, an effect of washing treatment solution ( $P < 0.05$ ) was observed in *E. coli* O157:H7 log populations after washing procedures with data pooled for contact time (Table 1).

**Table 1. Mean ± standard error *E. coli* O157:H7 populations (log<sub>10</sub>CFU/g) after application of washing treatments to leaf lettuce (n=6)**

Item	P-value
Washing treatment solution	0.01
<b>Washing treatment solution</b>	<b>Log<sub>10</sub> CFU/g</b>
Cold tap water **	5.50 ± 0.32 <sup>a</sup>
Commercial washing solution**	4.79 ± 0.33 <sup>b</sup>

\*\* data pooled for contact time (30, 60, 120 sec.); n= 18

<sup>ab</sup> Means with different superscripts within a column are significantly different ( $P < 0.05$ ).

The inoculated samples that were not treated with the washing treatments (n=6) showed an average *E. coli* O157:H7 mean population of 7.75 log<sub>10</sub> CFU/g, these data were used to estimate the average mean log<sub>10</sub> reductions. Average mean log<sub>10</sub> reductions for washing treatments applied to inoculated lettuce samples were 2.25 ± 0.34 log<sub>10</sub> CFU/g for cold tap water and 2.95 ± 0.34 log<sub>10</sub> CFU/g for the commercial wash solution ( $P < 0.05$ ).

For inoculated tomato samples, there were no significant ( $P > 0.05$ ) 2- or 3- way interactions or main effects for *Salmonella* spp populations. This may be due to recovery populations being below the detection limit. Therefore, to determine effectiveness of the washing treatments solution, an additional core from each treated sample was enriched in UPB to verify

for *Salmonella* spp. presence in the sample. After 24 h of incubation, *Salmonella* spp. was detected in the enriched samples (Table 2).

**Table 2. Presence (+) or absence (-) of *Salmonella* spp. on tomato samples treated with washing treatments and non-inoculated samples**

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

The inoculated tomato samples that were not treated with the washing treatments (n=6) showed an average mean population of  $3.55 \log_{10}$  CFU/cm<sup>2</sup>, these data were used to estimate the average mean  $\log_{10}$  reductions. Average mean  $\log_{10}$  reductions for washing treatments applied to inoculated tomato samples were  $2.50 \pm 0.49 \log_{10}$  CFU/g for cold tap water and  $2.96 \pm 0.49 \log_{10}$  CFU/g for the commercial wash solution.

## Conclusions

The application of the commercial wash solution was capable of significantly reducing *E. coli* O157:H7 and *Salmonella* spp. on inoculated green leaf lettuce and tomatoes, respectively. Overall, the commercial wash solution was capable of reducing approximately 3.0  $\log_{10}$  of these pathogens.

Although the efficacy of the washing treatments on the produce surface was not affected by contact times, the lowest mean population of recovered pathogens was observed when commercial wash solution was applied for 120 s. Therefore, it is recommended that commercial washing solution be applied for 120 s to obtain optimal log reductions on the surface of lettuce or tomatoes.

## Experiment 2

### Experimental design

Cantaloupes were inoculated with a five- or four-strain cocktail of *Salmonella* spp. or *Listeria monocytogenes*. Samples inoculated with *Salmonella* spp. were either treated with a commercial fruit and vegetable wash solution or regular cold tap water for three contact times (30, 60, 120 s), at room temperature ( $22 \pm 2^\circ\text{C}$ ), resulting in six treatment combinations: (1) commercial wash solution, 30 s contact time; (2) commercial wash solution, 60 s contact time; (3) commercial wash solution, 120 s contact time; (4) water, 30 s contact time; (5) water, 60 s contact time; and (6) water, 120 s contact time. Samples treated with *Listeria monocytogenes* were either treated with a commercial fruit and vegetable wash solution or regular cold tap water for 120 s. The experiment was replicated five times for cantaloupes inoculated with *Salmonella* spp. and three times for cantaloupes inoculated with *Listeria monocytogenes*.

## Materials and Methods

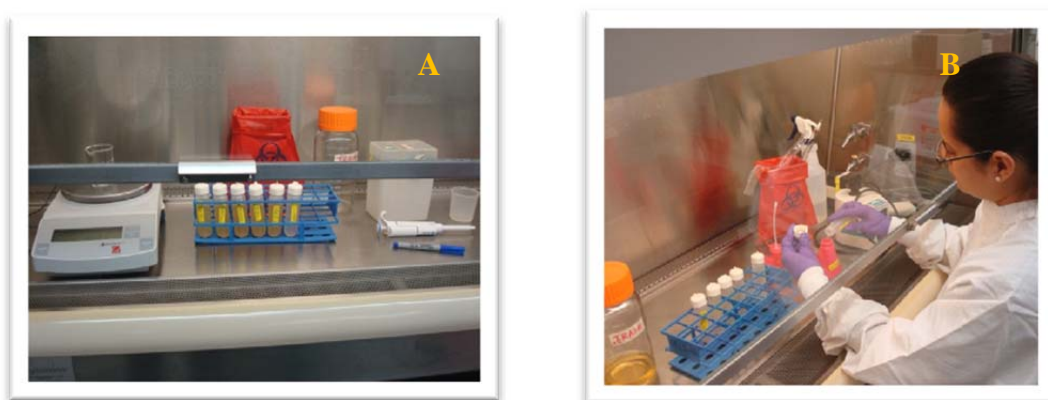
### Bacterial strains

Mixtures of each pathogen, isolated from different sources were used as inocula. *Salmonella* spp. strains were provided by Dr. Robert Mandrell (USDA ARS, Albany, CA), and

included RM33363 (serovar Poona), RM 6832 (serovar Newport), RM 2247 (serovar Baildon), RM 6825 (serovar Gaminara); and ATCC 13311 (*Salmonella* Thyphimurium, Manassas, VA); these strains have been associated with produce outbreaks. *Listeria monocytogenes* strains included RM 3818 (associated with cantaloupes outbreak), ATCC 19115 (serotype 4b, human isolate, Manassas, VA), ATCC 19118 (serotype 4e, chicken isolate, Manassas, VA), and SLR-2249 (laboratory strain with the *ActA* gene removed, St. Cloud, MN).

### **Inoculum preparation**

For *Salmonella* spp. and *Listeria monocytogenes* inocula preparation, one loopful of each strain was individually transferred into 9 ml of tryptic soy broth (TSB; Difco; Flankin Lakes, NJ) and incubated at 37°C for 24 h. Cells of each strain were collected by centrifugation (ca. 6000 × g, 15 min, 4°C) and then resuspended in 30 ml of sterile 0.1% peptone water and combined to form a five- or four-strain cocktail inoculum with (Figure 3) with a cell density of 8.54 CFU/ml (*Salmonella* spp.) and 8.52 CFU/ml (*Listeria monocytogenes*). The inoculum was maintained at 22 ± 2°C and applied to produce within 1 h of preparation.



**Figure 3. Inoculum preparation. A) weighing of cultures strains before centrifugation, B) combination of resuspended cell for mist inoculation**

## Procedure of inoculation

Cantaloupes were obtained from the K-State Dining Services and retail stores. Cantaloupes were stored at 4°C for no more than 1 day prior to inoculation; before inoculation, samples were tempered at room temperature ( $22 \pm 2^\circ\text{C}$ ). Cantaloupes were mist inoculated (ca. 8-10 ml), after inoculation cantaloupes were allowed to dry for 1 h to permit attachment of cells (Figure 4).



**Figure 4. Mist inoculation of cantaloupes performed under a biosafety cabinet**

## Washing procedures

Cantaloupes inoculated with *Salmonella* spp. as described above, were washed separately with a commercial wash solution or cold tap water for three contact times (30, 60, and 120 s), while cantaloupes inoculated with *Listeria monocytogenes* were washed with the commercial wash solution and cold tap water for 120 s. The commercial wash solution used to treat cantaloupes was prepared by mixing an antimicrobial powder (containing citric acid, sodium lauryl sulfate, sodium carbonate, magnesium carbonate, and grapefruit oil extract; 28 g) with 8 L of cold tap water according to the manufacturer's directions (HealthPro Brands, Cincinnati, OH).

Per treatment combination, one cantaloupe was washed by submerging and gently stirring it in the commercial wash solution or cold tap water. A disinfected metallic colander was used to hold cantaloupes during washing. After treatment cantaloupes were rinsed with tap water (1 L per unit) and then allowed to dry for 30 min before sampling (Figure 5).



**Figure 5. Washing procedures used for cantaloupes treatments. A) use of a disinfected metallic colander to remove cantaloupes from washing solution, B) rinsing step after washing treatment**

### **Sampling and enumeration procedures**

*Salmonella spp.* and *Listeria monocytogenes* population were determined. Five cores from each cantaloupe for each treatment combination were removed with a sterile scalpel (Figure 6). The procedure consisted in cutting around the core mark (11.34 cm<sup>2</sup>) and excising a circular area of tissue to a depth of 1 ± 0.5 mm resulting in a composite sample (56.7 cm<sup>2</sup>). The composite sample was placed in a sterile stomacher bag, 30 or 50 ml sterile 0.1% peptone water (Difco; Franklin Lakes, NJ) was added to the bags and then stomached on medium speed for 1 min (Seward 400 Stomacher, Seward Limited; Worthing, Great Britain). Samples were serially diluted by using 9 ml of 0.1% peptone water, and the mixtures were surface plated (0.1 ml) onto



xylose-lysine deoxycholate (XLD; Difco; Franklin Lakes, NJ) agar for *Salmonella* spp. recovery or modified oxford medium (MOX) for *Listeria monocytogenes*.

Additionally, non-inoculated cantaloupes were sampled for standard aerobic plate counts. Samples were diluted and plated on tryptic soy agar (TSA) before being incubated at 36°C for 24 h to estimate aerobic plate counts.



**Figure 6. Coring sampling of cantaloupes used for enumeration**

### Statistical analysis

A randomized complete block design (RCBD; with replication as block factor) was used to test the effects of washing treatments in combination with contact time on *Salmonella* spp. populations and a generalized RCBD with repetition day as block factor was used to test the effects of washing treatments on *Listeria monocytogenes* populations. *Salmonella* spp. and *Listeria monocytogenes* population data were analyzed using PROC MIXED in SAS version 9.2 (SAS institute, Cary, NC). Least squares means were determined and used to compare main effects and interactions at a significance level of  $P < 0.05$ . Mean  $\log_{10}$  reductions and associated standard errors were estimated from contrasts of the treatment combination minus the inoculated control treatment at each trial.

## Results and Discussions

Non-inoculated cantaloupes sampled for aerobic plate counts during *Salmonella* spp. and *Listeria monocytogenes* trials had total aerobic plate counts populations of 4.70 log<sub>10</sub> CFU/cm<sup>2</sup> and 4.80 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively. For cantaloupes inoculated with *Salmonella* spp., no interactions were observed between washing treatment solution and contact time for *Salmonella* spp. populations. However, significant (P < 0.05) main effects on *Salmonella* spp. populations were observed for wash treatment solution and contact time (Table 3).

**Table 3. Mean ± standard error *Salmonella* spp. population (log<sub>10</sub> CFU/cm<sup>2</sup>) after application of washing treatments on cantaloupes (n=6)**

Item	P-value
Washing treatment solution	0.0002
Contact time	0.04
<b>Washing treatment solution*</b>	<b>Log<sub>10</sub> CFU/cm<sup>2</sup></b>
Cold tap water	5.50 ± 0.22 <sup>a</sup>
Commercial washing solution	4.87 ± 0.22 <sup>b</sup>
<b>Contact times**</b>	
30 seconds	5.43 ± 0.23 <sup>x</sup>
60 seconds	5.19 ± 0.23 <sup>xy</sup>
120 seconds	4.94 ± 0.23 <sup>y</sup>

\*data pooled for contact time (30, 60, 120 sec.; n= 18)

\*\* data pooled for washing treatment (n=18)

<sup>ab</sup>Means or <sup>xy</sup>Means with different superscripts within a column section are significantly different (P < 0.05).

When inoculated cantaloupes were treated with cold tap water and commercial wash solution the average *Salmonella* spp. mean populations were 5.50 and 4.87 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively (Table 3). With respect to contact time, it was observed that 120 s contact time showed the lowest *Salmonella* spp. population recovery after either cold tap water or commercial wash solution treatment (Table 3).

Unwashed, *Salmonella* spp. inoculated cantaloupes (n=6) showed an average *Salmonella* spp. mean population of 6.13 log<sub>10</sub> CFU/cm<sup>2</sup>, these data were used to estimate the average mean log<sub>10</sub> reductions for washing treatment solutions. After commercial wash solution treatment, *Salmonella* spp. populations were reduced by 1.26 ± 0.20 log<sub>10</sub> CFU/cm<sup>2</sup>, whereas *Salmonella* spp. population after cold tap water treatment resulted in 0.62 ± 0.20 log<sub>10</sub> CFU/cm<sup>2</sup> reduction (P < 0.05).

Cantaloupes that were inoculated with *Listeria monocytogenes* were treated with washing treatment solutions for 120 s. This decision was based on the results obtained from the previous trial (Table 3) where application of washing treatment solutions for 120 s showed the lowest *Salmonella* spp. population recovery. A significant effect of washing treatment solutions (P < 0.05) was observed on *Listeria monocytogenes* populations after washing procedures (Table 4).

**Table 4. Mean ± standard error of *Listeria monocytogenes* populations (log<sub>10</sub> CFU/cm<sup>2</sup>) after application of washing treatments on cantaloupes for 120 s (n=9)**

Item	P-value
Washing treatment solution	0.0039
Item	Log <sub>10</sub> CFU/cm <sup>2</sup>
Cold tap water	5.41 ± 0.35 <sup>a</sup>
Commercial washing solution	4.92 ± 0.35 <sup>b</sup>

<sup>ab</sup>Means with different superscripts within a column section are significantly different (P < 0.05).

The initial population of *Listeria monocytogenes* on unwashed inoculated cantaloupes was 6.03 log<sub>10</sub> CFU/cm<sup>2</sup>, these data were used to estimate the average mean log<sub>10</sub> reductions. For *Listeria monocytogenes* inoculated cantaloupes after washing treatment, a significant reduction of 1.12 ± 0.19 log<sub>10</sub> CFU/cm<sup>2</sup> was achieved by applying commercial wash solution for 120 s. On

the other hand, a reduction of  $0.63 \pm 0.19 \log_{10}$  CFU/cm<sup>2</sup> was achieved by washing with cold tap water for 120 s.

## Conclusions

The application of the commercial wash solution was demonstrated to have an antimicrobial activity against *Salmonella* spp. and *Listeria monocytogenes* on the surface of cantaloupes. Overall the commercial wash solution was capable of reducing above 1.1 log<sub>10</sub> of *Salmonella* spp. and *Listeria monocytogenes* on inoculated cantaloupes. A main effect of contact time showed that the lowest mean population of recovered pathogens was observed when washing treatment solutions were applied for 120 s. This indicates that the antimicrobial effect of the commercial wash solution or cold tap water could be maximized when applied for 120 s. Therefore, it is recommended that the commercial wash solution, which showed the highest efficacy in reducing pathogens, be applied for 120 s to obtain optimal log reductions on the surface of cantaloupes.

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