Efficacy of Neutral pH Electrolyzed Water in Reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on Fresh Produce Items using an Automated Washer at Simulated Food Service Conditions

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Abstract: The objective of this study was to determine the efficacy of neutral pH electrolyzed (NEO) water (155 mg/L free chlorine, pH 7.5) in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on romaine lettuce, iceberg lettuce, and tomatoes washed in an automated produce washer for different times and washing speeds. Tomatoes and lettuce leaves were spot inoculated with 100 μL of a 5 strain cocktail mixture of either pathogen and washed with 10 or 8 L of NEO water, respectively. Washing lettuce for 30 min at 65 rpm led to the greatest reductions, with 4.2 and 5.9 log CFU/g reductions achieved for *E. coli* O157:H7 and *S.* Typhimurium respectively on romaine, whereas iceberg lettuce reductions were 3.2 and 4.6 log CFU/g for *E. coli* O157:H7 and *S.* Typhimurium respectively. Washing tomatoes for 10 min at 65 rpm achieved reductions greater than 8 and 6 log CFU/tomato on *S.* Typhimurium and *E. coli* O157:H7 respectively. All pathogens were completely inactivated in NEO water wash solutions. No detrimental effects on the visual quality of the produce studied were observed under all treatment conditions. Results show the adoption of this washing procedure in food service operations could be useful in ensuring produce safety.

Keywords: agitation, lettuce, neutral pH electrolyzed water, salad washer, tomatoes

Practical Application: Washing produce with NEO water in an automated washer at food service establishments can help reduce the occurrence of foodborne illnesses related to cross-contamination.

Introduction

Fresh fruit and vegetable consumption has been increasing significantly over recent years because of the various nutritional and health benefits that have been demonstrated from their consumption (Cook 2003). This trend however has coincided with an increasing number of foodborne illnesses and outbreaks in the United States as well as internationally leading to the need to develop means of tackling this worrying problem which can have detrimental effects on the health of consumers. According to the CDC, 1527 foodborne diseases were recorded in 2009 to 2010, resulting in 29444 illnesses, 1184 hospitalizations, and 23 reported deaths. Of these cases, *Salmonella spp.* and Shiga-toxin producing *Escherichia coli* accounted for the most outbreak-related hospitalizations with *Salmonella spp.* causing 49% of hospitalizations and 5 deaths and *E. coli* O157:H7 directly implicated in 16% of hospitalizations and 3 deaths (Gould and others 2013). Finally, it was determined that 48% of cases were caused by food consumed in a restaurant or food service operation (CDC 2013).

In most food service operations, fresh produce obtained is normally washed with running tap water to remove soil and debris, which has limiting effects on reducing or inactivating pathogenic microbes present (Koseki and others 2001). Food service establishments are now however commonly using chlorine-based chemicals as their main sanitizer (Monnin and others 2012).

Electrolyzed (EO) water, a modern antimicrobial treatment used in the fields of agriculture, dentistry, medicine, and the food industry (Hricova and others 2008), can be used as an effective sanitizer for fresh produce items. It is produced by the electrolysis of dilute salt solution (NaCl) through an electrolytic cell, with the anode and cathode separated by a membrane leading to the production of acidic EO water and electrolyzed reducing (ER) water. The EO water exhibits antimicrobial properties because of its low pH (2.3 to 2.7), high ORP (>1000 mV) and free chlorine with hypochlorous acid (HOCl) being the most active species (Huang and others 2008). EO water or acidic EO water has been shown to be lethal to many foodborne pathogens including *Salmonella* and *E. coli* 0157:H7 found on lettuce (Koseki and others 2004; Park and others 2001), tomatoes, lemons, and cabbage leaves (Pangloli and others 2009) as well as other produce items. Even though EO water is very effective, it also loses its antimicrobial activity relatively quickly because of 10% to 15% of the chlorine being in the form of chlorine gas at low pH levels (Len and others 2000). Near-neutral to neutral pH EO (NEO) water is generated by the electrolysis of NaCl solution in a sole-chamber system (without a separating membrane) or in a dual-chamber system as is the case with acidic EO water; however, part of the EO water formed at the anode is directed into the cathode chamber resulting in a near neutral pH solution also with antimicrobial properties

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(Abadias and others 2008; Waters and others 2012). NEO water shows less corrosiveness when compared with EO water and is also relatively more stable due to reduced chlorine loss at pH ranges between 6 and 9 (Ayebah and Hung 2005; Waters and Hung 2014). NEO water has also been shown to be effective in reducing and inactivating pathogens on fresh produce items. In a study comparing the effectiveness of NEO water containing 50 mg/L of free chlorine and chlorinated water at 120 mg/L free chlorine in inactivating *Salmonella spp.*, *E. coli* 0157:H7 and *Listeria innocua* on carrots, fresh-cut lettuce "endive" and corn salad, it was found that both solutions were equally effective (Abadias and others 2008). Deza and others (2003) also demonstrated 4 log reductions using NEO water on tomatoes that had been surface inoculated with *Salmonella enteritidis*, *E. coli* 0157:H7, nonpathogenic *E. coli* and *Listeria monocytogenes*. Treatment of lettuce by dipping in NEO water also reduced *E. coli* O157:H7 and *Salmonella* Typhimurium by 2.1 and 2.0 log CFU/g respectively (Yang and others 2003).

In some food service establishments, a salad washer is used in the washing and rinsing steps as well as for removal of excess water from some produce items including lettuce and cabbage. This step could be modified to include an antimicrobial wash-step with agitation to ensure produce safety. Wang and others (2007) demonstrated that increasing flow velocity and agitation rate led to increased reduction of *E. coli* O157:H7 on fruit surfaces when treated with peroxyacetic acid. The objective of this study was to determine the effects of treatment time and washing speed on the reductions of *E. coli* O157:H7 and *S.* Typhimurium DT 104 on different produce items using NEO water in an automated produce washer.

Materials and Methods

Inoculum preparation

The 5 nalidixic acid-adapted *E. coli* O157:H7 strains used were 1 (Beef isolate), 5 (human isolate), 932 (human isolate), E009 (Beef isolate), and E0122 (cattle isolate); and the five strains of *S.* Typhimurium DT 104 were H2662 (cattle isolate), 11942A (cattle isolate), 13068A (cattle isolate), 152N17-1 (dairy isolate), and H3279 (human isolate). All strains were activated from frozen stock cultures by transferring loopful culture into 10 ml tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md., U.S.A.) supplemented with 50 mg/L nalidixic acid for *E. coli* O157:H7 (TSBN) or TSB for *S.* Typhimurium DT 104 (which was not nalidixic acid adapted). Cultures were grown individually in TSBN or TSB for 24 h at 37 °C and then sedimented by centrifugation at 3000 x *g* and 20 °C for 15 min. The supernatant was then discarded and cells resuspended in phosphate-buffered saline (PBS, pH 7). Two different 5 strain mixtures were prepared by mixing 2 mL of individual strains of either pathogen. Bacterial populations of each mixture were then verified by plating 0.1 mL of the appropriate dilution on tryptic soy agar (TSA) supplemented with 50 mg/L nalidixic acid (TSAN) for *E. coli* O157:H7 or on TSA for *S.* Typhimurium DT 104 and incubated at 37 °C for 24 h. The use of nalidixic acid was to enable inhibition of the microflora naturally present on produce samples and to allow for selective isolation and enumeration of inoculated pathogens.

Preparation and inoculation of produce items

Romaine lettuce (*Lactuca sativa L.* var. *longifolia*), iceberg lettuce (*Lactuca sativa L.*), and round red tomatoes (*Lycopersicum esculentum* Mill) were obtained from a local restaurant, and stored at 4 °C and used within 24 h. The outer 2 or 3 damaged leaves of lettuce were discarded, with the next 3 to 4 leaves collected and placed with the abaxial side facing up on sanitized trays. Each whole leaf was spot inoculated with 100 μL (10 drops) of *E. coli* O157:H7 or *S.* Typhimurium DT 104 five strain mixtures using a micropipettor. Approximately 400 g of leaves (16 whole leaves) were inoculated for each time-washing speed treatment. For tomatoes, uniform sizes of red round tomatoes without damage or bruises were selected. Ten to twelve red round tomatoes were selected for each time-washing speed treatment, with the tomatoes weighing approximately 1 kg per treatment batch (80 to 100 g for each tomato) individually placed stem end down on sanitized trays and spot inoculated with 100 μL of either *E. coli* O157:H7 or *S.* Typhimurium DT 104 5-strain mixture per fruit. The inoculated produce was then allowed to dry under a laminar flow hood for 2 h to allow for attachment of pathogens. Trays with the air-dried inoculated produce items were then covered with aluminum foil and placed in a 4 °C cool room for 24 h to simulate produce handling practices in some restaurants and food service kitchens. Initial pathogen populations present on leaves and tomatoes were determined after the storage period.

Preparation of neutral EO water

NEO water was generated by electrolyzing a dilute NaCl solution (ca. 10%) using a GenEon^{TM} Instaflow generator (GenEon Technologies, San Antonio, Tex., U.S.A.) operating at about 11 V to obtain NEO water with free chlorine concentration of approximately 155 mg/L. The production capacity of the generator was approximately 3.0 L/min. The NEO water was then collected in screw-cap containers and stored at 4 °C before use. NEO water generated at a pH and free chlorine concentration above desired value was adjusted through the addition of drops of 1 N HCl (Waters and others 2014) and dilution with deionized (DI) water respectively. The ORP and pH of NEO water were measured using a dual-channel ACCUMET meter (model AR 50; Fisher Scientific, Pittsburgh, Pa., U.S.A.). The DPD-FEAS titrimetric method (Hach Co., Loveland, Colo., U.S.A.) was used in the determination of free chlorine concentrations. DI water was also collected and chilled at 4 °C and used as control.

Automated produce washer

A Dynamic^{TM} salad spinner (Model E20SC E004; Kitchen Equipment Australia, Thomaston, Australia) with a capacity of 20 L (diameter of 43 cm and height of 50 cm) was modified for this study. A Dayton electric gear motor system (Model 42726A; Dayton Electric Mfg. Co., Niles, Ill., U.S.A.) was attached to the spinner to enable automated rotation when in operation. The speed at which the spinner moves is determined using the dial (readings of 1 to 10) on the Dayton gear motor system. For this study, dial was set to 2 and 4 corresponding to spinner speeds of 40 and 65 rpm respectively. The automated produce washer apparatus is shown in Figure 1.

Procedure for washing produce items

A 3-step washing protocol was used for lettuce. First, each whole leaf was rinsed for 3 s under running DI water (control) or NEO water containing 155 mg/L free chlorine. A total of 400 g of leaves were then submerged and washed in either 1:20 w/v (8 L) chilled DI sterilized water (control) or NEO water (155 mg/L available chlorine) in the automated produce washer for various lengths of

time (1, 5, 10, 15, or 30 min) with varying levels of washing speed (40 and 65 rpm).

At the end of designated wash period, treatment solution was completely drained and replaced with 8 L of fresh chilled NEO water or DI water and washed for an additional 30 s. Drained wash solution from the designated wash period (2nd step) was collected for microbiological analysis. After draining and spinning to remove excess water, washed leaves were chopped using the EasyLettuceKutter lettuce chopper (Model 55650–2; Nemco Food Equipment, Hicksville, Ohio, U.S.A.) and three different 50 g samples of chopped leaves were combined with 200 mL of Dey-Engley (DE) neutralizing broth (Difco) in 1.5 L Whirl-Pak bags, whereas 25 mL of wash solution was added to 25 mL of double strength DE broth for microbiological analysis.

For tomatoes, a 2-step washing protocol was employed. Tomatoes were rinsed by rubbing the entire surface with gloved hands under running wash water (NEO water or DI water) for 3 s/tomato. After rinsing, tomatoes were submerged in either 1:10 w/v (10 L) chilled (4 °C) DI water (control) or NEO water (155 mg/L available chlorine) in the automated produce washer for various lengths of time (1, 5, and 10 min) with varying washing speed levels (40 and 65 rpm). At the end of designated wash period, treatment solution was completely drained. After treatment, three tomatoes were selected and individually placed in different 1.5 L Whirl-Pak bags containing 50 mL DE broth and 25 mL of treatment solution were collected separately and combined with 25 mL of double strength DE broth for microbiological analysis.

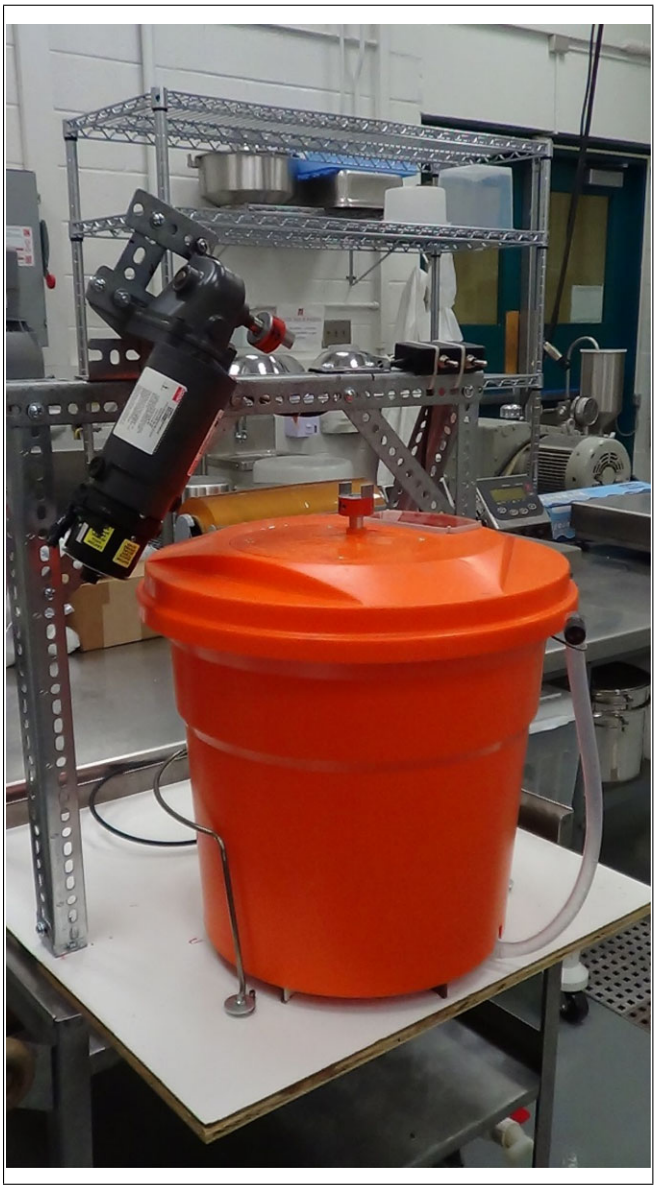

Figure 1–Modified automated produce washer apparatus with electric gear motor system.

Microbiological analysis

The Whirl-Pak bags containing lettuce samples and DE broth were pummeled in a stomacher (Stomacher® 80) for 2 min at 260 rpm speed while tomatoes in Whirl-Pak bags with DE broth were hand rubbed for 2 min. Wash solutions collected were also pummeled for 1 min at 260 rpm in the stomacher. The DE broths were then serially diluted in PBS and plated (in duplicates, 100 μL) on sorbitol MacConkey agar supplemented with 50 μg/mL nalidixic acid and 0.1% sodium pyruvate (SMACNP) for *E. coli* O157:H7 and XLD agar supplemented with 0.1% sodium pyruvate, 32 mg/mL ampicillin, 16 mg/mL tetracycline, and 64 mg/mL streptomycin for *S.* Typhimurium DT 104 (XLDASTP) (Jadeja and Hung 2014). After plating, plates were incubated at 37 °C for 24 h and counted afterwards using a colony counter (aColyte 7510/SYN; Microbiology Intl., Frederick, Md., U.S.A.).

To detect the presence of low numbers of pathogens that would not be detected by direct plating, 250 mL of double strength modified TSB supplemented with 50 mg/L nalidixic acid and 0.1% sodium pyruvate (dmTSBNP) was added to each stomacher bag containing romaine lettuce with 200 mL of DE broth. For low numbers of *S.* Typhimurium DT 104 enrichment, 250 mL of double strength lactose broth supplemented with 0.1% sodium pyruvate, 32 mg/mL ampicillin, 16 mg/mL tetracycline, and 64 mg/mL streptomycin (LBASTP) was used for enrichment. All enrichments were incubated at 37 °C for 24 h. If direct plating did not yield any colonies, incubated enrichment broth was streaked onto SMACNP or XLDASTP plates for *E. coli* O157:H7 and *S.* Typhimurium DT 104 respectively and incubated at 37 °C for 24 h. At the end of the incubation period, plates were examined for the presence of presumptive colonies of either *E. coli* O157:H7 (colorless) or *S.* Typhimurium DT 104 (black). Five presumptive-positive colonies were randomly selected from SMACNP and XLDASTP plates with the appropriate dilution and subjected to biochemical tests (API 20E assay; bioMe'reux, Hazelwood, Mo., U.S.A.) and latex agglutination assay (Oxoid, UK).

Statistical analysis

Experiments were replicated twice with each duplicate consisting of 3 different samples for each treatment. Microbial counts were expressed as log CFU/mL (wash solutions), log CFU/g (lettuce), and log CFU/tomato. Reported values of plate counts are the mean values of six samples ± standard deviations for treated produce samples and four samples ± standard deviations for wash solutions. Data were subjected to ANOVA with a completely randomized factorial design. These analyses were performed with the SAS software release 9.2 (SAS Institute, Cary, N.C., U.S.A.). The Tukey HSD method was used for multiple comparisons of means with the level of significance at 0.05.

Results and Discussion

NEO water properties

The properties of the NEO water were as follows: the pH at 7.52 ± 0.08, the ORP at 760 ± 19 mV and the free chlorine concentration at 155 ± 3 mg/L.

Romaine lettuce

Treatment time and washing speed were both significant factors ($P < 0.05$) in determining the reductions of initial populations on all lettuce leaves treated with NEO water. In the case of DI water, a higher washing speed did generally lead to increased reductions but these differences were not significant ($P < 0.05$) at the same treatment time for any of the 2 pathogens tested (Table 1 and 2).

Reductions between 2.0 and 5.9 log CFU/g were achieved in the case of *S.* Typhimurium DT 104 when treated with NEO water for 1 to 30 min while DI water treatment reductions between 1.6 and 3.0 log CFU/g were observed (Table 1). The differences in reductions observed for 1 min treatments at comparable speeds for DI water (1.6 and 2.0 log CFU/g) when compared with those for NEO water (2.0 and 2.3 log CFU/g) were far smaller than for treatments of 5 min and above. For treatments of 5 min and higher, a minimum of 1 log CFU/g further reductions were observed for NEO water than DI water with the greatest difference (2.8 log CFU/g) detected at 30 min treatment with 65 rpm speed. Five logarithmic reductions were obtained for 10, 15, and 30 min treatments at 65 rpm washing speed and the highest reduction was after NEO water treatment for 30 min at a washing speed of 65 rpm (5.9 log CFU/g).

For *E. coli* O157:H7 inoculated romaine lettuce, NEO water treatment reductions ranged from 1.2 to 4.2 log CFU/g, whereas those treated with DI water were between 0.9 and 2.4 log CFU/g (Table 2). The observed reduction trends were similar with those in *S.* Typhimurium DT 104 tests, with increasing time and washing speed leading to significantly greater reductions ($P < 0.05$). The highest reduction in the initial population of romaine lettuce leaves inoculated with *E. coli* O157:H7 was after treatment for 30 min at a washing speed of 65 rpm (4.2 log CFU/g reductions). At 40 rpm and identical time, a mean reduction of only 3.3 log CFU/g was observed. After 15 min of treatment, reductions ranging from 3.1 to 3.5 log CFU/g were observed, whereas the initial population decreased by 2.5 and 3.0 log CFU/g after 10 min of treatment for both speeds. Differences in reductions observed after 1 min treatments for NEO water and DI water treatments were minimal however, as was the case with *S.* Typhimurium DT 104, NEO water treatment reductions were always at least 1 log CFU/g higher than comparable reductions in DI water treatments for treatments at or above 5 min with the greatest difference of 1.8 log CFU/g being observed for 30 min at 65 rpm treatment.

For wash solutions analyzed after washing treatment, neither one of the two pathogens were detected through direct plating or enrichment in the case of NEO water, suggesting that its use in food service operations or restaurants can prevent cross contamination. In the case of DI water treatments, bacterial populations ranging from 1.8 to 5.1 log CFU/mL and 2.4 to 6.2 log CFU/mL were recovered for *S.* Typhimurium DT 104 and *E. coli* O157:H7 respectively signifying its susceptibility to cause cross contamination when used for washing. After treatment, all lettuce leaves were visually inspected and no noticeable damage to the overall leaf structure was observed.

Iceberg lettuce

As observed for romaine lettuce, reductions of *S.* Typhimurium DT 104 on iceberg lettuce leaf surfaces were generally greater than *E. coli* O157:H7 reductions for each comparable time-washing speed treatment combination (Table 3 and 4). The range of *S.* Typhimurium DT 104 reductions for NEO water treatment were from 1.8 to 4.6 log CFU/g (Table 3) with the highest reduction observed after treatment for 30 min at 65 rpm. At every treatment time except 1 min, the higher washing speed (65 rpm) always led to a significantly higher reduction ($P < 0.05$) when compared with the lower washing speed (40 rpm). Reductions above 4 log CFU/g were only observed for 15 and 30 min treatments at 65 rpm. DI water treatments showed significantly lower reductions of *S.* Typhimurium DT 104 than NEO water treatments under identical conditions (Table 3) with washing speed not having a significant effect ($P < 0.05$) on reductions for treatments at the same time.

For *E. coli* O157:H7, reductions between 0.9 and 3.2 log CFU/g were observed after iceberg lettuce treatments with NEO water treatments while DI water treatment reductions ranged from 0.5 to 1.8 log CFU/g (Table 4). Reductions for these treatments were generally the lowest when compared with other treatments at similar treatment conditions (Table 1 to 3). For *E. coli* O157:H7 inoculated iceberg lettuce, only the 30 min NEO water treatment in the salad washer at 65 rpm showed a reduction above 3 log CFU/g. A significant difference in reductions ($P < 0.05$) between washing speeds at the same treatment time was observed only at 30 min of treatment (3.2 and 2.4 log CFU/g for 65 and 40 rpm, respectively). NEO water treatments for 10 min at 65 rpm and 15 min and above were always at least 1 log CFU/g reductions greater than DI water treatments at similar conditions. Washing speed did not have a significant effect on DI water treatments ($P < 0.05$).

Both pathogens were completely inactivated in NEO water wash solutions recovered after treatments even after enrichment making it an appropriate solution to help ensure produce safety and prevent cross contamination (Table 3 and 4). The 2 pathogens tested were always recovered in the DI water wash solutions after treatment, with the populations ranging from 2.8 to 4.9 log CFU/ml for *S.* Typhimurium DT 104 and 3.2 to 4.8 log CFU/ml for *E. coli* O157:H7. As was the case with romaine lettuce, no noticeable damage to treated iceberg lettuce leaves was observed after visual inspection. The reductions of *S.* Typhimurium DT 104 and *E. coli* O157:H7 inoculated on romaine lettuce after treatment with NEO water were always greater when compared with reductions on iceberg lettuce.

Several studies have shown the efficacy of EO water in reducing pathogenic microbes on lettuce. Pangloli and Hung (2011) found that washing iceberg lettuce for 15 and 30 s with running slightly acidic EO (SAEO) water resulted in 1.4 to 2.3 log CFU/leaf reductions in *E. coli* O157:H7 with increased time leading to higher reductions as was the case in this study. A subsequent chill in SAEO water for 15 min after the 15 s wash increased reductions by up to 2.4 log CFU/leaf. Reductions of up to 3 log CFU/leaf were observed when a 15 or 30 s wash followed by 15 min iceberg lettuce treatment in chilled EO water (Pangloli and others 2009). Both studies had lower pH values and free chlorine concentrations when compared with NEO water, however as was the case in this study, reductions in initial populations all exceeded at least 2 logs in magnitude. Also, results for NEO water treatments of *E. coli* O157:H7 on iceberg lettuce after 1 min for the

Table 1–Mean log reduction of *Salmonella* Typhimurium DT 104 on romaine lettuce after washing treatment.

Water type	Time (min)	Washing speed (rpm)	Recovery (log CFU/g)	Reduction (log CFU/g)[a,b]	Recovery from wash solution (log CFU/mL)[c]
EO water	1	40	4.9 ± 0.3	2.0^{DE}	ND
		65	4.6 ± 0.4	2.3^{D}	ND
	5	40	3.6 ± 0.6	3.0^{C}	ND
		65	2.4 ± 0.8	4.2^{B}	ND
	10	40	2.9 ± 0.7	3.8^{C}	ND
		65	1.5 ± 1.3	5.1^{AB}	ND
	15	40	3.1 ± 0.6	4.4^{B}	ND
		65	1.9 ± 1.1	5.6^{A}	ND
	30	40	2.6 ± 0.5	4.8^{B}	ND
		65	1.4 ± 1.2	5.9^{A}	ND
DI water	1	40	5.2 ± 0.6	1.6^{E}	5.1
		65	4.9 ± 0.4	1.8^{DE}	5.2
	5	40	4.6 ± 0.4	2.0^{DE}	3.1
		65	4.6 ± 0.7	2.0^{DE}	4.6
	10	40	4.5 ± 0.8	2.1^{DE}	3.9
		65	4.4 ± 0.6	2.3^{D}	4.2
	15	40	4.8 ± 0.6	2.7^{DC}	2.6
		65	4.9 ± 0.9	2.5^{D}	2.8
	30	40	4.7 ± 1.0	2.5^{D}	1.3
		65	4.9 ± 0.9	3.0^{C}	1.8

[a]Values in this column represent the difference between the populations of *Salmonella* Typhimurium DT 104 present on lettuce surface before and after treatment with the initial population ranging between 6.1 and 7.9 log CFU/g with a mean of 7.2 log CFU/g (Detection limit was 1.7 log CFU/g; if pathogen was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was 1.6 log CFU/g).
[b]Mean values not followed by the same letter in a column are significantly different ($P < 0.05$).
[c]ND = not detected by direct plating and enrichment (detection limit of 0.3 log CFU/mL).

Table 2–Mean log reduction of *E. coli* O157:H7 on romaine lettuce after washing treatment.

Water type	Time (min)	Washing speed (rpm)	Recovery (log CFU/g)	Reduction (log CFU/g)[a,b]	Recovery from wash solution (log CFU/mL)[c]
EO water	1	40	5.9 ± 0.7	1.2^{DE}	ND
		65	5.9 ± 0.3	1.2^{DE}	ND
	5	40	5.1 ± 0.7	2.2^{C}	ND
		65	4.7 ± 1.1	2.5^{BC}	ND
	10	40	4.1 ± 0.6	2.5^{BC}	ND
		65	3.6 ± 0.8	3.0^{B}	ND
	15	40	3.9 ± 0.7	3.1^{B}	ND
		65	3.5 ± 0.9	3.5^{AB}	ND
	30	40	3.8 ± 0.4	3.3^{AB}	ND
		65	3.1 ± 1.1	4.2^{A}	ND
DI water	1	40	6.2 ± 0.7	0.9^{DE}	6.1
		65	6.3 ± 0.9	1.0^{DE}	6.1
	5	40	6.1 ± 0.6	1.1^{DE}	5.4
		65	5.9 ± 0.5	1.3^{D}	5.0
	10	40	4.9 ± 0.5	1.6^{D}	4.6
		65	5.0 ± 0.4	1.7^{CD}	4.2
	15	40	5.1 ± 0.4	1.8^{CD}	4.1
		65	4.9 ± 0.5	2.1^{C}	3.3
	30	40	4.5 ± 0.6	2.2^{C}	2.7
		65	4.8 ± 0.4	2.4^{BC}	2.4

[a]Values in this column represent the difference between the populations of *E. coli* O157:H7 present on lettuce surface before and after treatment with the initial population ranging between 6.3 and 7.6 log CFU/g with a mean of 7.1 log CFU/g (Detection limit was 1.7 log CFU/g; if pathogen was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was 1.6 log CFU/g).
[b]Mean values not followed by the same letter in a column are significantly different ($P < 0.05$).
[c]ND = not detected by direct plating and enrichment (detection limit of 0.3 log CFU/mL).

current study were consistent with EO water treatments observed by Koseki and others (2003) in achieving less than or just about 1 log CFU/g reductions with the dip method for *E. coli* O157:H7 and *Salmonella*. Keskinen and others (2009) also found the reductions of *E. coli* O157:H7 inoculated romaine lettuce were slightly higher than reductions for iceberg lettuce after treatment with EO water for 2 min. NEO water (pH 7, 30 °C, and 300 mg/L chlorine) treatment of iceberg lettuce for 5 min also reduced both *S.* Typhimurium and *E. coli* O157:H7 by 2 log CFU/g (Yang and others 2003).

The results from the use of the proposed produce washer for washing lettuce leaves shows greater reductions in the instance of *S.* Typhimurium than *E. coli* O157:H7 and this may be attributed to the constant agitation provided by the motion of the automated washer during treatment and the sites of attachment on lettuce by both pathogens (Takeuchi and others 2000). These

Table 3–Mean log reduction of *Salmonella* Typhimurium DT 104 on iceberg lettuce after washing treatment.

Water type	Time (min)	Washing speed (rpm)	Recovery (log CFU/g)	Reduction (log CFU/g)[a,b]	Recovery from wash solution (log CFU/mL)[c]
EO water	1	40	5.6 ± 0.2	1.8^{DE}	ND
		65	5.2 ± 0.3	2.3^{D}	ND
	5	40	5.4 ± 0.5	2.0^{DE}	ND
		65	4.6 ± 0.3	2.9^{C}	ND
	10	40	5.1 ± 0.3	2.3^{D}	ND
		65	4.1 ± 0.4	3.4^{B}	ND
	15	40	4.1 ± 0.3	3.4^{B}	ND
		65	3.2 ± 0.3	4.3^{A}	ND
	30	40	3.9 ± 0.2	3.6^{B}	ND
		65	2.8 ± 0.3	4.6^{A}	ND
DI water	1	40	6.6 ± 0.8	0.8^{F}	4.9
		65	6.7 ± 0.7	0.8^{F}	4.6
	5	40	6.4 ± 0.3	1.1^{F}	4.5
		65	6.3 ± 0.7	1.2^{F}	3.5
	10	40	6.1 ± 0.6	1.4^{EF}	3.8
		65	6.2 ± 0.6	1.3^{EF}	4.0
	15	40	5.9 ± 0.3	1.6^{E}	3.4
		65	5.7 ± 0.5	1.8^{DE}	3.2
	30	40	5.6 ± 0.5	1.9^{DE}	2.9
		65	5.4 ± 0.5	2.1^{DE}	2.8

[a]Values in this column represent the difference between the populations of *Salmonella* Typhimurium DT 104 present on iceberg lettuce surface before and after treatment with a mean initial population of 7.5 log CFU/g (Detection limit was 1.7 log CFU/g; if pathogen was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was 1.6 log CFU/g).
[b]Mean values not followed by the same letter in a column are significantly different ($P < 0.05$).
[c]ND = not detected by direct plating and enrichment (detection limit of 0.3 log CFU/mL).

Table 4–Mean log reduction of *E. coli* O157:H7 on iceberg lettuce after washing treatment.

Water type	Time (min)	Washing speed (rpm)	Recovery (log CFU/g)	Reduction (log CFU/g)[a,b]	Recovery from wash solution (log CFU/mL)[c]
EO water	1	40	6.7 ± 0.4	0.9^{DE}	ND
		65	6.6 ± 0.3	1.0^{D}	ND
	5	40	6.2 ± 0.4	1.4^{CD}	ND
		65	5.7 ± 0.5	1.9^{C}	ND
	10	40	5.6 ± 0.4	2.0^{C}	ND
		65	5.4 ± 0.3	2.2^{BC}	ND
	15	40	5.5 ± 0.5	2.1^{BC}	ND
		65	5.2 ± 0.4	2.4^{B}	ND
	30	40	5.2 ± 0.8	2.4^{B}	ND
		65	4.5 ± 0.4	3.2^{A}	ND
DI water	1	40	7.1 ± 0.4	0.5^{E}	4.6
		65	7.0 ± 0.3	0.6^{E}	4.7
	5	40	6.8 ± 0.8	0.8^{DE}	4.5
		65	6.7 ± 0.4	0.9^{DE}	4.7
	10	40	6.5 ± 0.4	1.1^{D}	4.0
		65	6.5 ± 0.6	0.9^{DE}	4.3
	15	40	6.2 ± 0.8	1.1^{D}	3.4
		65	6.2 ± 0.4	1.1^{D}	3.9
	30	40	6.1 ± 0.4	1.5^{CD}	3.0
		65	5.8 ± 0.6	1.8^{C}	3.2

[a]Values in this column represent the difference between the populations of *E. coli* O157:H7 present on iceberg lettuce surface before and after treatment with a mean initial population of 7.6 log CFU/g. (Detection limit was 1.7 log CFU/g; if pathogen was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was 1.6 log CFU/g).
[b]Mean values not followed by the same letter in a column are significantly different ($P < 0.05$).
[c]ND = not detected by direct plating and enrichment (detection limit of 0.3 log CFU/mL).

authors showed that *E. coli* O157:H7 attached more favorably to damaged tissues of cut edges of lettuce leaves than intact surfaces while *S.* Typhimurium attached similarly to both intact surfaces and damaged cut edges of lettuce leaves. However, in the study by Takeuchi and Frank (2000), it was observed that given a sufficient amount of time (24 h), *E. coli* O157:H7 cells were able to attach to both smooth and damaged tissues of leaves. Takeuchi and Frank (2000) also showed that *E. coli* O157:H7 cells penetrated deeper into leaves when they were held at 4 °C compared with higher temperatures before treatment, providing the cells greater protection from sanitizing solution. In this study, inoculated leaves were held at 4 °C for 24 h and this may explain why higher populations of *E. coli* O157:H7 were recovered after treatment with NEO water since the conditions were suitable to allow leaf penetration, resulting in cells being relatively more difficult to remove from leaf surfaces. Differences in reductions observed for the two types of lettuce may also be attributed to their varied leaf structure, with iceberg lettuce having a higher water content and relatively thinner

Table 5–Mean log reduction of *Salmonella* Typhimurium DT 104 on tomatoes after washing treatment.

Water type	Time (min)	Washing speed (rpm)	Recovery (log CFU/tomato)	Reduction (log CFU/tomato)[a,b]	Wash Solution Recovery (log CFU/mL)[c]
EO water	1	40	2.0 ± 1.6	6.5^{B}	ND
		65	1.8 ± 1.4	6.7^{B}	ND
	5	40	0.9 ± 1.3	7.6^{A}	ND
		65	0.4 ± 1.1	8.0^{A}	ND
	10	40	<0.1	8.5^{A}	ND
		65	<0.1	8.5^{A}	ND
DI water	1	40	3.2 ± 0.5	5.2^{C}	1.9 ± 0.4
		65	3.2 ± 0.3	5.3^{C}	1.8 ± 0.8
	5	40	3.2 ± 0.6	5.3^{C}	4/4
		65	2.8 ± 0.2	5.7^{C}	4/4
	10	40	2.3 ± 1.1	6.2^{BC}	4/4
		65	2.2 ± 1.1	6.2^{BC}	4/4

[a]Values in this column represent the difference between the populations of *Salmonella* Typhimurium DT 104 present on tomato surface before and after treatment with a mean initial population of 8.5 log CFU/tomato (Detection limit was 2.7 log CFU/tomato; if pathogen was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was 2.6 log CFU/tomato).
[b]Mean values not followed by the same letter in a column are significantly different ($P < 0.05$).
[c]ND = not detected by direct plating and enrichment (detection limit of 0.3 log CFU/mL) while 4/4 indicates samples were not detected by direct plating but all 4 samples were positive after enrichment.

leaves than the darker romaine lettuce leaves (Herda 2010). Bacterial cells may thus be able to penetrate further into the leaves of iceberg than romaine, giving better protection to penetrated cells in iceberg lettuce from NEO water. Takeuchi and Frank (2000) were able to show that *E. coli* O157:H7 cells inoculated on iceberg lettuce leaves were observed in the stomata and within the tissues of the leaves.

Increased washing speed or agitation also reduced significantly greater populations of microorganisms ($P < 0.05$) on lettuce leaves, with similar observations reported in other studies of produce wash with different sanitizers as well. Wang and others (2007) reported that increasing the flow velocity and agitation rate of peroxyacetic acid treatment of cantaloupe rind and cup apples improved the rate at which *E. coli* O157:H7 was being reduced on surfaces. It was also shown that agitation increased the efficacy of acetic acid to reduce *E. coli* O157:H7 on the surface of iceberg lettuce by reducing the time needed to achieve a 5-log CFU/g reduction from 10 min without agitation to 5 min when agitated (Vijayakumar and Wolf-Hall 2002). The increased reduction observed in our current study may be because of the increased shear forces as a consequence of increased flow or washing speed (Wang and others 2007). This may have resulted in greater amounts of attached cells on surfaces being detached into NEO water, which then inactivates the pathogens and prevents re-attachment. Agitation also enhances the ability of NEO water to penetrate the surfaces of lettuce to reach bacterial cells that may not have been readily accessible (Park and others 2002). Increased contact time and washing speed of salad washer generally helped improve NEO water efficiency in treating lettuce samples.

Tomatoes

NEO water was very efficient in removing *S.* Typhimurium DT 104 cells on tomato surfaces irrespective of the washing speed (Table 5) with NEO water being significantly more effective than DI water ($P < 0.05$). One min of NEO water treatment resulted in reductions greater than 6 log CFU/tomato for both washing speeds with reductions increasing to 8 log CFU/tomato after 5 min treatment at 65 rpm speed (Table 5). A 10 min treatment led to complete inactivation of the inoculated 8.5 log CFU/tomato *S.* Typhimurium DT 104 cells. DI water treatments of inoculated tomatoes also resulted in high reductions of up to 5.7 and 6.2 log CFU/tomato after 5 and 10 min, respectively. As was the case with lettuce, *S.* Typhimurium DT 104 was completely inactivated in all NEO water wash solutions even after enrichment. *S.* Typhimurium DT 104 was always detected in DI water wash solutions with populations of up to 1.9 log CFU/mL detected after 1 min treatment and detected after enrichment for DI water treatments of 5 and 10 min (detection limit 0.3 log CFU/mL).

Reductions for *E. coli* O157:H7 on tomato surfaces (Table 6) were generally lower than reductions observed for *S.* Typhimurium DT 104 (Table 5) after both NEO water and DI water treatments. One min of treatment resulted in reductions greater than 3 log CFU/tomato for both washing speeds with the highest reductions (6.8 log CFU/tomato) observed after the 10 min treatment with NEO water at 65 rpm speed which was significantly greater than the 40 rpm treatment at that same time and for all other treatments (Table 6). DI water treatments of inoculated tomatoes also led to reductions between 1.7 to 3.5 log CFU/tomato after 1 and 10 min treatments, respectively. As was the case with *S.* Typhimurium DT 104, *E. coli* O157:H7 was completely inactivated in all NEO water wash solutions (non-detectable after enrichment). For DI water treatments, up to 3.9 log CFU/mL of *E. coli* O157:H7 was recovered in wash solutions after the 1 min treatment. After 5 and 10 min of treatment, the pathogen was detected after enrichment in all 4 samples.

Tomato results were comparable with other reported findings from other studies. Pangloli and Hung (2011) found that *E. coli* O157:H7 reductions after 8 and 15 s washes with slightly acidic EO water were as high as between 5.4 and 7.6 log CFU/tomato. Washing with running EO water also reduced *E. coli* O157:H7 by 7.9 log CFU/tomato (Pangloli and others 2009) and *Salmonella* by up to 7.7 log CFU/g. In the case of NEO water (89 mg/L active chlorine, pH 8), reductions of up to 4.92 log CFU/cm^2 and 4.3 log CFU/cm^2 after 60 s of immersion for *E. coli* O157:H7 and *S. enteritidis* were observed (Deza and others 2003). For the same time periods of treatment in this study, reductions for all pathogens on tomato surfaces were always higher (Table 5 and 6) than those observed on lettuce surfaces (Table 1 to 4). This may be attributed to the differences in the nature of their surfaces, with tomatoes having a relatively smoother surface when compared with lettuce

Table 6–Mean log reduction of *E. coli* O157:H7 on tomatoes after washing treatment.

Water type	Time (min)	Washing speed (rpm)	Recovery (log CFU/tomato)	Reduction (log CFU/tomato)[a,b]	Wash Solution Recovery (log CFU/mL)[c]
EO water	1	40	5.7 ± 0.8	3.0^{CD}	ND
		65	5.1 ± 0.9	3.6^{C}	ND
	5	40	5.0 ± 0.8	3.7^{C}	ND
		65	4.3 ± 0.5	4.4^{B}	ND
	10	40	4.1 ± 0.7	4.6^{B}	ND
		65	1.9 ± 1.5	6.8^{A}	ND
DI water	1	40	7.1 ± 0.6	1.7^{E}	3.5
		65	6.8 ± 0.3	1.9^{E}	3.9
	5	40	6.9 ± 0.3	1.8^{E}	4/4
		65	6.1 ± 0.6	2.6^{D}	4/4
	10	40	5.9 ± 0.5	2.8^{D}	4/4
		65	5.2 ± 0.3	3.5^{C}	4/4

[a]Values in this column represent the difference between the populations of *E. coli* O157:H7 present on tomato surface before and after treatment with a mean initial population of 8.7 log CFU/tomato (Detection limit was 2.7 log CFU/tomato; if pathogen was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was 2.6 log CFU/tomato).
[b]Mean values not followed by the same letter in a column are significantly different ($P < 0.05$).
[c]ND = not detected by direct plating and enrichment (detection limit of 0.3 log CFU/mL) while 4/4 indicates samples were not detected by direct plating but all 4 samples were positive after enrichment.

leaves which have folds and crevices that bacterial cells can adhere to, thus preventing inactivation by sanitizing solution (Takeuchi and others 2000).

Conclusions

The use of an automated produce washer along with NEO water led to significant reductions of populations of *S.* Typhimurium DT 104 and *E. coli* O157:H7 on tomatoes and lettuce. Increased washing speed and agitation resulted in greater reductions on lettuce and the treatment was generally more effective in reducing pathogens on tomato surfaces. This treatment procedure can therefore be incorporated in food service kitchens to ensure the safety of produce items.

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