Journal of Food Protection, Vol. 72, No. 9, 2009, Pages 1854–1861

Reduction of *Escherichia coli* O157:H7 on Produce by Use of Electrolyzed Water under Simulated Food Service Operation Conditions

PHILIPUS PANGLOLI,[1] YEN-CON HUNG,[1]* LARRY R. BEUCHAT,[1,2] C. HAROLD KING,[3] AND ZHI-HUI ZHAO[4]

[1]Department of Food Science and Technology and [2]Center for Food Safety University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797, USA; [3]Food and Product Safety, Chick-Fil-A, Inc., 5200 Buffington Road, Atlanta, Georgia 30349-2998, USA; and [4]Shanghai Academy of Agricultural Sciences, 2901 Beidi Road, Shanghai 201106, China

MS 08-554: Received 5 November 2008/Accepted 14 April 2009

ABSTRACT

Treatment of fresh fruits and vegetables with electrolyzed water (EW) has been shown to kill or reduce foodborne pathogens. We evaluated the efficacy of EW in killing *Escherichia coli* O157:H7 on iceberg lettuce, cabbage, lemons, and tomatoes by using washing and/or chilling treatments simulating those followed in some food service kitchens. Greatest reduction levels on lettuce were achieved by sequentially washing with 14-A (amperage) acidic EW (AcEW) for 15 or 30 s followed by chilling in 16-A AcEW for 15 min. This procedure reduced the pathogen by 2.8 and 3.0 log CFU per leaf, respectively, whereas washing and chilling with tap water reduced the pathogen by 1.9 and 2.4 log CFU per leaf. Washing cabbage leaves for 15 or 30 s with tap water or 14-A AcEW reduced the pathogen by 2.0 and 3.0 log CFU per leaf and 2.5 to 3.0 log CFU per leaf, respectively. The pathogen was reduced by 4.7 log CFU per lemon by washing with 14-A AcEW and 4.1 and 4.5 log CFU per lemon by washing with tap water for 15 or 30 s. A reduction of 5.3 log CFU per lemon was achieved by washing with 14-A alkaline EW for 15 s prior to washing with 14-A AcEW for 15 s. Washing tomatoes with tap water or 14-A AcEW for 15 s reduced the pathogen by 6.4 and 7.9 log CFU per tomato, respectively. Application of AcEW using procedures mimicking food service operations should help minimize cross-contamination and reduce the risk of *E. coli* O157:H7 being present on produce at the time of consumption.

Consumption of fresh produce in the United States has increased in recent years as a result of the active promotion of vegetables and fruits as an important part of healthier diets *(26)*. Concurrent with this increase, the frequency of outbreaks of foodborne illness associated with consumption of contaminated produce has also increased. The increased involvement of produce in outbreaks of foodborne illnesses not only is due to the contamination of produce but also may be related to improving epidemiological systems used to determine the source of foodborne illness outbreaks such as PulseNet of the Centers for Disease Control and Prevention. However, these outbreaks have raised public concerns about the safety of produce and caused economic losses in the produce industry.

Salmonella and *Escherichia coli* O157:H7 have proven to be most problematic in fresh produce, with these two bacterial pathogens having been respectively responsible for about 50 and 20% of produce-related outbreaks documented in the United States from 1998 to 2002 *(18)*. In 2005 and 2006, four multistate outbreaks of salmonellosis associated with eating tomatoes in restaurants sickened at least 450 people in 21 states *(6)*. In 2006, outbreaks of *E. coli* O157:H7 infections linked to bagged spinach affected at least 183 people in 26 states *(5)* and outbreaks associated with consumption of lettuce in fast-food restaurants sickened 81 individuals in 3 states *(27)*. In 2008, an outbreak of salmonellosis implicating consumption of jalapeno peppers contaminated with *Salmonella* Saintpaul involved more than 1,400 infected people in 43 states, the District of Columbia, and Canada *(7)*. Initially this outbreak was suspected to have been caused by the consumption of contaminated tomatoes, resulting in restaurants and food service operations removing certain types of tomatoes from menus and causing economic losses of approximately 250 million dollars *(1)*.

Contamination of produce with pathogens can occur during production, harvesting, processing, storage, and handling or during preparation in food service kitchens or at home. Vegetables and fruits such as lettuce, cabbage, tomatoes, lemons, and oranges used to make salads and fresh-squeezed juices or sandwiches in restaurant kitchens often require washing with water before serving. However, this washing step may be ineffective in completely removing all pathogenic microorganisms from produce *(19)*.

Electrolyzed water (EW) is produced through electrolysis of a mild salt (NaCl) solution in a chamber with cathode and anode electrodes *(25)*. Acidic EW (AcEW), generated from the anode side, is lethal to most foodborne bacterial pathogens *(12, 28)* due to its low pH, high oxidation-reduction potential, and the presence of hypochlorous acid. Alkaline EW (AkEW), generated from the cathode side, has a strong cleaning effect. Koseki et al. *(14)* reduced populations of aerobic bacteria by 2 log CFU/g by washing

* Author for correspondence. Tel: 770-412-4739; Fax: 770-412-4748; E-mail: yhung@uga.edu.

lettuce with AkEW for 1 min followed by AcEW for 1 min. Several studies have shown that AcEW is effective in killing or reducing foodborne pathogens attached to the surface of lettuce *(16, 20, 21, 29),* cabbage *(11, 17),* spinach *(29),* leafy greens *(24),* tomatoes *(2, 9, 22),* alfalfa sprouts *(13),* and green onions *(22)*.

Most studies examining the efficacy of EW as a produce sanitizer have been conducted under conditions not considering unique situations at food service practices. A study on reduction of bacteria on spinach and lettuce in food service area was reported by Guentzel et al. *(10)*. However, they did not mimic food service washing practices. They also did not recover bacteria on the produce after treatment. The objective of our study was to evaluate the efficacy of EW in killing or removing *E. coli* O157:H7 attached to the surface of produce under simulated food service operation conditions. The efficacy of EW in killing the pathogen during washing and/or chilling of iceberg lettuce and washing of cabbage, tomatoes, and lemons was determined.

MATERIALS AND METHODS

Strains used and preparation of inoculum. A mixture of five strains of nalidixic acid–adapted *E. coli* O157:H7 was used as an inoculum. The five strains consisted of CDC-658 (isolated from human feces in a cantaloupe-associated outbreak), E-19 (calf feces isolate), F-4546 (human feces, alfalfa sprout–associated outbreak), H-1730 (human feces, lettuce-associated outbreak), and LJH-557 (apple cider isolate). Each strain was grown in tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) supplemented with 50 μg/ml nalidixic acid (TSBN) at 37°C for 24 h. At least two successive transfers to TSBN at 24-h intervals were made before cells were harvested to prepare the inoculum. Each strain was plated individually on tryptic soy agar (Difco) supplemented with 50 μg/ml nalidixic acid (TSAN), which resulted in approximately equal populations (8.8 log CFU/ml), indicating that the five strains grew equally in TSBN. Stock cultures of all strains were maintained at 4°C on TSAN slants and occasionally (not more than 4 wk) transferred to new TSA slants after reculturing in TSBN at 37°C for 24 h. Each strain was cultured in 10 ml of TSBN at 37°C for 24 h, and cells were sedimented by centrifugation (2,000 × *g* at 22°C for 15 min). Cells were resuspended in 2 ml of sterile 0.1% peptone water. Equal volumes (2 ml) of each strain suspension were combined to obtain 10 ml of an inoculum containing approximately 9 log CFU/ml and equal populations of each strain. Populations in the individual cultures and the five-strain suspension were determined by serial dilution in 0.1% peptone water followed by spread plating on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted.

Source, preparation, and inoculation of produce. Iceberg lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* L.), lemons (*Citrus limonum* Risso.), and round red tomatoes (*Lycopersicum esculentum* Mill.) were obtained from a local restaurant, stored at 4°C, and used within 48 h. The outer two or three damaged or green wrapper leaves of lettuce and cabbage were discarded. The next three or four whole leaves, weighing 40 to 50 g per leaf for washing treatments or 50 ± 1 g total weight for chilling or sequential washing and chilling treatments, were placed on sanitized trays with the outer (abaxial) side facing up. Only three or four leaves from each head were used in experiments, thereby minimizing variability of leaves that may occur at outer and inner locations in the heads. Each whole leaf was inoculated with 100 μl of the mixed-strain suspension of *E. coli* O157:H7 by placing drops onto 15 to 20 locations of the leaf surface by use of a micropipettor to obtain a population of about 8 log CFU per leaf.

Lemons (150 ± 10 g) at 22°C were held with the stem end facing down, using sanitized tongs, and spot inoculated with 100 μl of the cell suspension. The inoculum was applied at 18 to 22 locations on the top surface of the lemons using micropipettor. Care was taken to avoid applying inoculum onto the blossom scar tissue. The inoculated lemons were then placed stem scar end down in sanitized ice-maker trays, which functioned as holders to prevent movement of the lemons.

Tomatoes (150 to 200 g) at 22 ± 2°C were placed stem scar end down on sanitized trays. The cell suspension (100 μl) was spot inoculated onto the top surface of tomatoes by a micropipettor. The inoculum was deposited on 18 to 22 locations to prevent running off the side and to facilitate drying. Care was taken to avoid applying inoculum onto the stem scar tissue or blossom end.

The inoculated lettuce, cabbage, lemons, and tomatoes were air dried in a biosafety laminar flow hood for 2 h at 22°C to allow attachment of *E. coli* O157:H7. Trays containing the inoculated produce were then covered with aluminum foil and stored at 4°C for 22 ± 2 h to simulate handling of produce in preparation areas in food service kitchens.

Preparation of EW and chilled water. EW was generated by electrolyzing a diluted NaCl solution (ca. 0.1%) using a Hoshizaki generator (model ROX-20TA, Hoshizaki Electric Co., Ltd., Toyoake, Aichi, Japan) at 14 and 16 A (amperage) to obtain AcEW with free chlorine levels of 42.0 ± 2.0 and 52.0 ± 2.0 μg/ml, respectively. A 12% NaCl solution and deionized water were simultaneously pumped into the generator to give a 0.1% NaCl solution passing through the electrodes in the generator chamber. AcEW was generated from the anode side, while AkEW was generated from the cathode side simultaneously.

Chilled tap water and AcEW were prepared by mixing 1 liter of tap water or AcEW produced at 16 A with 300 g of ice (30%, wt/vol). The chilled 16-A AcEW had properties similar to those of 14-A AcEW at room temperature. The pH and oxidation-reduction potential of AcEW and tap water were measured using a dual channel ACCUMET meter (model AR50, Fisher Scientific, Pittsburgh, PA). Free chlorine levels were determined using the DPD-FEAS method (Hach Co., Loveland, CO).

Procedures for treating produce. Procedures for treating produce were designed to simulate conditions that may exist in preparation areas in food service kitchens. Treatment of lettuce was performed in two phases. In the first series of experiments, the inoculated whole lettuce leaves (40 to 50 g) were washed with running tap water (ca. 2.0 ± 0.2 liter/min) (control), 14-A AcEW (42.0 ± 2.0 μg/ml free chlorine), or 14-A AkEW for 15 or 30 s, or the inoculated whole leaves (50 ± 1 g) were cut into 2- to 3-cm-long pieces and chilled at 4°C in tap water or 16-A AcEW (40.0 ± 0.2 μg/ml free chlorine) for 15 min. The whole leaf was held at the butt end, washed uniformly under running tap water or EW for designated times, cut into 2- to 3-cm-long pieces, and placed into a 1.5-liter round-bottom Whirl-Pak bag (Nasco, Fort Atkinson, WI). Immediately after treatments, 200 ml of Dey-Engley (DE) neutralizing broth (Difco, Becton Dickinson) was added to the bag in preparation for microbiological analyses. Wash solution (25 ml) separately collected in a 532-ml Whirl-Pak bag was combined with 25 ml of double-strength DE broth (dDE) and subjected to microbiological analyses.

For the chilling treatment, the inoculated leaf (50 ± 1 g) was cut into 2- to 3-cm-long pieces and placed into a 1.5-liter round-

bottom Whirl-Pak bag with 175 ml of chilled (4°C) tap water or 16-A AcEW. The bag was closed and positioned in a basket such that the lettuce was submerged and then held at 4°C for 15 min. After the chilling treatment, tap water and AcEW were decanted into a 710-ml Whirl-Pak bag. DE broth (200 ml) was added to the bag containing the lettuce, and the mixture was subjected to microbiological analysis. Chilling solution (25 ml) was combined with 25 ml of dDE in a 532-ml Whirl-Pak bag and also subjected to microbiological analyses.

In the second series of experiments, inoculated lettuce leaves (50 ± 1 g) were washed with running tap water, 14-A AcEW, or 14-A AkEW as previously described, cut into 2- to 3-cm-long pieces, and chilled in tap water or 16-A AkEW at 4°C for 15 min. Lettuce washed with tap water followed by chilling in tap water served as the control. The washed, chilled lettuce was combined with 200 ml of DE broth, whereas 25 ml of chilling water was combined with 25 ml of dDE for microbiological analyses.

Inoculated whole cabbage leaves (40 to 50 g) and lemons were washed with running (ca. 2.0 ± 0.2 liter/min) tap water (control) or 14-A AcEW for 15 or 30 s or with running 14-A AkEW for 15 s followed by 14-A AcEW for 15 s. The procedures for washing cabbage were the same as those described for lettuce. The washed cabbage leaves were cut into 2- to 3-cm-long pieces to facilitate pummeling, transferred to 1.5-liter round-bottom Whirl-Pak bags, combined with 200 ml of DE broth, and subjected to microbiological analyses. Wash solutions (25 ml) from cabbage treatments were transferred to 532-ml Whirl-Pak bags, combined with 25 ml of dDE broth, and subjected to microbiological analyses. Lemons were washed by rubbing the entire surface with gloved hands under running wash water for designated times. The washed lemons were placed in 1.5-liter Whirl-Pak bags, and 50 ml of DE broth was added, while their respective wash solutions (25 ml) were combined with 25 ml of dDE in 532-ml bags for microbiological analyses.

Tomatoes were treated by washing the inoculated samples with running tap water (control), 14-A AcEW, or 14-A AkEW for 8 or 15 s, or with 14-A AkEW for 7 s followed by 14-A AcEW for 8 s. Washing times for tomatoes was reduced compared with the times used for other produce because *E. coli* O157:H7 was not recovered (detection limit, <1.0 log CFU per tomato) by direct plating from the tomatoes washed for 30 s. Tomatoes were washed by thoroughly rubbing them under running wash water as described for lemons. Care was taken while rubbing tomatoes to prevent damage to the skin. The washed tomatoes were placed in 1.5-liter round-bottom Whirl-Pak bags to which 50 ml of DE broth was added before being subjected to microbiological analyses. Wash solutions (25 ml) in 532-ml Whirl-Pak bags were combined with 25 ml of dDE and also subjected to microbiological analyses. Treatments resulting in substantial reductions in the number of *E. coli* O157:H7 were applied to produce inoculated at approximately 3 log CFU to determine their efficacy in killing the pathogen at more realistic contamination levels.

Microbiological analyses. Populations of *E. coli* O157:H7 on untreated and treated produce and in wash and chill solutions after treatment were determined. The Whirl-Pak bags containing lettuce or cabbage samples and DE broth were pummeled in a stomacher (Stomacher 400 Circulator, Seward, London, UK) for 1 min at normal (230-rpm) and high (260-rpm) speed, respectively, while lemons or tomatoes in Whirl-Pak bags with DE broth were hand rubbed for 2 min. The DE wash broth was serially diluted in 0.1% peptone water and plated (0.1 ml, in duplicate) on sorbitol-MacConkey agar (Difco, Becton Dickinson) containing 50 μg/ml nalidixic acid and 0.1% sodium pyruvate (SMACNP) and on TSAN supplemented with 0.1% sodium pyruvate (TSANP) using a spiral plater (WSAP 2, Microbiology International, Frederick, MD). Undiluted samples (0.25 ml, in quadruplicate) were also plated on SMACNP and TSANP. The Whirl-Pak bags containing wash or chilling solutions and dDE broth were pummeled for 1 min at normal speed in a stomacher (Seward). Homogenates were serially diluted and plated on SMACNP and TSANP as described above. Plates were incubated at 37°C for 24 h before colonies were counted.

To detect the presence of low numbers of *E. coli* O157:H7 that would not be detected by direct plating, 250 ml of double-strength modified TSB supplemented with 50 μg/ml nalidixic acid and 0.1% sodium pyruvate (dmTSBNP) was added to each stomacher bag containing lettuce or cabbage leaves and 200 ml of DE broth for enrichment. To bags containing a single lemon or tomato and 50 ml of DE broth and to bags containing 25 ml of wash or chill solutions and 25 ml of dDE, 50 ml of dmTSBNP was added. Mixtures of enrichment broth and produce or enrichment broth and wash or chill solutions were incubated at 37°C for 24 h. When no colonies were recovered by direct plating, the enrichment broth was streaked onto SMACNP and TSANP plates and incubated at 37°C for 24 h. Plates were examined for the presence of presumptive colonies of *E. coli* O157:H7.

Presumptive-positive colonies (10 to 20 per treatment) were randomly selected from SMACNP and TSANP plates for biochemical and serological confirmation. Colonies were picked with sterile 2.1-mm-diameter wooden applicators and spot inoculated onto MacConkey agar (Difco, Becton Dickinson) plates containing lactose and supplemented with 4-methylumbelliferyl-β-D-glucuronide (MUG) (0.1 g/liter). Plates were incubated at 37°C for 24 h. Colonies positive for lactose (pink color) and negative for MUG (nonfluorescent) were subjected to the Dryspot *E. coli* O157 latex agglutination test (Oxoid, Basingstoke, UK).

Statistical analysis. Lettuce experiments were replicated four times, and each replicate consisted of two samples for each treatment. Four replicate experiments (three samples per replicate) were conducted for cabbage and tomatoes. Experiments with lemons were replicated three times (three samples per replicate). Data were subjected to analysis of variance with a randomized block design, block on replication. Statistical analysis was performed with the SAS Mixed Procedures using SAS Software Release 8.2 (SAS Institute Inc., Cary, NC). Significant differences among means were determined by the least-square-means method with P value for differences (PDIFF) option *(23)*.

RESULTS AND DISCUSSION

Water properties. The properties of tap water and EW used to treat produce were as follows. The pH values of tap water and chilled tap water were 7.3 and 7.4, with oxidation-reduction potential values of 720 and 740 mV and free chlorine contents of 1.0 and 1.3 μg/ml, respectively. AcEW and chilled AcEW had pH values of 2.4 and 3.0, oxidation-reduction potential values of 1170 and 1176 mV, and free chlorine contents of 42.0 and 40.0 μg/ml, respectively. The pH value of 16-A AkEW was 11.9.

Lettuce. Washing lettuce leaves with running 14-A AcEW or 14-A AkEW for 15 s reduced the number of *E. coli* O157:H7 organisms on lettuce by 1.6 and 1.5 log CFU per leaf, respectively, which was significantly greater ($P \leq 0.05$) than the 1.2-log reduction achieved by washing with

TABLE 1. *Population of* E. coli *O157:H7 recovered from lettuce leaves and wash and chilling solutions after treatment*

Type of treatment	Treatment time	Mean *E. coli* O157:H7 population[a]		
		On lettuce (log CFU/leaf)		In solutions (log CFU/ml)
		Recovered	Reduction	
None (untreated whole leaf)		8.13 A		
Washing with tap water	15 s	6.97 C	1.16	0.85
	30 s	5.97 F	2.16	0.30
Washing with 14-A AcEW	15 s	6.51 DE	1.62	ND[b]
	30 s	6.54 DE	1.59	ND
Washing with 14-A AkEW	15 s	6.64 D	1.49	4.99
	30 s	6.48 DE	1.65	4.73
Chilling in tap water	15 min	7.39 B	0.74	5.56
Chilling in 16-A AcEW	15 min	6.32 E	1.81	ND

[a] Mean values not followed by the same letter are significantly different ($P \leq 0.05$).
[b] ND, not detected by direct plating (detection limit, 0.3 log CFU/ml) or enrichment.

tap water (Table 1). Prolonging washing time with running tap water to 30 s significantly increased ($P \leq 0.05$) this reduction to 2.2 log CFU per leaf, but increasing washing time with AcEW or AkEW did not significantly affect reductions. Chilling cut lettuce with 16-A AcEW for 15 min reduced the pathogen by 1.8 log CFU per cut leaf sample, but chilling with tap water reduced the pathogen by only 0.7 log CFU per cut leaf sample. *E. coli* O157:H7 was recovered from tap water and 14-A AkEW after washing and chilling treatments. However, the pathogen was not detected in 14-A or 16-A AcEW solutions by enrichment after washing or chilling lettuce, which makes it advantageous in reducing the potential for cross-contamination during preparation of produce and other foods in food service kitchen environments.

The 1.8-log reduction of *E. coli* O157:H7 achieved by chilling lettuce in 16-A AcEW for 15 min was similar to reductions reported for lettuce *(15),* Romaine lettuce *(29),* and leafy greens *(24).* Subsequent immersion of dip-inoculated lettuce (15 to 25 g) in 1.5 liters of AcEW at 20°C for 5 min twice followed by rinsing twice with 1.0 liter of distilled water reduced the pathogen by 1.5 log CFU/g *(15).* When the lettuce was submerged in AcEW at 4°C for 5 min followed by the same rinsing procedure, the pathogen was reduced by 1.3 log CFU/g. Yang et al. *(29)* immersed dip-inoculated fresh-cut Romaine lettuce (25 g) in 800 ml of EW (300 μg/ml available chlorine; pH 4) for 5 min and reduced *E. coli* O157:H7 by 2.2 log CFU/g. Stopforth et al. *(24)* reduced the pathogen on spray-inoculated leafy greens by 2.5 log CFU/g by immersing samples (10 g) in 1,500 ml of acidic EW (30 to 35 μg/ml free chlorine) for 90 s.

Park et al. *(20, 21)* and Koseki et al. *(16)* reported more substantial reductions of *E. coli* O157:H7 on lettuce by using AcEW (ca. 35 to 40 μg/ml free chlorine). Park et al. *(20)* immersed spot-inoculated iceberg lettuce (ca. 50 g) in 1.5 liters of AcEW for 3 min with continuous shaking. *E. coli* O157:H7 was reduced by 4.2 log CFU per leaf. Similarly, Park et al. *(21)* were able to reduce the pathogen by more than 5 log CFU/g by immersing 10 g of spot-inoculated lettuce in 500 ml of AcEW for 3 min. Koseki et al. *(16)* reduced the pathogen by 4.7 log CFU/g by immersing dip-inoculated lettuce (12.5 g) in 1.5 liters of AcEW for 1 min. These relatively high reductions might be due in part to the high ratios of produce to EW, coupled with continuous shaking during treatment. Tissue fluids released from cut produce undoubtedly react with chlorine, thereby reducing its lethality. The high ratios of produce to EW may result in prolonged availability of free chlorine in solution, resulting in higher lethality to *E. coli* O157:H7. Continuous shaking may enhance bacterial cells contact with chlorine. The use of high ratios of produce to water and continuous shaking might not be practical, however, in food service kitchens. In our study, we used a produce-to-water ratio of 1:3.5 to simulate food service operations. In preliminary experiments, ratios of 1:3.5, 1:5.0, and 1:6.0 and shaking at 5-min intervals during chilling were applied. However, these treatments did not increase the reduction of *E. coli* O157:H7 on lettuce (data not shown).

Sequential washing in 14-A AcEW or 14-A AkEW and chilling in 16-A AcEW increased the reduction of *E. coli* O157:H7 on lettuce (Table 2). Washing lettuce with tap water, 14-A AcEW, or 14-A AkEW followed by chilling for 15 min in 16-A AcEW reduced the pathogen by 2.5 to 3.0 log CFU per leaf. The pathogen survived in tap water and 14-A AkEW wash solutions, which would increase the possibility of cross-contamination. Chilling in tap water for 15 min after washing with tap water for 30 s reduced the pathogen by 2.4 log CFU per leaf, which was significantly greater ($P \leq 0.05$) than the reduction (1.9 log CFU per leaf) resulting from washing for 15 s followed by chilling for 15 min. Regardless of the type of water used to wash lettuce for 15 s, subsequent chilling in 16-A AcEW killed higher numbers of the pathogen than chilling in tap water ($P \leq 0.05$). Washing lettuce leaves with 14-A AcEW for 30 s, followed by chilling in 16-A AcEW for 15 min resulted in the highest reduction of *E. coli* O157:H7. This treatment combination reduced *E. coli* O157:H7 on lettuce by 3.0 log CFU per leaf, which was significantly greater ($P \leq 0.05$) than the reductions of 1.9 and 2.4 log CFU per leaf achieved

TABLE 2. *Population of* E. coli *O157:H7 recovered from inoculated lettuce leaves and solutions after sequential washing and chilling*

Type of washing treatment	Washing time (s)	Type of chilling water[a]	Mean *E. coli* O157:H7 population			
			On lettuce (log CFU/leaf)		In solution (log CFU/ml)	
			Recovered[b]	Reduction	Wash	Chilling
None			8.14 A			
Tap water	15	Tap water	6.28 B	1.86	1.76	4.16
	30	Tap water	5.77 C	2.37	1.99	4.00
Tap water	15	16-A AcEW	5.59 CD	2.55	NA[c]	ND
	30	16-A AcEW	5.48 CD	2.66	NA	ND
14-A AcEW	15	16-A AcEW	5.32 CD	2.82	ND[d]	ND
	30	16-A AcEW	5.17 D	2.97	ND	ND
14-A AkEW	15	16-A AcEW	5.21 D	2.93	5.17	ND
	30	16-A AcEW	5.65 CD	2.49	4.86	ND

[a] Washed lettuce was chilled at 4°C for 15 min.
[b] Mean values not followed by the same letter are significantly different ($P \leq 0.05$).
[c] NA, not analyzed.
[d] ND, not detected by direct plating (detection limit, 0.3 log CFU/ml) or enrichment.

by washing with tap water for 15 and 30 s, respectively, followed by chilling in tap water for 15 min. Additionally, this process would minimize survival of the pathogen in chilling solutions, which otherwise might cause cross-contamination. Reductions of *E. coli* O157:H7 achieved in our study were higher than those reported for lettuce by Koseki et al. *(15)*. In the latter study, *E. coli* O157:H7 populations were reduced by 1.8 log CFU/g of dip-inoculated lettuce by sequential 5-min immersion in AkEW and AcEW.

Cabbage. Washing cabbage leaves with running tap water for 15 s decreased the populations of *E. coli* O157:H7 by 2.0 log CFU per leaf, which was not significantly different ($P > 0.05$) than the 2.5-log reduction achieved by washing with running 14-A AcEW for 15 s (Table 3). Increasing the washing time to 30 s increased the reduction to 3.0 log CFU per leaf. Lin et al. *(17)* soaked cabbage leaves in AcEW for 9 min with continuous shaking and reduced the number of naturally occurring aerobic microorganisms by 1.1 log CFU/g. Inatsu et al. *(11)* reduced *E. coli* O157:H7 on Chinese cabbage by 2.5 to 3.0 log CFU/g with a 15-min immersion treatment of dip-inoculated leaves in 1.0 liter of acidified sodium chlorite. Soaking cabbage in water is not normally practiced in food service operations because it can cause the cabbage to appear soapy.

Washing cabbage leaves with 14-A AkEW for 15 s prior to washing with 14-A AcEW for 15 s did not increase the reduction of the pathogen significantly ($P > 0.05$) and allowed the pathogen to survive in two of six wash solution samples by enrichment (Table 3). Washing cabbage with tap water for 15 or 30 s resulted in the survival of the pathogen in six of six wash solutions. Lin et al. *(17)* increased the reduction of aerobic microorganisms on leafy cabbage from 1.1 log CFU/g to 1.6 log CFU/g by soaking the samples in AkEW for 3 min after a 9-min soaking treatment in AcEW.

TABLE 3. *Population of* E. coli *O157:H7 recovered from inoculated cabbage leaves and wash solution after treatment*

Type of washing treatment	Washing time (s)	*E. coli* O157:H7 population		
		On cabbage (log CFU/leaf)		In wash solution (log CFU/ml)
		Recovered[a]	Reduction	
None		8.01 A		
Tap water	15	5.99 B	2.02	6/6[b]
	30	5.02 C	2.99	6/6[b]
14-A AcEW	15	5.48 BC	2.53	ND[c]
	30	5.01 C	3.00	ND
14-A AkEW for 15 s, followed by 14-A AcEW for 15 s	30	5.21 C	2.80	2/6[d]

[a] Mean values not followed by the same letter are significantly different ($P \leq 0.05$).
[b] Not detected by direct plating (detection limit, 0.3 log CFU/ml), but six of six samples were positive by enrichment.
[c] ND, not detected by direct plating or enrichment.
[d] Not detected by direct plating (detection limit, 0.3 log CFU/ml), but two of six samples were positive by enrichment.

TABLE 4. *Population of* E. coli *O157:H7 recovered from inoculated lemons and wash solutions after treatments*

Type of washing treatment	Washing time (s)	*E. coli* O157:H7 population		
		On lemons (log CFU/lemon)		In wash solution (log CFU/ml)
		Recovered[a]	Reduction	
None	0	7.24 A		
Tap water	15	3.14 B	4.10	2/9[b]
	30	2.73 BC	4.51	1/9[c]
14-A AcEW	15	2.58 C	4.66	ND[d]
	30	2.53 C	4.71	ND
14-A AkEW for 15 s, followed by 14-A AcEW for 15 s	30	1.99 D	5.25	ND

[a] Mean values not followed by the same letter are significantly different ($P \leq 0.05$).
[b] Not detected by direct plating (detection limit, 0.3 log CFU/ml), but two of nine samples were positive by enrichment.
[c] Not detected by direct plating (detection limit, 0.3 log CFU/ml), but one of nine samples was positive by enrichment.
[d] ND, not detected by direct plating or enrichment.

Lemons. Washing lemons with running tap water or 14-A AcEW for 15 s reduced the populations of *E. coli* O157:H7 by 4.1 and 4.7 CFU per lemon, respectively (Table 4). Regardless of the type of wash water, prolonging washing time to 30 s did not have a significant effect ($P > 0.05$) on further reducing the pathogen. In addition to inactivating *E. coli* O157:H7 in wash solutions, washing lemons with running 14-A EW generally reduced the pathogen more than washing in tap water. Washing lemons with running 14-A AkEW for 15 s prior to washing with 14-A AcEW for 15 s significantly reduced ($P \leq 0.05$) the number of *E. coli* O157:H7 by 5.3 log CFU per lemon. *E. coli* O157:H7 was not detected in EW wash solutions and some of the tap water solutions by enrichment. This may be because of the antibacterial activities of essential oils and other compounds released from the lemon skin during rubbing. Lemons and other citrus fruits contain flavonoids and terpenes with antimicrobial activities *(8)*.

Tomatoes. Regardless of the washing treatment, the number of *E. coli* O157:H7 cells removed from the surface of tomatoes (ca. 5.7 to 7.4 log CFU per tomato) was generally higher than those removed from other test produce, even though the washing time for tomatoes was one-half of that used for the other produce (Table 5). Washing tomatoes with 14-A AcEW for 15 s achieved the highest reduction, 7.4 log CFU per tomato. This reduction was significantly higher than those of all other treatments. Washing tomatoes with 14-A AkEW for 7 s prior to washing with 14-A AcEW for 8 s was significantly more effective than the other non-AcEW treatments. However, this washing treatment did not eliminate all viable *E. coli* O157:H7 organisms in the washing solution, as one of nine solution samples was positive for the pathogen by enrichment. Overall, considering reductions on tomatoes as well as in wash solutions, the most effective treatment was washing tomatoes with 14-A AcEW for 15 s, which reduced

TABLE 5. *Population of* E. coli *O157:H7 recovered from inoculated tomatoes and wash solutions after treatment*

Type of washing treatment	Washing time (s)	*E. coli* O157:H7 population		
		On tomatoes (log CFU/tomato)		In wash solution (log CFU/ml)
		Recovered[a]	Reduction	
None		7.90 A		
Tap water	8	2.22 B	5.68	1.71
	15	2.12 B	5.78	1.82
14-A AcEW	8	2.07 BC	5.83	ND[b]
	15	0.53 D	7.37	ND
14-A AkEW	8	2.31 B	5.59	3.58
	15	2.14 B	5.76	3.39
14-A AkEW for 7 s, followed by 14-A AcEW for 8 s	15	1.77 C	6.13	1/9[c]

[a] Mean values not followed by the same letter are significantly different (P 0 $\leq$.05). The detection limit was 1.7 log CFU per tomato. If *E. coli* O157:H7 was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was less than the detection limit, e.g., 1.6 log CFU per tomato.
[b] ND, not detected by direct plating (detection limit, 0.3 log CFU/ml) and enrichment.
[c] Not detected by direct plating, but one of nine samples was positive by enrichment.

TABLE 6. *Population of* E. coli *O157:H7 recovered from lettuce, cabbage, lemons, and tomatoes inoculated at ca. 3 log CFU per lettuce or cabbage leaf, lemon, or tomato after treatment*

Type of washing treatment	Washing time (s)	Type of chilling water[a]	*E. coli* O157:H7 population (log CFU/sample)	
			Recovered[b]	Reduction
Lettuce				
None		None	2.42	
14-A AcEW	15	16-A AcEW	1.37	1.05
	30	16-A AcEW	1.07	1.35
Cabbage				
None		None	2.58	
14-A AcEW	15	None	1.86	0.72
	30	None	1.33	1.25
Lemons				
None		None	0.53	
14-A AcEW	15	None	ND[c]	0.53
	30	None	ND	0.53
Tomatoes				
None		None	1.28	
14-A AcEW	8	None	ND	1.28
	15	None	ND	1.28

[a] Washed lettuce was chilled at 4°C for 15 min.
[b] Detection limit was 1.70 log CFU per sample. If *E. coli* O157:H7 was not detected by both direct plating and enrichment, the recovery was 0; if the pathogen was only detected by enrichment, the recovery was less than the detection limit, e.g., 1.6 log CFU per sample.
[c] Not detected by direct plating or enrichment.

the highest number of the pathogen on the surface of tomatoes and eliminated the pathogen in the wash solution after treatment.

The results of our study with tomatoes are in line with those reported in other studies. Bari et al. *(2)* achieved a 7.7-log reduction of *E. coli* O157:H7 by rubbing spot-inoculated tomatoes in 20 ml of AcEW (ca. 30 μg/ml free chlorine) for 20 s. Treatment with chlorinated water (ca. 200 μg/ml free chlorine) reduced the pathogen by only 4.6 log CFU per tomato. Shaking dip-inoculated tomatoes immersed in 100 ml of neutral EW (ca. 89 μg/ml free chlorine) for 30 or 60 s removed *E. coli* O157:H7 by 4.4 and 4.9 log CFU/cm^2 of tomato surface, respectively *(9)*. Similarly, treating tomatoes by immersing in 500 ml of AcEW for 30 s reduced *E. coli* O157:H7 by 4.16 log CFU/g *(22)*. Reductions increased with increased immersion time. The last study (Park et al. *(22)*) indicated that the reduction of the pathogen achieved by immersion in AkEW for 10 s prior to the treatment with AcEW was less than the reduction achieved by direct treatment with AcEW.

Treatments of produce with low inocula. At a high inoculum level (ca. 8 log CFU per leaf), the most effective treatments for lettuce and cabbage reduced *E. coli* O157:H7 by approximately 3.0 log CFU per leaf (Tables 2 and 3, respectively). However, these treatments did not completely remove the pathogen from lettuce and cabbage with lower inoculum levels (Table 6). At a 3-log CFU per leaf inoculum level, sequentially washing lettuce for 15 s in 14-A AcEW and chilling in 16-A AcEW for 15 min reduced the pathogen by only 1.3 log CFU per leaf. At this inoculum level, the pathogen was not recovered by direct plating after washing lettuce with 14-A AcEW for 30 s followed by chilling in 16-A AcEW for 15 min; however, it was detected in four of six samples by enrichment. Koseki et al. *(16)* reported that treatment of lettuce containing a high inoculum (5 to 6 log CFU/g) with AcEW reduced *E. coli* O157:H7 by 3.2 log CFU/g, but at a low inoculum level (3 to 4 log CFU/g) the reduction was only about 1.9 log CFU/g. They theorized that the pathogen penetrated into the interior of lettuce tissue through stomata regardless of the inoculation site, which could prevent complete removal. Beuchat *(3)* reported that a low inoculum level of *E. coli* O157:H7 (ca. 2 log CFU/g) on lettuce was not eliminated by treatment with chlorinated water (200 μg/ml).

Washing cabbage inoculated at ca. 3 log CFU per leaf with 14-A AcEW for 15 s reduced *E. coli* O157:H7 by only 0.7 log CFU per leaf (Table 6). The pathogen was not recovered by direct plating after washing cabbage for 30 s, but it was detected in five of six samples by enrichment.

E. coli O157:H7 was not detected on lemons and tomatoes treated with 14-A AcEW for 15 or 30 s and 8 or 15 s, respectively. At a 3-log CFU inoculum level, the pathogen was not detected by direct plating or enrichment after washing lemons for 15 or 30 s and tomatoes for 8 or 15 s (Table 6). However, *E. coli* O157:H7 was also not detected in unwashed lemons containing low inoculum by direct plating. Two of six lemons were positive by enrichment. These reductions may be due in part to the antimicrobial activities of flavonoids, terpenes, and other components in lemon rind.

At the low inoculum level (ca. 3 log CFU per tomato), *E. coli* O157:H7 was recovered in only two samples by direct plating and in one of six samples by enrichment of inoculated tomatoes stored at 4°C for 22 ± 2 h. Beuchat et

al. *(4)* reported that *E. coli* O157:H7 was adversely affected by drying, which reduced the initial population of 6.9 log CFU per tomato to 3.9 log CFU per tomato (ca. 3-log reduction) within 2 h at 22 ± 2°C.

In conclusion, application of AcEW by a process mimicking that used in a restaurant or food service operation can reduce the risk of *E. coli* O157:H7 being present on produce at the time of consumption. In addition, application of AcEW may minimize or eliminate the pathogen in wash or chilling solutions, and therefore, it may minimize cross-contamination during preparation of foods in food service kitchens.

ACKNOWLEDGMENTS

This research was funded by the State of Georgia through a FoodPAC project in collaboration of the Department of Food Science and Technology, The University of Georgia, Hoshizaki America, Inc., and Chick-Fil-A, Inc. This research could not have been done without the technical support of Frank Sack from Hoshizaki and Joy Adams, Kim Hortz, Matt Goss, Sue Ellen McCullogh, and Sandra Walker from The University of Georgia. We are grateful for their dedication.

REFERENCES

1. Anonymous. 2008. CDC: *Salmonella* outbreak appears to be over. Available at: http://www.msnbc.msn.com/id/26439766/. Accessed 3 October 2008.
2. Bari, M. L., Y. Sabina, S. Isobe, T. Uemura, and K. Isshiki. 2003. Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* on the surfaces of tomatoes. *J. Food Prot.* 66:542–548.
3. 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.
4. 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.
5. Centers for Disease Control and Prevention. 2006. Ongoing multistate outbreaks of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach—United States, September 2006. *Morb. Mortal. Wkly. Rep.* 55:1045–1046.
6. Centers for Disease Control and Prevention. 2007. Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants—United States, 2005–2006. *Morb. Mortal. Wkly. Rep.* 56:909–911.
7. Centers for Disease Control and Prevention. 2008. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items—United States, 2008. *Morb. Mortal. Wkly. Rep.* 57: 929–934.
8. Conte, A., B. Speranza, M. Sinigaglia, and M. A. D. Nobile. 2007. Effect of lemon extract on foodborne microorganisms. *J. Food Prot.* 70:1896–1900.
9. Deza, M. A., M. Araujo, and M. J. Garrido. 2003. Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. *Lett. Appl. Microbiol.* 37:482–487.
10. Guentzel, J. L., K. L. Lam, M. A. Callan, S. A. Emmons, and V. L. Dunham. 2008. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiol.* 25:36–41.
11. Inatsu, Y., M. L. Bari, S. Kawasaki, K. Isshiki, and S. Kawamoto. 2005. Efficacy of acidified sodium chlorite treatments in reducing *Escherichia coli* O157:H7 on Chinese cabbage. *J. Food Prot.* 68: 251–255.
12. Kim, C., Y.-C. Hung, and R. E. Brackett. 2000. Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. *J. Food Prot.* 63: 19–24.
13. Kim, C., Y.-C. Hung, R. E. Brackett, and C. Y. Lin. 2003. Efficacy of electrolyzed water in activating *Salmonella* on alfalfa seeds and sprouts. *J. Food Prot.* 66:208–214.
14. Koseki, S., K. Yoshida, S. Isobe, and K. Itoh. 2001. Decontamination of lettuce using acidic electrolyzed water. *J. Food Prot.* 64:652–658.
15. Koseki, S., K. Yoshida, Y. Kamitani, S. Isobe, and K. Itoh. 2004. Effect of mild heat pre-treatment with alkaline electrolyzed water on the efficacy of acidic electrolyzed water against *Escherichia coli* O157:H7 and *Salmonella* on lettuce. *Food Microbiol.* 21:559–566.
16. Koseki, S., K. Yoshida, Y. Kamitani, and K. Itoh. 2003. Influence of inoculation method, spot inoculation site, and inoculation size on the efficacy of acidic electrolyzed water against pathogens on lettuce. *J. Food Prot.* 66:2010–2016.
17. Lin, C.-S., C. Wu, J.-Y. Yeh, and F. K. Saalia. 2005. The evaluation of electrolyzed water as an agent for reducing microorganisms on vegetables. *Int. J. Food Sci. Technol.* 40:495–500.
18. Lynch, M., J. Painter, R. Woodruff, and C. Braden. 2006. Surveillance for foodborne-disease outbreaks—United States, 1998–2002. *Morb. Mortal. Wkly. Rep. Surveill. Summ.* 55:1–38.
19. Parish, M. E., L. R. Beuchat, T. V. Suslow, L. J. Haris, E. H. Garret, J. N. Farber, and F. F. Busta. 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce, chap. 5. *In* Analysis and evaluation of preventive control measure for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. *Comp. Rev. Food Sci. Food Saf.* 2(Suppl.):161–173.
20. Park, C. M., Y.-C. Hung, M. P. Doyle, G. O. I. Ezeike, and C. Kim. 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. *J. Food. Sci.* 66:1368–1372.
21. Park, E. J., E. Alexander, G. A. Taylor, R. Costa, and D.-H. Kang. 2008. Effect of electrolyzed water for reduction of foodborne pathogens on lettuce and spinach. *J. Food Sci.* 73:M268–M272.
22. Park, E. J., E. Alexander, G. A. Taylor, R. Costa, and D.-H. Kang. 2008. Fate of foodborne pathogens on green onions and tomatoes by electrolyzed water. *Lett. Appl. Microbiol.* 46:519–525.
23. Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed, p. 1243–1246. *In* Proceedings of the 23rd SAS Users Group International. SAS Institute, Cary, NC.
24. Stopforth, J. D., T. Mai, B. Kottapalli, and M. Samadpour. 2008. Effect of acidified sodium chlorite, chlorine, and acidic electrolyzed water on *Escherichia coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* inoculated onto leafy greens. *J. Food Prot.* 71:625–628.
25. Su, Y.-C., C. Liu, and Y.-C. Hung. 2007. Electrolyzed water: principles and applications, chap. 14. *In* P. C. Zhu (ed.), New biocides development: the combined approach of chemistry and microbiology. American Chemical Society, Washington, DC.
26. U.S. Food and Drug Administration. 2001. Outbreaks associated with fresh and fresh-cut produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce, chap. 4. *In* Analysis and evaluation of preventive control measures for the control and education/elimination of microbial hazards on fresh and fresh-cut produce. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Available at: http://www.cfan.fda.gov/~comm/ift3-4a.html. Accessed 3 July 2008.
27. U.S. Food and Drug Administration. 2007. FDA and states closer to identifying sources of *Escherichia coli* contamination associated with illnesses at Taco John's restaurants. U.S. Food and Drug Administration. Available at: http://www.fda.gov/bbs/topics/NEWS/2007/NEW01546.html. Accessed 3 July 2008.
28. Venkitanarayanan, K. S., G. O. I. Ezeike, Y.-C. Hung, and M. P. Doyle. 1999. Efficacy of electrolyzed oxidizing water for inactivation of *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 65:4276–4279.
29. Yang, H., B. L. Swem, and Y. Li. 2003. The effect of pH on inactivation of pathogenic bacteria on fresh-cut lettuce by dipping treatment with electrolyzed water. *J. Food Sci.* 68:1013–1017.