#### Keyla Lopez,<sup>1</sup> Kelly J.K. Getty<sup>1\*</sup> and Christopher I. Vahl<sup>2</sup>

<sup>1</sup>Food Science Institute and Dept. of Animal Sciences and Industry <sup>2</sup>Dept. of Statistics, Kansas State University, Manhattan, KS 66506, USA

#### **PEER-REVIEWED ARTICLE**

Food Protection Trends, Vol 35, No. 5, p.377–384 Copyright®2015, International Association for Food Protection 6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864



Validation of Washing Treatments to Reduce *Escherichia coli* 0157:H7 and *Salmonella* spp. on the Surface of Green Leaf Lettuce and Tomatoes

#### ABSTRACT

Outbreaks associated with consumption of fresh produce have been linked to Escherichia coli 0157:H7 and Salmonella contamination. The objective was to determine the efficacy of a chemical wash treatment (citric acid, sodium lauryl sulfate, sodium carbonate, magnesium carbonate, and grapefruit oil extract) in reducing pathogens on the surface of leaf lettuce and tomatoes. Lettuce (25 ± 0.3 g) and whole tomatoes were inoculated with E. coli 0157:H7 (~7.8 log<sub>10</sub> CFU/ml) and Salmonella spp. (9.39 log<sub>10</sub> CFU/ml) cocktails, respectively. Samples were treated with cold tap water (negative control) or the chemical wash treatment for various exposure times (30, 60, and 120 s), and then rinsed with tap water. Samples then were plated on selective media. The chemical wash treatment was capable of reducing by ca. 3.0 log<sub>10</sub> units of *E. coli* O157:H7 and Salmonella spp. populations on the surface of leaf lettuce and tomatoes, respectively. Even though there were no significant differences

among results with different exposure times (P > 0.05), application of the chemical wash treatment for 120 s lowered the mean populations of recovered pathogens by 0.1 to 0.66 log<sub>10</sub> CFU. Therefore, it is recommended that the chemical wash treatment be applied for 120 s to obtain optimal log reductions on the surface of leaf lettuce and tomatoes.

#### **INTRODUCTION**

Increasing demand for year-round availability of fresh produce, accessibility to ready-to-eat vegetables (pre-prepared or bagged produce), a changing ethnic composition of the population, and an emphasis on increasing consumption of fresh produce for a healthier lifestyle have contributed to increased per capita consumption of fresh produce in the United States (U.S.) (*5*, *12*, *24*). Concurrently with the increase in consumption, the U.S. Food and Drug Administration (FDA) has responded to several foodborne illness outbreaks linked to fresh produce. The increase in reported outbreaks associated with fresh produce is strongly linked to increased consumption of these commodities, and the improved epidemiological systems used to determine the source of a foodborne illnesses outbreak, such as PulseNet at the Centers for Disease Control and Prevention (13, 23), have enabled these associations to be made.

In a review of U.S. outbreaks from 1973 through 1997, Sivapalasingam et al. (28) reported an eightfold increase in the proportion of illness attributed to produce. In addition, the authors (28) found that 190 produce-associated outbreaks caused 16,058 illnesses, 598 hospitalizations, and 8 deaths in 32 states during that time. Painter et al. (22) recently analyzed data from documented outbreaks in 1998 through 2008 and estimated the number of annual U.S. foodborne illnesses attributable to each of 17 commodities; their results attributed 46% of the illnesses to produce. Among the 17 commodities analyzed, more illnesses were associated with leafy vegetables (22%) than with any other commodity. According to the Centers for Disease Control and Prevention (11), the percentage of outbreaks associated with leafy vegetables increased, during 2006 through 2008, from 6 to 11%. Analysis of the settings of food preparation and consumption associated with recognized foodborne outbreaks in the U.S. showed that the largest outbreaks occur in institutional settings such as schools, prisons, and camps (11).

Fresh produce such as tomatoes, lettuce, and cantaloupes has been associated repeatedly with food outbreaks connected to various *Salmonella* serovars, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. In 2005 and 2006, four multistate outbreaks of *Salmonella* infections that were linked to the consumption of raw tomatoes in restaurants resulted in 450 confirmed cases in 21 states (9). A multistate outbreak of *E. coli* O157:H7 infections linked to romaine lettuce affected 58 people from nine states in 2012 (10).

Contamination of fresh produce can occur at any point in the food chain (production, harvesting, transportation, processing, or preparation in food service or home kitchens) (23). To maintain organoleptic characteristics, fresh produce is usually exposed to minimal processing, which increases the potential risk of contamination (2). Washing produce with tap water is recommended to reduce potential microbial contamination on the produce surface, but this technique cannot be relied on to remove pathogenic contamination completely (6). Therefore, the aim of this study was to determine the efficacy of a chemical wash treatment in reducing pathogens on the surface of green leaf lettuce and tomatoes.

#### **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 a 2006 spinach outbreak; clinical isolations), both of which were kindly provided by Dr. Robert Mandrell (USDA ARS, Albany, CA). The *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 provided by Dr. Robert Mandrell, included RM33363 (serovar Poona), RM 6832 (serovar Newport), RM 2247 (serovar Baildon), RM 6825 (serovar Gaminara), and ATCC 13311 (*Salmonella* Typhimirum); these strains have been associated with produce outbreaks. All culture strains were maintained in tryptic soy agar (TSA; Difco; Franklin Lakes, NJ) slants and then transferred to tryptic soy broth (TSB; Difco; Franklin Lakes, NJ) prior to preparation of inoculum.

#### **Inoculum preparation**

For green leaf lettuce E. coli O157:H7 inoculum preparation, one loopful of each culture strain was used to inoculate 9 ml of TSB and each broth was incubated at 37°C for 24 h. The cocktail was prepared by mixing the five strains in a sterile beaker to deliver a final volume of 50 ml of inoculum with a final E. coli O157:H7 cell density of 7.86 log<sub>10</sub> CFU/ml. For tomato Salmonella spp. inoculum preparation, 100 µl of each strain was used to inoculate 100 ml of TSB and then incubated at 37°C for 24 h. A fivestrain cocktail was prepared by transferring 20 ml of each inoculated broth 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<sub>10</sub> CFU/ml. Inoculum suspensions were maintained at  $22 \pm 2$ °C and applied to produce within 1 h of preparation.

#### Inoculation procedure

Unwashed green leaf lettuce and unwaxed ripe tomatoes were obtained from the Kansas State University Dining Services and local retail stores (Manhattan, KS). Produce was stored at  $4 \pm 1^{\circ}$ C for no more than 2 days prior to inoculation, and samples were tempered at room temperature  $(22 \pm 2^{\circ}C)$  prior to inoculation. Inoculum suspensions containing E. coli O157:H7 and Salmonella spp. were used to inoculate green leaf lettuce and tomatoes, respectively. Lettuce samples  $(25 \pm 0.3 \text{ g}, 2 \text{ leaves})$  were placed on a sterile surface in a biosafety cabinet, and 1 ml of the fivestrain E. coli O157:H7 cocktail was spot-inoculated with a micropipettor onto 10 sites on the adaxial side of lettuce leaves. Tomato surfaces were inoculated by submerging tomatoes in Salmonella spp. suspension for 30 s. After inoculation, produce was allowed to dry for 1 h at room temperature to permit attachment of cells.

#### Washing procedures

Green leaf lettuce and tomatoes were washed separately with a chemical wash sanitizer (antimicrobial powder containing citric acid, sodium lauryl sulfate, sodium carbonate, magnesium carbonate, and grapefruit oil extract, pH 3.6 ([HealthPro Brands Inc., Cincinnati, OH]) or with cold tap water (as negative control, 22.4°C, 0 ppm free chlorine, and 50 mg/l of chloride ions) for three exposure 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 food service operation. For green leaf lettuce, chemical wash treatment was prepared according to manufacturer's directions by mixing 14 g antimicrobial powder with 4 l of cold tap water to achieve an antimicrobial concentration of 0.35% (HealthPro Brands Inc., Cincinnati, OH). For tomatoes, chemical wash treatment was prepared by mixing 28 g of antimicrobial powder with 8 l of cold tap water (0.35% antimicrobial concentration).

Two inoculated lettuce samples  $(25 \pm 0.3 \text{ g per sample};$ 2 leaves per sample) or two inoculated whole tomatoes per treatment combination were washed by submerging/dipping and gently stirring the produce item in the chemical wash treatment or cold tap water for 30, 60, or 120 s. A disinfected metal colander was used to hold produce during washing. After application of washing procedures, lettuce or tomato samples were rinsed with tap water. During the rinsing step, each lettuce leaf was held with sterile tweezers and 50 ml of tap water was dispensed with a pipettor onto the adaxial and abaxial side of each lettuce leaf. Each tomato was held in a disinfected metal colander and 100 ml of tap water was dispensed with a pipettor onto the tomato surface (tomatoes were rotated to ensure coverage of the entire surface). Produce was allowed to air dry for at least 5 min after rinsing prior to enumeration.

#### Sampling, enumeration, and enrichment procedures

E. coli O157:H7 and Salmonella spp. populations on treated leaf lettuce and tomatoes were determined. Lettuce and tomatoes from all treatment combinations were sampled within 10 min after washing procedures. Lettuce samples (25  $\pm$  0.3 per sample; 2 leaves per sample) were transferred to a sterile stomacher bag; 225 ml of sterile 0.1% peptone water (Bacto; Franklin Lakes, NJ) was added to the bag, which was then stomached on medium speed for 1 min (Seward 400 Stomacher, Seward Limited; Worthing, Great Britain). Samples were serial diluted 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 Inc., Lenexa, KS) for E. coli O157:H7 enumeration. In addition, non-inoculated samples to which 225 ml of E. coli enrichment broth (Difco; Franklin Lakes, NJ) was added were incubated for 18 to 24 h at 37°C. After enrichment, 0.1 ml aliquot was plated onto CTSMAC to verify absence of E. coli O157:H7 in background flora of the sample.

Surface tissue samples from two whole tomatoes were removed with a sterile scalpel. The procedure consisted

of cutting around a core mark  $(11.34 \text{ cm}^2)$  and excising a circular area of tissue to a depth of  $1.5 \pm 0.5$  mm. Each sample was placed in a sterile stomacher bag to which 30 ml sterile 0.1% peptone water (Difco; Franklin Lakes, NJ) was added, then stomached on medium speed for 1 min. Samples were subsequently surface-plated (0.1 ml aliquots in duplicate or 0.25 ml aliquots in quadruplicate (totaling 1 ml)) onto xylose-lisine deoxycholate (XLD; Difco; Franklin Lakes, NJ) agar for *Salmonella* spp. enumeration. An additional surface tissue sample from treated and noninoculated tomatoes had 30 ml of universal preenrichment broth (UPB; Difco; Franklin Lakes, NJ) added and were incubated for 24 h at 37°C. After enrichment, a 0.1 ml aliquot was plated onto XLD to test for *Salmonella* spp. presence or absence in the sample.

After washing treatments were applied, the residual water from wash solutions was sampled to determine the bacterial load transferred from produce to water. Samples were surfaceplated (0.1 ml in duplicate and 0.25 ml in quadruplicate) onto CTSMAC and XLD media for enumeration of *E. coli* O157:H7 and *Salmonella* spp., respectively. The detection limits for lettuce and tomato residual water were 1.95 and 0.95 log<sub>10</sub> CFU/ml, respectively.

Inoculated samples (n = 6) were surface plated onto CTSMAC and XLD media for enumeration of *E. coli* O157:H7 and *Salmonella* spp. attached to lettuce and tomato samples, respectively. Additionally, non-inoculated lettuce and tomato samples (n = 6) were prepared and plated onto TSA to estimate aerobic plate counts.

#### Statistical analysis

A split-plot design (replication day as the whole-plot blocking factor) with three replications was used to test the effectiveness of washing treatments in combination with exposure time on E. coli O157:H7 and Salmonella spp. populations in lettuce and tomatoes, respectively. Two samples of lettuce and two whole tomatoes within each treatment combination [washing solution × exposure time] and replication were collected to determine the effectiveness of the washing procedure, resulting in n = 6 per treatment combination, or 2 samples for each of 3 replications. Washing treatment and exposure time were considered whole-plot factors, and washing order of the two samples was the subplot factor. Data were analyzed using PROC MIXED in SAS version 9.2 (SAS institute, Cary, NC), with washing treatment, exposure time, and sample order being treated as fixed effects and replicate day and replicate day × washing treatment × exposure time treated as random. The 3-way (washing treatment  $\times$ exposure time  $\times$  sample order) and 2-way (exposure time  $\times$ sample order, washing treatment × sample order, or washing treatment × exposure time) interactions were tested first at a significance level of P = 0.05, followed by tests of main effects. The appropriate corresponding least squares means were determined, and pairwise comparisons were conducted using Fisher's protected LSD. Mean  $\log_{10}$  reductions and associated standard errors were estimated by contrasts of the washing treatment combination minus the inoculated samples at each trial.

#### **RESULTS AND DISCUSSION**

#### Non-inoculated samples

Enrichment of non-inoculated samples was performed for detection of *E. coli* O157:H7 and *Salmonella* spp. on the background flora of lettuce and tomato surfaces, respectively. Following 24 h of enrichment, none of the noninoculated lettuce and tomato samples had *E. coli* O157:H7 or *Salmonella* spp. populations present. Mean aerobic populations for non-inoculated lettuce samples (n = 6) were ca. 5.3 log<sub>10</sub> CFU/g, whereas mean aerobic populations for non-inoculated tomatoes (n = 6) were ca. 1.2 log<sub>10</sub> CFU/cm<sup>2</sup>.

#### Green leaf lettuce

Inoculated samples not treated with the washing treatments (n = 6) showed an *E. coli* O157:H7 mean population of ca. 7.75  $\pm$  0.2 log<sub>10</sub> CFU/g, and this value was used to estimate log<sub>10</sub> reductions. *E. coli* O157:H7 populations were not affected by 3- or 2- way interactions, exposure time, and sample order; however, populations were significantly affected by the chemical washing treatment (*Table 1*). Overall, *E. coli* O157:H7 population reductions in green leaf lettuce were greater (*P* < 0.05) for chemical washing treatment (2.95 log<sub>10</sub> CFU/g) than for cold tap water washing (2.25 log<sub>10</sub> CFU/g; *Table 2*). Mean log<sub>10</sub> reductions in green leaf lettuce washed with the chemical wash treatment for various exposures times ranged from 2.53 to 3.21  $\log_{10}$  CFU/g, whereas mean  $\log_{10}$  reductions with cold tap water applied for the same exposure times ranged from 2.16 to 2.34  $\log_{10}$  CFU/g (*Table 2*).

Sampling of residual water solutions indicated that *E. coli* O157:H7-contaminated lettuce transferred the pathogenic load to regular tap water by  $4.92 \log_{10} \text{CFU/ml}$ . However, *E. coli* O157:H7 recovery from the chemical wash treatment residual water was below the detection limit of  $1.95 \log_{10} \text{CFU/ml}$  (*Table 3*).

#### Tomatoes

Inoculated tomatoes not treated with the washing treatments (n = 6) showed *Salmonella* spp. populations of ca.  $3.55 \pm 0.57 \log_{10} \text{CFU/cm}^2$ . *Salmonella* spp. populations on the surface of tomatoes were not significantly (P > 0.05) affected by 3- or 2- way interactions (exposure time, sample order, and washing treatments). *Salmonella* spp. reductions of 2.50 log<sub>10</sub> CFU/cm<sup>2</sup> were achieved for cold tap water and 2.96 log<sub>10</sub> CFU/cm<sup>2</sup> for the chemical wash treatment (P > 0.05; *Table 2*). However, 16 out of 18 tomatoes washed with the chemical wash treatment had contamination levels below the detection limit (0.42 log<sub>10</sub> CFU/cm<sup>2</sup>), whereas only 8 out of 18 tomatoes washed with cold tap water had *Salmonella* spp. populations below the detection limit.

Samples with *Salmonella* spp. populations below the detection limit were enriched in UPB to verify the presence or absence of *Salmonella* spp. remaining on the surface of tomatoes after application of washing treatments. After 24 h of incubation, 15 of 18 (83.3 %) tomatoes treated with

	<i>P</i> -values		
Effect	<i>E. coli</i> O157:H7 on green leaf lettuce	Salmonella spp. on tomatoes	
Washing treatment	0.0131 <sup>a</sup>	0.2410	
Exposure time	0.4594	0.6764	
Sample order	0.3981	0.4767	
Washing treatment × Exposure time	0.5295	0.7259	
Washing treatment × Sample order	0.3502	0.8748	
Exposure time × Sample order	0.8793	0.1404	
Washing treatment × Exposure time × Sample order	0.6731	0.5180	
<sup><i>a</i></sup> Main and/or interaction effect was significant $(P < 0.05)$			

 TABLE 1. P-values of the main effects and interaction effects for viable E. coli 0157:H7

 and Salmonella spp. after application of washing treatments

the chemical wash treatment tested positive for *Salmonella* spp., while all tomatoes (n = 18) treated with cold tap water tested positive for *Salmonella* spp.

Sampling of residual wash solutions resulted in recovery of 2.73  $\log_{10}$  CFU/ml of *Salmonella* spp. from the cold tap water solution and populations below the detection limit (0.95  $\log_{10}$  CFU/ml) for the chemical wash treatment (*Table 3*). Overall, the chemical wash treatment was slightly more effective in reducing the potential transmission of pathogens from inoculated tomatoes than the cold tap water wash was.

#### **DISCUSSION**

#### Green leaf lettuce

Velaquez et al. (29) studied the efficacy of 0.1 mg/ml benzalkonium chloride and 0.2% lactic acid against *E. coli* O157:H7 on lettuce. Bezalkonium chloride reduced *E. coli* O157:H7 by 1.71 log<sub>10</sub> CFU/g, while lactic acid reduced *E. coli* O157:H7 by 0.4 log<sub>10</sub> CFU/g. Keeratipibul et al. (15) reported that lettuce leaves dipped for 10 min in 75 ppm hypochlorous acid and 50 ppm peracetic acid reduced *E. coli* by 1.3 and 2.5 log<sub>10</sub> CFU/g, respectively. Ölmez (21) found that treatment of lettuce with 1.5 ppm aqueous ozone and a

# TABLE 2. Mean log<sub>10</sub> reductions ± standard error in populations of *E. coli* 0157:H7on green leaf lettuce and *Salmonella* spp. on tomatoes after chemical washtreatment or cold tap water wash

Effect	Treatment	Exposure time (s)	<i>E. coli</i> O157:H7 Log <sub>10</sub> Reduction (CFU/g) <sup>b</sup>	Salmonella spp. Log <sub>10</sub> Reduction (CFU/cm <sup>2</sup> ) <sup>c</sup>
Main effects <sup>a</sup>	Cold tap water	-	$2.25 \pm 0.34^{x}$	$2.50 \pm 0.49$
Main effects"	Chemical wash treatment	-	$2.95 \pm 0.34^{\circ}$	$2.96\pm0.49$
Interaction effect	Cold tap water	30	$2.16\pm0.41$	$2.47\pm0.60$
	Cold tap water	60	$2.24\pm0.41$	$2.26\pm0.60$
	Cold tap water	120	$2.34\pm0.41$	$2.78\pm0.60$
	Chemical wash treatment	30	$3.11 \pm 0.41$	$2.62\pm0.60$
	Chemical wash treatment	60	$2.53 \pm 0.44$	3.13 ± 0.60
	Chemical wash treatment	120	$3.21 \pm 0.41$	$3.13 \pm 0.60$

<sup>*a*</sup>Data pooled for exposure time (30, 60, 120); n = 18.

<sup>b</sup>E. coli O157:H7 inoculated samples mean population was  $7.75 \pm 0.37$  (SD)  $\log_{10}$  CFU/g.

<sup>c</sup>Salmonella spp. inoculated samples mean population was  $3.55 \pm 0.57$  (SD)  $\log_{10}$  CFU/cm<sup>2</sup>.

<sup>xy</sup>Means  $\pm$  standard error (SE) with different superscripts within a column are significantly different (P < 0.05).

### TABLE 3. Mean ± standard error Escherichia coli 0157:H7 and Salmonella spp.populations recovered from residual water after wash treatments (n = 9)

Produce	Pathogen	Treatment	Log <sub>10</sub> CFU/ml
T etterer	E. coli 0157:H7	Cold tap water	$4.92 \pm 0.23$
Lettuce	<i>E. coll</i> O15/:H/	Chemical wash treatment	< 1.95 DL <sup>a</sup>
	C 1 11	Cold tap water	$2.73 \pm 0.25$
Tomatoes	Salmonella spp.	Chemical wash treatment	< 0.95 DL

<sup>a</sup>Detection limits (DL) for lettuce and tomato samples were 1.95 and 0.95 log<sub>10</sub> CFU/ml, respectively.

mixture of organic acids (0.25% citric acid + 0.50% ascorbic acid) for 2 min reduced *E. coli* by 1.19 and 1.40  $\log_{10}$  CFU/g, respectively. Various studies have reported that chlorine solutions reduce *E. coli* by < 1 to 3  $\log_{10}$  CFU/g on lettuce. These results are highly dependent on inoculation method, method of application, exposure time, and free chlorine concentrations (1, 4, 15, 16, 21). In some cases, reductions achieved by chlorine solutions were the same as reductions achieved by water alone (4).

Similar reductions of *E. coli* O157:H7 on leaf lettuce were obtained in our study. Although reductions using different exposure times were not significantly different, it is recommended that the chemical wash treatment be used for 120 s to reduce microbial load from the lettuce surface and to reduce possible cross-contamination in the washing tank.

#### Tomatoes

Beuchat et al. (7) reported reductions (>  $6.83 \log_{10}$ ) of *Salmonella* populations on tomatoes when a prototype wash (containing citric acid and distilled grapefruit oil, among other ingredients) was applied. In a scaled-up study using the same commercial prototype wash, reductions in *Salmonella* were greater than those achieved with sterile water or with Dey and Engley (D/E) broth (14). In both studies, *Salmonella* reductions achieved by the prototype wash were obtained by sampling the rinse and residual wash solutions used to wash tomatoes.

In our study, *Salmonella* spp. and *E. coli* O157:H7 reductions (ca.  $3 \log_{10}$ ) were obtained by sampling the tissue/skin of each treated tomato or lettuce leaf. Therefore, it is difficult to compare the reductions obtained in our study to those obtained in these studies, because of differences in treatment application and methods used for recovery of *Salmonella*. However, in our study, *Salmonella* counts in the residual wash (*Table 3*) were consistent with those reported by Beuchat et al. (7) and Harris et al. (14), who reported *Salmonella* reductions in rinse and residual water 2 to 4 logs<sub>10</sub> greater than for controls (water and D/E broth), respectively.

Various studies have reported the efficacy of different sanitizers in reducing populations of Salmonella on the surface of tomatoes. Sapers et al. (27) reported 2.59 log<sub>10</sub> reductions of Salmonella in tomatoes treated with 5% hydrogen peroxide at  $60^{\circ}$ C for 2 min. Long et al. (17), who investigated the efficacy of ozone washing systems in reducing Salmonella and E. coli on tomatoes, reported that ozone systems did not significantly reduce the pathogenic load attached on tomato surfaces, but ozone application did significantly reduce Salmonella and E. coli (> 1  $log_{10}$ ) in wash water. Wei et al. (30) and Zhuang et al. (31) reported Salmonella Montevideo reductions between 1 to  $2 \log_{10}$  for tomato skin dipped for up to 2 min in 60 to 350 ppm free chlorine solutions; however, Salmonella populations were not eliminated. These results are similar to the results obtained in the current study, in which Salmonella reductions were between 2 to 3 log<sub>10</sub>.

Multiple studies have investigated the microbiological quality of produce. In Canada, two surveys testing over 600 lettuce samples reported generic E. coli populations that ranged from < 1 to  $3 \log_{10} CFU/g(3, 8)$ . Moreover, two surveys in United States (U.S.) reported coliform counts from 1.5 to 4.1  $\log_{10}$  MPN/g for lettuce and 1.8 to 2.3  $\log_{10}$ MPN/g for tomatoes (19, 20). Additionally, Mukherjee et al. (20) reported lettuce samples with generic *E. coli* populations of 2.2 to 2.4 log<sub>10</sub> MPN/g. Despite the prevalence of *E. coli*, the serotype O157:H7 was not detected on any lettuce samples (3, 8, 19, 20). In various surveys of retail markets of United Kingdom (428 samples), Canada (120 samples) and the U.S. (108 samples), Salmonella was not isolated from tomato samples (8, 19, 25). However, in a survey in Canada that tested Roma tomatoes (148 samples), one sample tested positive for Salmonella spp.; however, although Salmonella spp. was detected, the population recovered from the sample was not reported (3).

If the initial population of E. coli O157:H7 and Salmonella spp. in naturally contaminated fresh produce is  $\leq 3 \log$ , reduction levels (ca.  $3 \log_{10}$ ) obtained with the chemical wash treatment for both Salmonella and E. coli O157:H7 may reduce the risk of foodborne illnesses. This might be applicable for produce (lettuce and tomatoes) exposed to contamination prior to being washed with this product. However, it is important to note that this treatment might not be able to ensure produce safety if pathogens are present in populations  $> 3 \log 3$  on the surface or internalized in produce. Contamination can occur at numerous points along the farm-to-table food chain because produce is grown in open fields, handled by humans or automatized equipment prior, during, and post harvest, and eaten raw (18). To reduce contamination of produce, multiple interventions (i.e., Good Agricultural Practices, GAP; Good Manufacturing Practices, GMPs; and Sanitation Standard Operating Procedures, SSOPs) at different points of the food chain (i.e., field production, harvesting, transportation, processing, or preparation in food service or home kitchens) need to be implemented.

Limitations of the effectiveness of the washing treatments used in our study may be the results of the specific surface characteristics of the produce (i.e., green leaf lettuce irregular surface, unwaxed or waxed tomatoes), time interval between inoculation and treatment, strong attachment of the pathogens to inaccessible sites, biofilm formation, and background microflora (26). However, our observations indicate that using the chemical wash treatment during the washing procedure will reduce foodborne pathogens on the surface of produce and also reduce cross-contamination that occurs when new produce is introduced into a washing tank.

#### **CONCLUSIONS/RECOMMENDATIONS**

Data from this study expands knowledge of the chemical wash treatment as an alternative for produce decontamina-

tion and its potential value for preventing cross-contamination during produce washing. Overall, application of the chemical wash treatment was capable of reducing *E. coli* O157:H7 and *Salmonella* spp. by about  $3 \log_{10}$  units on the surface of green leaf lettuce and tomatoes, and post-treatment residual water with the chemical wash treatment contained populations below detection limits. Application of the chemical wash treatment (0.35%) by immersing the produce in the wash solution and gently stirring for 2 min, followed by rinsing with tap water, represents a potential intervention strategy for reducing pathogens on green leaf lettuce and tomato surfaces and in the wash water. However, further research exploring different microorganisms, levels of initial contamination, time intervals between produce inoculation and treatment application, application methods, and different antimicrobial concentrations are advisable to determine the effectiveness of the antimicrobial solution under different conditions.

#### ACKNOWLEDGMENTS

This is contribution number 14-248-J from the Kansas Agricultural Experiment Station. This research was conducted by Kansas State University and was funded at least in part with Federal funds from the U.S. Department of Agriculture. We also thank Kyle McLean and Krista McKay for laboratory work assistance during this research.

#### REFERENCES

- Akbas, M. Y., and H. Ölmez. 2007. Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatment in organic acids. *Lett. Appl. Microbiol.* 44:619–624.
- Allende, A., F. A. Tomas-Barberan, and M. I. Gil. 2006. Minimal processing for healthy traditional foods. *Trends Food Sci. Technol.* 17:513–519.
- Arthur, L. A., S. Jones, M. Fabri, and J. Odumeru. 2007. Microbial survey of selected Ontario-grown fresh fruits and vegetables. *J. Food Prot.* 70:2864–2867.
- Beuchat, L. R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and effectiveness of chlorinated water as a disinfectant. *J. Food Prot.* 62:845–849.
- Beuchat, L. R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microb. Infect.* 4:413–423.
- Beuchat, L. R., J. M. Farber, E. H. Garrett, L. J. Harris, M. E. Parish, T. V. Suslow, and F. F. Busta. 2001. Standarization of a method to determine the efficacy of sanitizers in inactivating human pathogenic microorganisms on raw fruits and vegetables. *J. Food Prot.* 64:1079–1084.
- Beuchat, L. R., L. J. Harris, T. E. Ward, and T. M. Kajs. 2001. Development of a proposed standard method for assessing the efficacy of fresh produce sanitizers. *J. Food Prot.* 64:1103–1109.
- Bohaychuk, V. M., R. W. Bradbury, R. Dimock, M. Fehr, G. E. Gensler, R. K. King, R. Rieve, and P. Romero Barrios. A microbiological survey of selected Alberta-grown fresh produce from farmers' markets in Alberta, Canada. J. Food Prot. 72:415–420.
- Centers for Disease Control and Prevention. 2007. Multistate outbreaks of Salmonella infections associated with raw tomatoes eaten in restaurants — United States, 2005–2006. MMWR. Mob. Mortal. Wkly. Rep. 56:909–911.

- Centers for Disease Control and Prevention. 2012. Multistate outbreak of *E. coli* O157:H7 infections linked to romaine lettuce (Final Update). Available at: http://www.cdc. gov/ecoli/2011/ecolio157/romainelettuce/032312/index.html. Accessed 18 June 2014.
- Centers for Disease Control and Prevention. 2013. Surveillance for foodborne disease outbreaks — United States, 1998–2008. Mob. Mortal. Wkly. Rep. 62 (SS02):1–34.
- Cook, R. 2011. Tracking demographics and U.S. fruit and vegetable consumption patterns. Available at: http://agecon.ucdavis. edu/people/faculty/roberta-cook/docs/Articles/BlueprintsEoEConsumptionCookFinal-Jan2012Figures.pdf Accessed 25 June 2014.
- Doyle, M. P., and M. C. Erickson. 2008. Summer meeting 2007—the problems with fresh produce: an overview. J. App. Microbiol. 105:317–330.
- Harris, L. J., L. R. Beuchat, T. M. Kajs, T. E. Ward, and C. H. Taylor. Efficacy and reproducibility of a produce wash in killing *Salmonella* on the surface of tomatoes assessed with a proposed standard method for produce sanitizers, *J. Food Prot.* 64:1477–1482.
- Keeratipibul, S., A. Phewpan, and C. Lursinsap. 2011. Prediction of coliforms and *Escherichia coli* on tomato fruits and lettuce leaves after sanitizing by using artificial neural networks. *LWT Food Sci. Technol.* 44:130–138.
- Keskinen, L. A. A. Burke, and B. A. Annous. 2009. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157:H7 from lettuce leaves. *Int. J. Food Microbiol.* 132:134–140.
- Long III, W. P., S. Pao, P. Inserra, E. Westbrook, and S. Ahn. 2011. Efficacy of ozone produce washers in reducing natural and artificially inoculated microorganisms on Roma tomatoes and green onions. *J. Food Safety* 31:268–275.

- Mandrell, R. E. 2009. Microbial contamination of fresh produce, p. 4–42. *In* X. Fan, B.A. Niermira, C. J. Doona, F. E. Feeherry, R. B. Gravani (ed.), Microbial safety of fresh produce. IFT Press, Wiley-Blackwell, Hoboken, NJ.
- Mukherjee, A., D. Speh, E. Dyck, and F. Diez-Gonzales. 2004. Preharvest evaluation of coliforms, *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.* 67:894–900.
- Mukherjee, A., D. Speh, A. T. Jones, K. M. Buesing, and F. Diez-Gonzales. 2006. Longitudinal microbiological survey of fresh produce grown by farmers in the upper Midwest. J. Food Prot. 69:1928–1936.
- Ölmez, H. 2010. Effect of different sanitizing methods and incubation time and temperature on inactivation of *Escherichia coli* on lettuce. J. Food Safety 30:288–299.
- 22. Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerg. Infect. Dis.* 19:407–415.
- 23. Pangloli, P., Y. C. Hung, L. R. Beuchat, C. H. King, and Z.H. Zhao. 2009. Reduction of *Escherichia coli* O157:H7 on produce by use of electrolyzed water under simulated food service operation conditions. *J. Food Prot.* 72:1854–1861.
- Pollack, S. L. 2001. Consumer demand for fruit and vegetables: The U.S. example. Available at: http://www.ers.usda.gov/media/293609/wrs011h\_1\_.pdf. Accessed 15 July 2013.
- Sagoo, S. K., C. L. Little, and R. T. Mitchell. 2001. The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. *Lett. Appl. Microbiol.* 33:434–439.
- Sapers, G. M. 2001. Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technol. Biotechnol.* 39:305–311.

- Sapers, G. M., and D. M. Jones. 2006. Improved sanitizing treatments for fresh tomatoes. J. Food Sci. 71:M252–M256.
- 28. Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. T. Tauxe. 2004. Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. 67:2342–2353.
- Velazquez, L. C., N. B. Barbini, M. E. Escudero, C. L. Estrada, and A. M. S. de Guzman.
   2009. Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yesinia enterocolitica* on fresh vegetables. *Food Control* 20:262–268.
- Wei, C. I., T. S. Huang, J. M. Kim, W. F. Lin, M. L. Tamplin, and J. A. Bartz. 1995. Growth and survival of *Salmonella montevideo* on tomatoes and disinfection with chlorinated water. J. Food Microbiol. 62:123–131.
- Zhuang, R. Y., L. R. Beuchat, and F. J. Angulo. 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. *Appl. Environ. Microbiol.* 61:2127–2131.

## Call for Secretary Nominations



A representative from the education sector will be elected in March of 2016 to serve as IAFP Secretary for the year 2016–2017. Letters of nomination, along with a biographical sketch, are now being accepted by the Nominations Chairperson:

Karl Matthews c/o IAFP 6200 Aurora Ave., Suite 200W Des Moines, IA 50322-2864

The Secretary-Elect is determined by a majority of votes cast through a vote taken in March of 2016. Official Secretary duties begin at the conclusion of IAFP 2016. The elected Secretary serves as a Member of the Executive Board for a total of five years, succeeding to President, then serving as Past President.

For information regarding requirements of the position, contact David Tharp, Executive Director,

at +1 800.369.6337 or +1 515.276.3344; E-mail: dtharp@foodprotection.org.

### Nominations Close October 1, 2015