ARTICLES

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Efficacy of Various Consumer-Friendly Produce Washing Technologies in Reducing Pathogens on Fresh Produce

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ABSTRACT

Disease outbreaks associated with consumption of fresh produce have led to increased demand for technologies that can be used in the home kitchen to decrease pathogen exposure. Produce treatment technologies currently being marketed to consumers include use of electrolyzed oxidizing (EO) water, ozone, and commercial vegetable wash (food-grade soap). In this study, we determined the ability of these technologies, along with chlorine bleach (70 ppm free chlorine) and running tap water, to remove *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* inoculated onto tomatoes, broccoli, cantaloupe, lettuce, spinach, and green onions. Some treatments were more effective than running tap water for specific pathogen-produce combinations, but no treatment produced greater reductions than tap water for all tested combinations. EO water treatment exhibited more consistent effectiveness than the ozone, vegetable wash or tap water and was more effective than chlorine for treating lettuce but less effective than chlorine for treating cantaloupe. The degree of pathogen reduction achieved with the consumer-friendly technologies (1 to 3 log decrease) was similar to reductions achieved in studies using laboratory-generated active agents.

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INTRODUCTION

Consumption of fresh fruits and vegetables in the United States is increasing *(18)*. This has resulted in an increased number of foodborne outbreaks associated with fresh produce. Pathogen contamination of fresh produce can occur at numerous points in the farm-to-fork continuum, including in the field, during packing and shipping, or during storage *(9, 12, 23, 24, 41, 47, 48)*. Over the past six years, the FDA has reported 14 multi-state bacterial outbreaks associated with fresh produce, and in 2011 alone there were 41 recalls of fresh produce due to bacterial contamination *(49)*. The main bacterial pathogens of concern include *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. These pathogens have been linked to outbreaks involving lettuce, spinach, cabbage, peppers, apple cider, alfalfa sprouts, tomatoes, papayas, and cantaloupes *(13–17, 19–21)*.

As outbreaks continue to occur, the adequacy of consumer washing of fresh produce under running tap water has come into question. This has led to the development of home use washing and decontamination treatments. Although the most common method of washing

TABLE 1. *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enterica* strains used in study

Strain	Reference Number	Source
L. monocytogenes	LCDC	Cabbage associated outbreak
L. monocytogenes	G3982	Clinical isolate from a Jalisco cheese outbreak
L. monocytogenes	Scott A	Human isolate
L. monocytogenes	LM254	From drain of chicken processing plant
L. monocytogenes	LM311	From raw chicken product
E. coli O157:H7	H1730	Lettuce associated outbreak
E. coli O157:H7	F4556	Alfalfa sprouts associated outbreak
E. coli O157:H7	#994	Salami isolate
E. coli O157:H7	SEA13B88	Apple juice associated outbreak
E. coli O157:H7	CDC-658	Cantaloupe associated outbreak
S. Baildon	Not Available	Tomato associated outbreak
S. Montevideo	G4639	Tomato associated outbreak
S. Poona	01A3923	Cantaloupe associated outbreak
S. Stanley	H1256	Alfalfa sprouts associated outbreak
S. Typhimurium	DT104:H3380	Clinical isolate

fresh produce in the home remains rinsing under running tap water, treatments such as aqueous ozone, electrolyzed oxidizing water, commercial produce washes, and dilute chlorine bleach have been proposed for home use.

Aqueous ozone is considered generally recognized as safe (GRAS) and is able to inactivate a broad spectrum of microorganisms, including Gram-negative bacteria, Gram-positive bacteria, spores, fungi, viruses, and parasites. Ozone is a naturally occurring molecule that decomposes into oxygen, leaving no residues *(26, 32)*. Ozone generators have been designed specifically for treating fresh produce at home.

Electrolyzed oxidizing (EO) water is created by passing a dilute salt solution through an electric current. This produces water with a high oxidation reduction potential (ORP), low pH, and free chlorine. The low pH of EO water is not harmful to the skin, but, in combination with the high ORP and free chlorine, is effective in inactivating a wide range of microorganisms, including Gram-negative bacteria, Gram-positive bacteria, fungi, viruses, algae, and protozoa *(28, 31, 43)*. There are EO generators specifically designed for home use, although they are not currently available in the United States.

Commercial produce washes, widely available for consumer use, are typically made from natural oils and surfactants and are promoted for their cleaning ability. They may enhance soil removal during rinsing but are usually not formulated to have disinfection efficacy *(43)*.

Chlorine-based sanitizers are commonly used to treat water for industrial-scale washing of fresh produce. The maximum allowable level for food contact without a rinse step is 200 ppm available chlorine. The average treatment is 1 to 2 minutes in a 50 to 200 ppm solution *(38, 44)*. Although chlorine-based sanitizers may provide greater reduction of pathogen levels on fresh produce than water alone, they are not recommended for use in the home because of the potential toxicity of hypochlorite, if misused.

Previous work on the efficacy of antimicrobial wash treatments for fresh produce has been directed toward industrial applications. This study aims to provide an evaluation of the efficacy of produce washing treatments designed for consumer use. The treatments to be evaluated include ozonated water, electrolyzed oxidizing water, a commercial produce wash, and running tap water. Treatment with diluted chlorine bleach is also included in this study for comparison purposes. These treatments were evaluated for removal of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on tomatoes, broccoli, cantaloupe, lettuce, spinach, and green onions.

MATERIALS AND METHODS

Bacterial strains

Five strains each of *Salmonella, E. coli* O157:H7 and *L. monocytogenes* were used in this study. The list of strains, reference numbers, and sources are presented in Table 1. Prior to use, each strain was made resistant to 100 µg/mL rifampicin (Sigma-Aldrich Chemical Co., St. Louis, MO), to allow for consistent recovery of the inoculated pathogens in the presence of competing microflora *(10, 22)*. The concentration of 100 µg/mL rifampicin was selected because this was the lowest concentration that completely inhibited growth of background microflora and allowed growth of resistant mutants *(35, 42)*. Sufficient rifampicin was added to tryptic soy agar (TSA; Difco Laboratories, Becton, Dickinson and Company Sparks, MD) to achieve a final concentration of 100 µg/mL rifampicin. Initial stock wild-type cultures were streaked onto rifampicin-TSA (TSA-R100), incubated at 37°C for 24 h, and then checked for spontaneous mutations. Mutated isolates were tested for species identity,

TABLE 2. Initial and post-treatment physicochemical properties of test solutions

Treatment	Initial Temperature (°C)[a]	ORP (mV)		Free Chlorine Concentration (ppm)		Initial pH
		Initial	Post-treatment	Initial	Post-treatment	
Ozone[b]	14.6 ± 1.0	ND[c]	ND	ND	ND	4.92 ± 0.23
Bleach	14.5 ± 0.6	648.1 ± 4.2	6.47 ± 29.4	71 ± 5	70 ± 4	NA
EO Water	16.0 ± 1.0	1097 ± 21	1092 ± 22	13 ± 5	10 ± 3	2.89 ± 0.13
Veggie Wash	14.3 ± 0.8	ND	ND	ND	ND	ND
Running Tap	23 ± 1.0	ND	ND	0-1	ND	ND

[a]n = eighteen replications

[b]Initial ozone concentration: 0.75 ± 0.4 mg/L

[c]ND, not determined

growth similar to that of the wild-type strain, and growth in the absence of antibiotic *(10, 46)*. Stock cultures were maintained at -80ºC on Microbank™ Bacterial and Fungal Preservation System beads (Pro-Lab Diagnostics, Round Rock, TX).

Preparation of inoculum

Prior to use, each strain was subcultured at least twice in tryptic soy broth (TSB; Difco) containing 100 µg/mL rifampicin (TSB-R100) at 37ºC for 24 h. Each strain was centrifuged at 4,345 x g and 15°C for 15 minutes (Beckman Coulter Allegra 21R Refrigerated High Speed Table Top Centrifuge). The pellet was then washed once in 0.1% peptone water (PW; Difco) and resuspended in PW, using an absorbance standard obtained with between 10^9 and 10^{10} CFU/mL of each strain. Equal volumes (1 mL) of each culture were combined to form a five-strain cocktail. The cell concentration of the cocktail was determined by plate count, using TSA-R100 incubated for 24 h at 37ºC.

Produce

Uncoated tomatoes (*Solanum lycopersicum*; 142 – 265 g, mean weight 198 g), broccoli (*Brassica oleracea*; 50 – 129 g; mean weight 92 g), cantaloupes (*Cucumismelo* var. cantalupensis; 1324 – 1947 g; mean weight 1558 g), romaine lettuce (*Lactuca sativa* var. longifolia; 0.30 – 0.55 g; mean weight 0.47 g (cut to size), spinach (*Spinacia oleracea*; 0.55 – 0.90 g; mean weight 0.71 g), and green onions (*Allium fistulosum*; 0.45 – 0.85 g; mean weight 0.61 g) were purchased at retail and stored at 4ºC for no longer than 7 days. Each item was inspected to ensure that no rotten or damaged tissue was present. Prior to inoculation, each piece of produce was rinsed under running deionized water (DW) for 15 seconds to remove soil. Following rinsing, the lettuce, spinach, and green onions were dried by use of a ratchet salad spinner (Progressive International® Corp, Kent, WA). Three pieces of one type of produce were treated together and considered one replication, with three replications per treatment. Weights equivalent to two serving sizes (170 g of lettuce, 170 g of spinach, 50 g of green onions) were included in treatments, in addition to the inoculated item, when lettuce, spinach, and green onions were evaluated.

Produce inoculation

Produce was inoculated by the procedure described by Beuchat et al. *(11)*. Briefly, tomatoes were inoculated with 50 µl of the bacterial cocktail in 10 spots around the blossom scar, but avoiding the scar itself. Broccoli was inoculated with 50 µl of the bacterial cocktail in 10 spots on the floret. A 5 cm x 5 cm square was marked on the cantaloupe with a permanent marker and was then inoculated as previously described *(11)*. Lettuce leaves for analysis were trimmed into pieces (ca. 4.5 cm x 4.0 cm), using a sterile carbon steel surgical blade (REF 4-121, Miltex® Inc., York, PA), while the rest of the leaves were kept intact. The trimmed leaves were inoculated with 50 µl of the bacterial cocktail in 10 spots on the abaxial surface of each leaf *(11)*. Spinach leaves for analysis were trimmed to remove stalks and inoculated as previously described. The inoculated leaves were marked with a red dye (Testors® 1103 Enamel Paint Red) in order to allow the inoculated leaves to be distinguished from the rest of the leaves during treatment. Roots and peels of the green onions were removed, and the remaining hollow upper green tissues were trimmed and inoculated as previously described. The produce was then left to dry in a laminar flow class II biosafety hood at 22ºC for 1 h to allow for attachment *(10, 11, 34, 39)*. The produce was inoculated no earlier than 2 hours prior to use. The bacterial cocktail was held at 4ºC between inoculations.

Treatments

Five treatments were tested to determine efficacy: ozonated water, electrolyzed oxidizing water, dilute chlorine bleach water, a commercial produce wash, and running tap water. All treatments were applied at 15°C ± 2°C. For all treatments, pH was determined by using a Thermo Scientific Orion combination pH electrode on an Orion 3-Star Plus Benchtop pH/mV Meter (Thermo Scientific, Beverly, MA), and temperature was determined by using a Fisher Scientific Traceable® Memory/Waterproof Thermometer (Fisher Scientific).

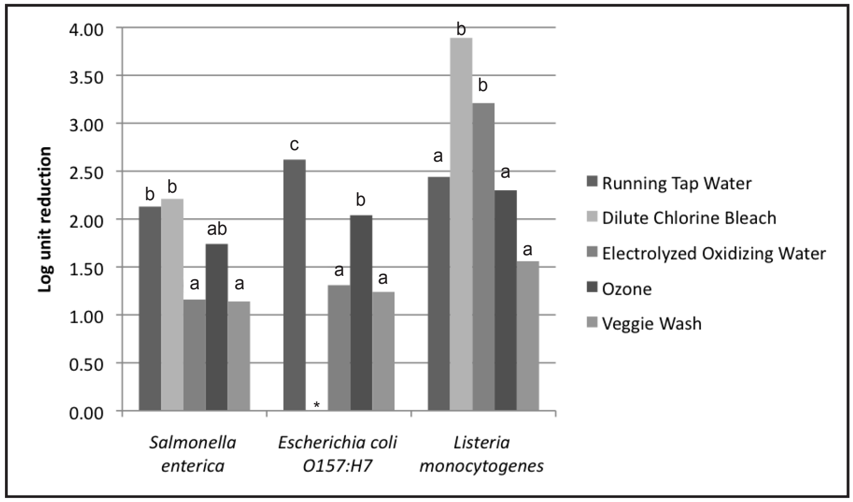
Ozonated water was produced by using the Lotus Sanitizing System (Model LSR 100, Tersano Int., Buffalo, NY). Initial ozone concentrations were determined via the Hach Indigo Colorimeter method (Hach Co., Loveland, CO). The pH was adjusted to 5 by adding distilled white vinegar (0.5% acidity, Publix®, Lakeland, FL) in order to dissolve the ozone more efficiently.

Electrolyzed oxidizing (EO) water was produced by use of a Bion-Tech EO generator (BTM-3000, Bion-Tech Co., Seoul, South Korea). The company provided a standardized scoop that held 0.85 g of table salt (NaCl; Publix® Table Salt), which is added to produce acidic EO water. Treatment water and salt were added to both chambers of the unit to obtain a final salt concentration of 0.043% (w/v). After a generation time of 20 min, 2 L of water was collected from the acidic chamber. Acidic EO water (4 L) was generated on each test day, no earlier than 1 h prior to treatment. Oxidation reduction potential was determined by using an Epoxy Sure-Flow Combination Redox/ORP Electrode with the Orion meter (Fisher Scientific). Free chlorine concentration was determined by using an Iodine-Chlorine Kit (#101; Ecolab Center, St. Paul, MN).

Dilute chlorine bleach water was prepared by combining a sufficient amount (approximately 5 ml) of household bleach (containing 6% sodium hypochlorite, Clorox Co., Oakland, CA) with 3.785 L (one gallon) of treatment water to obtain approximately 70 ppm free chlorine. Free chlorine and ORP were determined as previously described.

Veggie Wash® (Beaumont Products, Inc., Kennesaw, GA) was prepared according to the manufacturer's directions. Produce wash solution (60 ml) was added to 3.785 L of treatment water and thoroughly mixed to ensure homogeneity.

Figure 1. Log reduction of bacterial pathogens on tomatoes treated using home use washing technologies. Initial pathogen counts ranged from 7.5 to 8.0 log CFU/tomato. Bars within one pathogen with same letter are not significantly different ($P < 0.05$). *Reduction of *E. coli* O157:H7 by bleach not shown on graph—see Table 3 for data.



Treatment exposure

Ozonated water was used immediately after preparation to ensure that the concentration was at the maximum level during treatment. For cantaloupe, tomatoes, and broccoli, DW water (3.785 L) was added to the sterilized bowl and vegetable retainer system provided with the equipment. For leafy greens and green onions, water was added to achieve a ratio of 12 times the amount of water per weight of produce being treated, e.g., 170 g of lettuce was immersed in 2040 mL of solution. This process eliminated the effect of treatment solution amount on the results, as these vegetables were treated in quantities equivalent to two servings rather than equivalent weights. The produce items were added to the water and submerged using the retainer sold with the unit, after which the unit was started. The cycle took approximately 3 min until the digital display reached 100%, followed by a 2-min hold period. For tomatoes and cantaloupe, 3 L DW was used because these items displaced too much water to allow use of the manufacturer recommended 3.785 L. Because the ozone apparatus could not contain three cantaloupes at once, each cantaloupe was treated separately. The water was reused, but ozone was generated for each cantaloupe. Within 30 s of the end of the hold period, the produce items were removed from the water with sterilized tongs and immediately placed into a Whirl-Pak bag (710 mL size, Nasco, Fort Atkinson, WI) containing 50 mL of sterile neutralizing buffer. The neutralizing buffer used in this study was formulated to provide the concentration of chlorine neutralizing agents in Dey-Engley broth: 1 g sodium thioglycolate (Sigma Aldrich), 6 g sodium thiosulfate (Acros Organics, Beverly, MA), and 2.5 g sodium bisulfite (Acros Organics) were added to 1 L of DW. EO water was prepared no earlier than 1 h prior to use. For cantaloupe, tomatoes, and broccoli, treatment water (3.785 L) was added to a sterilized 5.678 L stainless steel bowl. For leafy greens and green onions, the amount of water used was based on a 1:12 produce weight to water ratio as previously described. The produce was submerged for 2 min by covering with a sterile Ziploc freezer bag (Double zipper freezer bags, S.C. Johnson, Racine, WI) containing DW. The produce then was removed and placed in the neutralizing buffer. Dilute chlorine bleach water was prepared immediately prior to use to ensure maximum free chlorine levels. The test items were treated with the dilute chlorine bleach by submersion, as previously described.

The commercial produce wash was prepared immediately prior to use and the test items were treated as previously described. For the tap water treatment, tap water was run continuously over the produce at a rate of approximately 2 L/min for 15 s. The produce was positioned under the running tap water so that the inoculated section was neither directly hit by the water nor positioned

TABLE 3. Frequency of *Escherichia coli* O157:H7 recovered from tomatoes after treatment with chlorine bleach diluted to 71 ppm free chlorine.[a] Initial counts ranged from 7.5 to 8.0 log CFU/tomato

Mean *E. coli* O157:H7 Population		
Number of Tomatoes[b]		
Below Detection Limit (2 log CFU/sample)	Between 2 and 4.5 log CFU/sample	Above 4.5 log CFU/sample
6	12	3

[a]No pathogens detected in any residual treatment solution by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

[b]n = seven replicates with three tomatoes per replicate

where the water ran off the produce item. Following treatment, the produce was neutralized as previously described.

Microbial analysis

Following neutralization, produce items (including untreated inoculated controls and uninoculated controls) were either macerated, shaken, or hand rubbed for 1 min to separate microorganisms from the tissue. Tomatoes were hand rubbed for 1 min *(10, 34)*. The marked portion of the rind of cantaloupes was excised with a flame-sterilized scalpel *(2, 3, 7)*. The excised rind was checked to ensure that all remaining meat was removed, returned to the Whirl-Pak bag, and rubbed by hand for 1 min *(2)*. One person was responsible for rubbing all tomatoes and cantaloupe skins in each experiment to reduce experimental variation. Broccoli was stomached for 1 min in a Stomacher 400 (Tekmar Co., Cincinnati, OH) at the normal speed setting prior to analysis *(5, 30)*. Lettuce and spinach were macerated by hand for 1 min. Green onions were shaken rather than macerated for 1 min to avoid the disruption of cells and the release of antimicrobials from the tissue. The rinse solution was then tested for pathogens. This solution was serially diluted (1:10) in PW and then plated in duplicate on TSA-R100. *Salmonella* and *E. coli* O157:H7 treatment plates were incubated at 37°C for 24 h prior to enumeration, while *L. monocytogenes* treatment plates were incubated at 37°C for 48 h prior to enumeration. Presumptive colonies were randomly selected by the Harrison's disc method *(27)* and streaked on Xylose Lysine Desoxycholate (XLD; Difco), MacConkey II Agar with Sorbitol (SMAC; Difco), or Oxford agar (OX; Difco) to confirm presence of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Selective media were incubated at 37°C for 24 h for colony confirmation.

Residual treatment water

Residual treatment water was tested for the presence of pathogens. Treatment water (1 mL) was serially diluted (1:10) in PW and plated in duplicate on TSA-R100. For treatments resulting in low bacterial counts, 1 mL of treatment water was added to 9 mL of TSB-R100 and incubated at 37°C for 48 h for enrichment. Positive enrichments were confirmed by streaking on TSA-R100.

Statistical analysis

Data obtained from analysis of three produce items were averaged to generate one replication. Three replications were performed per treatment, with replicate data being obtained on different days. Microbial data (CFU/sample) were analyzed after log transformation. Data were analyzed separately for each produce item by use of Analysis of Variance, and means were separated using Tukey's comparison method (significant level of $\alpha = 0.05$). Statistical analysis was accomplished by use of Minitab® Statistical Software (Minitab Inc., State College, PA).

RESULTS

Treatment solutions

Properties of the treatment solutions are presented in Table 2. These data indicate that treatment solutions with efficacy based on active chlorine (EO water and bleach) maintained residual active chlorine after produce treatment.

Tomatoes

Results from tomato decontamination treatments are presented in Figure 1. All treatments on tomatoes produced a significant ($P < 0.05$) reduction in pathogen levels compared with levels of the untreated inoculated samples. Running tap water produced 2.13, 2.62, and 2.44 log unit reductions for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Treatments with ozone and the commercial produce wash were either of similar effectiveness or less effective than running tap water. EO water was more effective than running tap water only for one pathogen, *L. monocytogenes*, reducing the level of this pathogen on the tomatoes by 3.21 log units. Dilute chlorine bleach produced a moderate reduction in *Salmonella* of 2.21 log units, but this was not significantly better than that achieved with running tap water. However, dilute chlorine bleach was more effective than running tap water at reducing levels of *L. monocytogenes* (3.21 log unit reduction). Treatment of tomatoes with dilute chlorine bleach produced inconsistent results in reducing *E. coli* O157:H7. For the 7 replications, which included 21 tomatoes, the treatment reduced *E. coli* O157:H7 to below the detection limit (2 log CFU/tomato) for 6 tomatoes, between 2 and 4.5 log CFU/tomato were recovered from 12 tomatoes, and greater than 4.5 log CFU/tomato were recovered from 3 tomatoes (Table 3).

Similar results were obtained with the three pathogens for most of the tomato treatments. However, the EO water treatment was approximately 2 log units more effective at removing *L. monocytogenes* than at removing *E. coli* O157:H7 or *Salmonella*. The dilute chlorine bleach treatment was 1.68 log units more effective at removing *L. monocytogenes* than *Salmonella* on tomatoes.

Figure 2. Log reduction of bacterial pathogens on broccoli treated using home use washing technologies. Initial pathogen counts ranged from 8.6 to 8.7 log CFU/broccoli. Bars within one pathogen with same letter are not significantly different (P < 0.05).

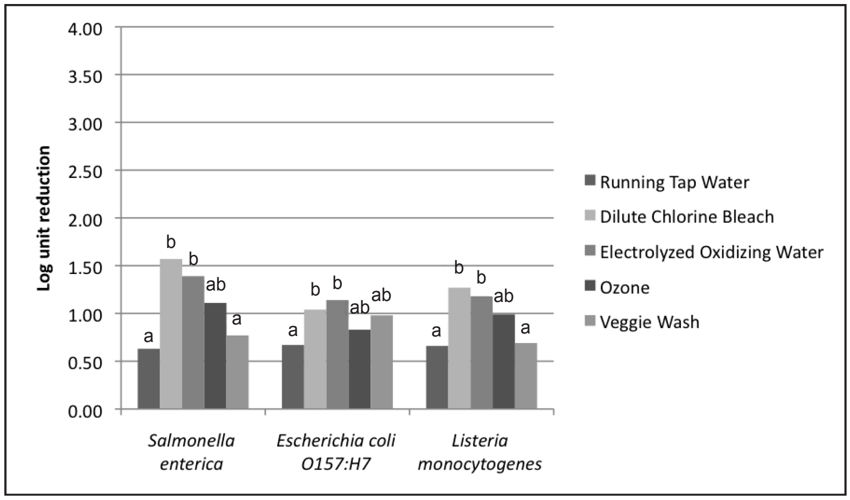
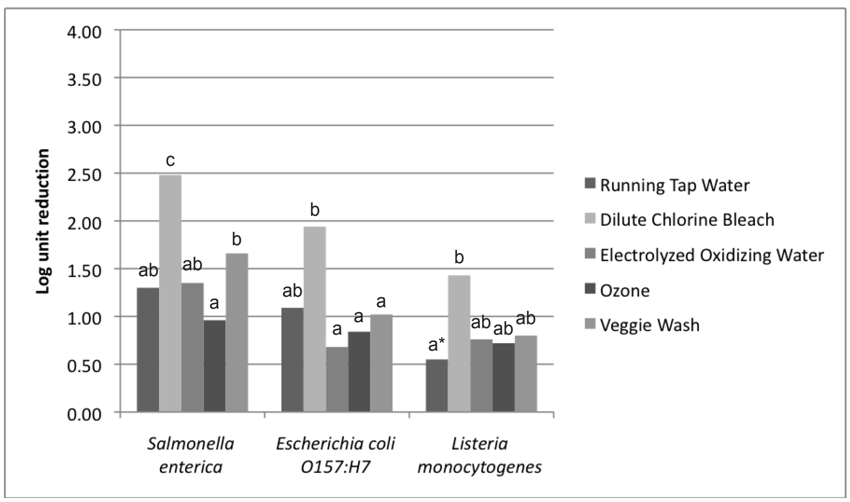


Figure 3. Log reduction of bacterial pathogens on cantaloupes treated using home use washing technologies. Initial pathogen counts ranged from 7.4 to 8.0 log CFU/cantaloupe. Bars within one pathogen with same letter are not significantly different (P < 0.05). **L. monocytogenes* running tap water did not produce pathogen counts significantly different from the initial pathogen count.



Broccoli

Results from broccoli decontamination treatments are shown in Figure 2. All treatments on broccoli produced a significant (P < 0.05) reduction in pathogen levels compared to the untreated inoculated samples. Running tap water produced reductions of 0.63–0.67 log units in pathogen levels. Treatments with dilute chlorine bleach and EO water, while providing reductions greater than running tap water, reduced counts of *Salmonella* by only 1.57 and 1.39 log units, and were even less effective at removing *E. coli* O157:H7 and *L. monocytogenes* from broccoli. Ozone and the commercial produce wash produced results similar to running tap water for all the pathogens present on broccoli.

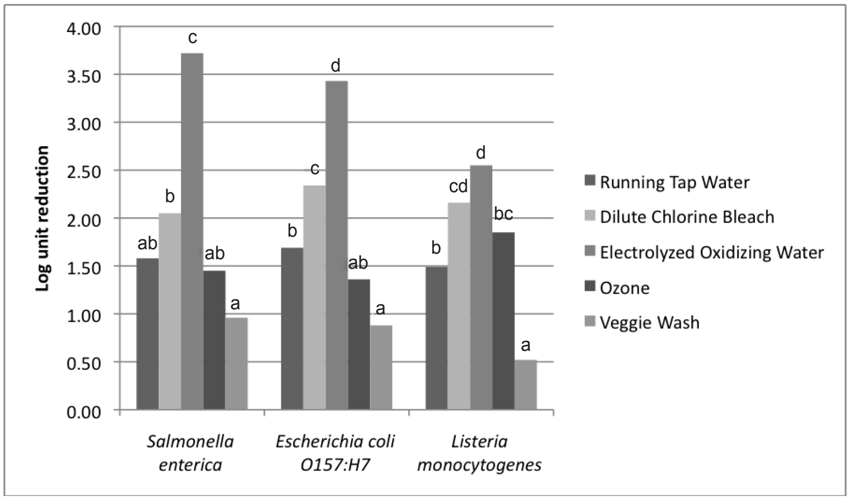
Cantaloupe

Results from cantaloupe decontamination treatments are shown in Figure 3. All treatments on cantaloupes produced a significant (P < 0.05) reduction in *Salmonella* and *E. coli* O157:H7 levels, compared with levels on the untreated inoculated samples. Running tap water produced a 1.30 log unit reduction in *Salmonella*, a 1.09 log unit reduction in *E. coli* O157:H7, and a 0.55 log unit reduction in *L. monocytogenes*. This 0.55 log unit reduction in *L. monocytogenes* produced by running tap water was not significantly different from the untreated inoculated controls. EO water, ozone, and the commercial produce wash provided reductions similar to running tap water for these pathogens. Dilute chlorine bleach provided a moderate reduction of *Salmonella*, reducing counts by 2.48 log units but producing lower reductions in counts of *E. coli* O157:H7 and *L. monocytogenes* (1.94 and 1.43 log units, respectively). These lower reductions were not significantly different from those achieved by running tap water. The decontamination treatments did not, in general, produce different results for the three pathogens when these were present on cantaloupe.

Lettuce

Results from lettuce decontamination treatments are shown in Figure 4. All treatments on lettuce produced a significant (P < 0.05) reduction in pathogen levels. Running tap water produced similar reductions in *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* (1.58, 1.69, and 1.49 log units, respectively). Ozone produced pathogen reductions similar to those produced by running tap water. The commercial produce wash treatment was less effective than running tap water at removing *E. coli* O157:H7 and *L. monocytogenes* and similar to running tap water in removing *Salmonella*.

Figure 4. Log reduction of bacterial pathogens on lettuce treated using home use washing technologies. Initial pathogen counts ranged from 7.0 to 7.1 log CFU/ lettuce. Bars within one pathogen with same letter are not significantly different ($P < 0.05$).



Dilute chlorine bleach reduced *Salmonella* by 2.05 log units on lettuce, but this reduction was not significantly different from that produced by the running tap water treatment. However, dilute chlorine bleach was more effective at reducing levels of *E. coli* O157:H7 and *L. monocytogenes* than running tap water, providing reductions of 2.34 and 2.16 log units, respectively. Treatment with EO water was more effective than running tap water at removing all pathogens on lettuce, providing reductions of 3.72 log units for *Salmonella*, 3.43 log units for *E. coli* O157:H7 and 2.55 log units for *L. monocytogenes*.

Spinach

Results from spinach decontamination treatments are shown in Figure 5. Treatments on spinach produced a significant ($P < 0.05$) reduction in *E. coli* O157:H7 and *L. monocytogenes* levels compared with the untreated samples, with the exception of the inability of the produce wash treatment to significantly reduce levels of *L. monocytogenes*. Running tap water provided approximately 1.0 log unit reductions for all pathogens tested. Dilute chlorine bleach and EO water did not produce additional pathogen reductions over those achieved by running tap water. Ozone and the commercial produce wash were less effective than running tap water in removing the pathogens from spinach.

Green onions

Results from green onion decontamination treatments are shown in Figure 6. All treatments on green onions produced a significant ($P < 0.05$) reduction in pathogen levels compared with the untreated inoculated samples. Running tap water produced a 1.0 log unit reduction in *Salmonella* and *L. monocytogenes* levels and a 1.45 log unit reduction in *E. coli* O157:H7. The commercial produce wash produced pathogen reductions similar to those produced by tap water. Dilute chlorine bleach, EO water, and ozone were all more effective than running tap water, reducing pathogen levels by more than 2 log units; EO water was the most effective treatment, reducing levels of *E. coli* O157:H7 by 3.1 log units and *L. monocytogenes* by 3.59 log units on green onions.

Pathogens in residual treatment water

No *Salmonella*, *E. coli* O157:H7 or *L. monocytogenes* were detected by enrichment in the residual treatment water of the EO or dilute chlorine bleach treatments after any of the produce treatments. Viable pathogens (*Salmonella* and *L. monocytogenes*) were detected in ozone post-treatment wash water by using enrichment in 1 of 3 replicates for broccoli, cantaloupe and spinach, but not for tomatoes, lettuce, or green onions. The residual wash liquid from all produce treatments using commercial produce wash contained viable pathogens ranging from 2.47 log CFU/mL to 4.97 log CFU/mL. The commercial produce wash is not promoted as being antimicrobial.

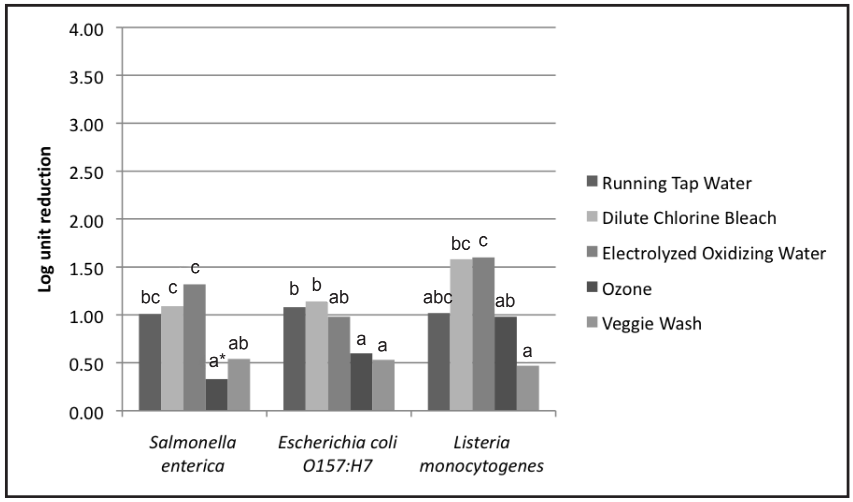
DISCUSSION

Although many studies have examined the ability of produce washing treatments to remove pathogens, the majority of those studies focused on industrial applications. This study determined the efficacy of various washing techniques promoted for at-home consumer use. Technologies using ozone, electrolyzed oxidizing water, and food-grade cleaning agents have been developed in consumer-friendly formats and therefore merit investigation.

Rinsing in dilute chlorine bleach was generally the most effective treatment for pathogen removal. This treatment was most effective for tomatoes, cantaloupe inoculated with *Salmonella*, lettuce, and green onions, with reductions ranging from 2.05 to 3.89 log units; in contrast, for broccoli, cantaloupe inoculated with *E. coli* O157:H7 and *L. monocytogenes*, and spinach, pathogen reductions were less, ranging from 1.04 to 1.94 log units. These results are similar to those of Behrsing et al. *(8)* who treated broccoli and lettuce inoculated with *E. coli* O157:H7 with a 100 ppm chlorine solution and observed a reduction of 2.5 log units and 2.7 log units, respectively. Albrecht et al. *(1)* also evaluated treatment of broccoli with a 50 ppm chlorine solution and observed a reduction in coliform populations of approximately 1 log unit. Pirovani et al. *(40)* investigated background populations on fresh-cut spinach and found a reduction of 2.4 log units when the spinach was soaked in a 75 ppm chlorine solution for 2 minutes. Zhuang et al. *(50)* determined the reduction of *Salmonella* on tomatoes that were soaked in a 50 ppm or 100 ppm chlorine solution for 2 minutes and found a reduction of 0.8 and 1.4 log units, respectively. Baur et al. *(6)* found that washing lettuce in a 100 ppm chlorine solution caused a reduction in the background microflora of approximately 0.7 to 1.5 log units. Results of treating tomatoes inoculated with *E. coli* O157:H7 with dilute chlorine bleach were variable. Six of the 21 replications produced counts below the detection limit. However, the average reduction in *E. coli* O157:H7 level for this treatment was 4.37 log units (Table 4). Free chlorine levels did not decrease significantly during treatment (Tables 2 and 3), because of the high proportion (12:1 by weight) of wash solution per produce item treated.

The EO water treatment was also generally effective, although results were pathogen and produce dependent. This treatment was more effective on tomatoes, lettuce, and green onions, with reductions ranging from 1.16 to 3.72 log units, than on broccoli, cantaloupe, and spinach, which exhibited reductions ranging from 0.68 to 1.60 log units. These results are similar to Hung et al. *(29),* who tested *E. coli* O157:H7 on strawberries and broccoli and found a reduction of 1.28 log units and of 1.78 log units, respectively. The results contrast with those of Bari et al. *(4),* who found a 7.7 log unit reduction in *E. coli* O157:H7, a 7.4 log unit reduction in *Salmonella*, and a 7.6 log unit reduction in *L. monocytogenes* on tomatoes after washing in EO water for 20 seconds. Park et al. *(39)* also observed a marked reduction in *E. coli* O157:H7 and *L. monocytogenes* on lettuce. Their results showed a 4.2 log unit reduction of *E. coli* O157:H7 and a 3.9 to 4.4 log unit reduction in *L. monocytogenes* after exposure for up to 3 minutes. Pangloli et al. *(37)* observed a range of reductions in their produce treatments with EO water. For a 30-second wash, they found a 1.6, 3.0, 4.7 log unit reduction of *E. coli* O157:H7 in lettuce, cabbage, and lemons, respectively. For a 15-second wash on tomatoes, they found a 7.4 log unit reduction in *E. coli* O157:H7. The EO water used by Bari et al. *(4)*, Park et al. *(39)*, and Pangloli et al. *(37)* had free chlorine levels of 30 to 45 ppm, while the EO water used in this study had a free chlorine level of 13 ± 5 ppm. This indicates that the chlorine concentration of the EO water may be an important factor in the effectiveness of this treatment. It is possible that newer versions of EO water generators designed for consumer use will produce higher levels of free chlorine and therefore be more effective. The decrease in chlorine concentration of the EO water during treatment was slight with a beginning concentration of 13 ± 5 ppm and a final concentration of 10 ± 3 ppm free chlorine.

Figure 5. Log reduction of bacterial pathogens on spinach treated using home use washing technologies. Initial pathogen counts ranged from 6.9 to 7.3 log CFU/spinach. Bars within one pathogen with same letter are not significantly different (*P* < 0.05). **Salmonella* counts with use of ozone were not significantly different from the initial pathogen count.

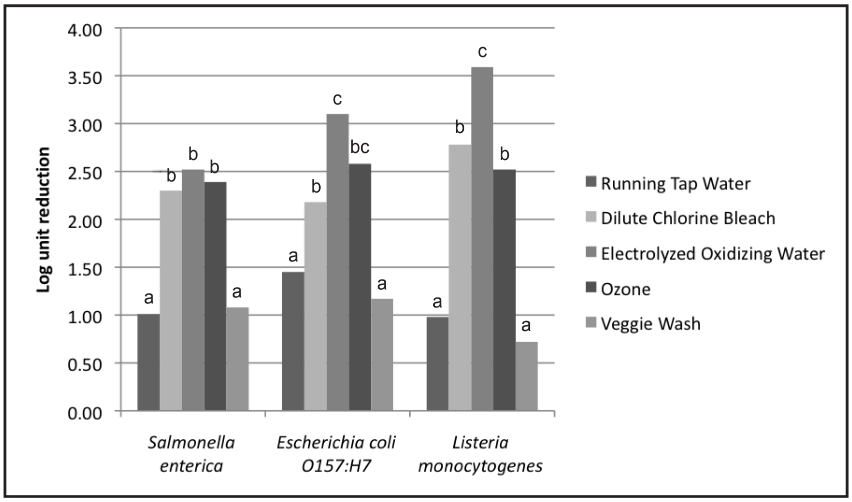


The efficacy of the consumer-friendly ozone treatment differed, depending on the pathogen and produce item combination. This treatment was more effective on tomatoes, lettuce, and green onions, with reductions ranging from 1.36 to 2.58 log units, than on broccoli, cantaloupe, and spinach, which had reductions ranging from only 0.33 to 1.11 log units. These results are similar to those of Singh et al. *(45)*, who treated *E. coli* O157:H7 inoculated lettuce in 200 mL of 10 mg/L aqueous ozone for 10 minutes and found a reduction of 2.81 log units. Koseki et al. *(33)* also found a log unit reduction between 1 and 1.5 in aerobic and coliform bacteria on lettuce that was soaked in 5 mg/L aqueous ozone for 10 minutes.

The commercial vegetable wash was generally the least effective treatment for removing pathogens on fresh produce, producing reductions of < 2 log units. The highest reduction this treatment produced was a 1.56 log unit reduction of *L. monocytogenes* on tomatoes. However, a combination of the commercial vegetable wash with a running tap water rinse was not tested in this study and could produce a greater reduction in pathogens than the wash treatment alone. Kilonzo-Nthenge et al. *(30)* used a 2 minute commercial treatment to reduce *L. innocua* counts on tomatoes by 2.9 log units, on broccoli by 1.5 log units, on lettuce by 1.7 log units, and on apples by 2.28 log units. The results of both this study and the study done by Kilonzo-Nthenge et al. *(33)* appear to show that this product works better on smooth-skinned produce, such as tomatoes and apples, than on produce with a complex surface structure. It should be noted that this vegetable wash product is not marketed as having antimicrobial properties.

The ability of running tap water to remove pathogens differed, depending on the produce item and the pathogen combination. Although running tap water reduced pathogens on tomatoes by up to 2.62 log units, it was less effective on broccoli, cantaloupe, lettuce, spinach, and green onions, reducing pathogens between 0.55 and 1.69 log units. The inefficacy of running tap water on broccoli might be due to the highly hydrophobic and irregular surface of the vegetable. Treatments involving submersion of the broccoli were more effective at pathogen removal. Treating cantaloupes inoculated with *L. monocytogenes* with running tap water did not produce any reduction in the pathogen, compared with the untreated inoculated controls. Running tap water was also not effective at removing pathogens on the leafy vegetables, possibly because of the overlapping leaves protecting the inoculation sites. In general, our results with running tap water are similar to those of Kilonzo-Nthenge et al. *(30)*, who used a 15-second running tap water treatment to remove *L. innocua* counts on produce and they found that the pathogen was reduced by 1.4 log

Figure 6. Log reduction of bacterial pathogens on green onions treated using home use washing technologies. Initial pathogen counts ranged from 7.1 to 7.2 log CFU/green onion. Bars within one pathogen with same letter are not significantly different ($P < 0.05$).



units on lettuce and broccoli and by 2.1 log units on tomatoes.

The microbial populations in the residual wash solutions indicate the potential for cross-contamination. Results obtained from diluted bleach and EO water indicate no significant risk for cross contamination, whereas treatment with ozone produced occasional pathogen-positive wash water, indicating a potential for cross contamination. Running water and vegetable wash treatments are not bacteriocidal, so the potential for cross contamination during use would depend on whether the consumer takes appropriate precautions.

The efficacy of the produce washing treatments evaluated in this study often varied with the pathogen and the produce item being tested. Pathogen reductions were generally greater for tomatoes than for broccoli or cantaloupe, probably because broccoli and cantaloupe have surface characteristics that protect pathogens from removal and inactivation *(25)*. Leafy greens also have different surface characteristics. The smooth surface of lettuce and green onions is protected by a relatively thick waxy cuticle with hydrophobic properties that repels water and possibly bacterial adhesion, while the abaxial side of spinach appears rougher and differs in other microstructure characteristics (such as cuticle hydrophobicity and thickness), which may affect the level of protection afforded to attached bacteria *(8, 30, 36)*.

The results from this study suggest that each of the washing treatments tested has the potential to reduce surface bacterial contamination on specific items of fresh produce, but none produced significantly greater reductions than tap water rinse for all tested items. EO water and dilute chlorine bleach were the most consistently effective of the treatments tested, with EO water showing greater effectiveness in treating lettuce and chlorine greater effectiveness in treating cantaloupe. However, consumer washing of fresh produce with diluted bleach is not recommended by the USDA. The effectiveness of other treatments depended on the item of produce and the target pathogen. For some produce/pathogen combinations, running tap water was as effective as the commercial technologies. A limitation of this study is that we did not test commercial treatments in combination with running tap water. Such combinations may result in increased pathogen removal. Overall, the results of this study indicate that consumers may achieve marginal decreases in risk by employing the consumer-oriented commercial produce washing technologies tested in this study.

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