EFFICACY OF ELECTROLYZED OXIDIZING (EO) WATER AND CHLORINATED WATER FOR INACTIVATION OF *ESCHERICHIA COLI* O157:H7 ON STRAWBERRIES AND BROCCOLI

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ABSTRACT

Inoculated strawberries were treated with deionized water (control), electrolyzed oxidizing (EO) water (23 and 55 mg/L of residual chlorine), and chlorinated water (55 mg/L of residual chlorine), either with or without ultrasonication. Inoculated broccoli was treated with EO water containing 55 and 100 mg/L of residual chlorine and chlorinated water with 100 mg/L of residual chlorine. Treatments were conducted for 1 and 5 min at temperatures of 4 and 24C, respectively. Dipping strawberries and broccoli into EO water or chlorinated water significantly ($P < 0.05$*) reduced the* Escherichia coli *O157:H7 counts compared with inoculated controls. Dipping inoculated strawberries with chlorinated water or EO water with ultrasonication reduced* E. coli *O157:H7 cells by 0.7 to 1.9 log cfu/g depending on the treatment time and treatment solution temperature. Dipping inoculated broccoli into chlorinated water or EO water with ultrasonication for 1 or 5 min reduced the bacterial population by 1.2 to 2.2 log cfu/g. Significant (*$P < 0.05$*) reductions in pathogen populations were observed when produce was treated with EO water in conjunction with ultrasonication. Results revealed that EO water was either more than or as effective as chlorinated water in killing* E. coli *O157:H7 cells on strawberries and broccoli.*

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PRACTICAL APPLICATIONS

Electrolyzed oxidizing (EO) water could be used as a washing treatment to help ensure the safety of strawberries and broccoli. EO water is also more effective than chlorinated water with the same residual chlorine content to inactivate *Escherichia coli* O157:H7 on strawberries and broccoli.

INTRODUCTION

Americans are increasing their demand for and consumption of fresh produce, which is increasingly grown on large industrial-scale farming operations or is imported from foreign countries (Brandl 2006; Johnston *et al.* 2006). Outbreaks of human illnesses associated with the consumption of raw vegetables and fruits have also increased worldwide in the past decade (Nguyen-the and Carlin 1994; Brackett 1999; Mead *et al.* 1999). Changes in agronomic, harvesting, distribution, processing and consumption patterns and practices have contributed to this increase. Food-borne illness have been associated with *Escherichia coli*, *Salmonella*, *Listeria*, *Shigella*, *Bacillus*, *Aeromonas*, *Clostridium* and *Campylobacter* (Beuchat 1996; Fain 1996; Little *et al.* 1997), which are naturally present in some soils and have been isolated from fresh produce. Brandl (2006) stated that factors involved in the emergence of produce-linked outbreaks include intensification and centralization of production, wider distribution of produce over longer distances, increased importation of fresh produce, increased consumption of meals outside of the home, increased popularity of salad bars and increased consumption of fresh fruits, vegetables and fresh fruit juices.

The use of chlorine and chlorine-containing compounds (hypochlorite ions [OCl^-], chlorine [Cl_2] and hypochlorous acid [HOCl]) has been the most common disinfecting method since the 19th century (Nagawara *et al.* 1998; Nunes and Edmond 1999). Today, hypochlorite dips are commonly used for sanitizing fruits and vegetables during postharvest, particularly in the fresh-cut industry (Sapers and Simons 1998; Xu 1999; Park *et al.* 2001). However, the production of some trihalomethane (THM) byproducts, known to be carcinogenic, occur when chlorine reacts with organic matter (Richardson *et al.* 1998). Moreover, Park *et al.* (2001) reported that other chemical disinfectants have been used to reduce the populations of pathogens on fresh produce, but many of these chemicals have minimal effect on inactivating pathogens on fresh produce. Therefore, safer and more effective methods to inactivate pathogens on fresh produce are needed (Beuchat 1996, 1999).

Several scientists (Park *et al.* 2001; Su *et al.* 2007) stated that electrolyzed oxidizing (EO) water is nonthermally produced by electrolyzing sodium chloride solution through an electrolysis chamber, where the anode and cathode electrodes are separated by a diaphragm. Major advantages of using EO water for fresh produce antimicrobial treatments include the efficacy of EO water treatments. The EO water apparatus is easy to operate, relatively inexpensive and environmentally friendly due to the production of the disinfectant using only water with sodium chloride; thus, there is no need for handling potentially dangerous chemicals. In addition, the properties of EO water can be controlled at the site of production.

One of the studies done by Kim *et al.* (2000b) investigated the three main properties (pH, oxidation-reduction potential [ORP] and residual chlorine) of acidic EO water and reported that although both ORP and residual chlorine contributed to the bacterial inactivation, the ORP may be the primary property responsible for its bactericidal effect. Efficacy of EO water to inactivate *E. coli* O157:H7 and *Listeria monocytogenes* on lettuce (Park *et al.* 2001), *Campylobacter jejuni* on poultry (Park *et al.* 2002a; Kim *et al.* 2005), *Salmonella enteritidis* and *L. monocytogenes* on shell eggs (Park *et al.* 2005) and seafoods (Huang *et al.* 2005, 2006) have also been reported. In addition, EO water is effective in sanitizing different contact surfaces commonly found in food processing facilities (Park *et al.* 2002b; Ayebah and Hung 2005), and in eliminating *L. monocytogenes* biofilms on stainless steel (Kim *et al.* 2001; Ayebah *et al.* 2005, 2006). Applications of EO water for microbial inactivation on fresh produce have been reported on lettuce (Park *et al.* 2001; Koseki *et al.* 2003; Delaquis *et al.* 2004; Ongeng *et al.* 2006), tomatoes (Bari *et al.* 2003), cucumbers (Koseki *et al.* 2004) and strawberries (Koseki *et al.* 2003; Udompijitkul *et al.* 2007). Koseki *et al.* (2004) reported that EO water reduced aerobic mesophiles by less than one log unit and coliform bacteria and fungi by 1.0 to 1.5 log cfu/strawberry. Udompijitkul *et al.* (2007) found EO water achieved 1.5 log and 1.1 to 1.8 log cfu/strawberry additional reductions on *L. monocytogenes* and *E. coli* O157:H7 than water, respectively.

Ultrasonication has been shown to be a complementary way to enhance inactivation of *E. coli* O157:H7 with sanitizers (Peterson and Pitt 1999; Seymour *et al.* 2002; Kim *et al.* 2003). Seymour *et al.* (2002) reported that an additional one-log unit reduction was achieved by applying ultrasound to a chlorinated water wash on fresh produce. Kim *et al.* (2003) found that application of EO water in conjunction with ultrasonication enhanced the bactericidal effectiveness of EO water on alfalfa sprouts by 80%. Ultrasonic cavitation has been demonstrated to be the mechanism responsible for the destruction of bacterial cells during ultrasonication (Shukla 1992). When ultrasonication was applied in conjunction with a sanitizer during poultry processing, bacterial cells on poultry skin have become detached, resulting in microorganisms that are more susceptible to the sanitizer (Lillard 1993). It is expected that *E. coli* O157:H7 inactivation by ultrasonication and the addition of a sanitizer will most likely occur from the synergistic effects of physical and chemical mechanisms which occur during acoustic cavitation.

The study reported here was undertaken to investigate the efficacy of EO and chlorinated water treatments at various temperatures and for various lengths of time in inactivating *E. coli* O157:H7 on strawberries and broccoli. Additional application of ultrasonication to enhance the effectiveness of the sanitizer treatment was also investigated.

MATERIALS AND METHODS

Preparation of Inoculum

Five strains of *E. coli* O157:H7 (F500 [human fecal isolate], E0019 [calf fecal isolate], 932 [human fecal isolate], H1730 [lettuce outbreak isolate] and F4546 [sprout outbreak isolate]) were used. Each bacterial strain was cultured individually by transferring 10 µL loop inocula into 10 mL of tryptic soy broth (TSB, pH 7.3, Difco Laboratories, Detroit, MI) at 37 ± 2C for 24 h. Each strain was cultured successively at least three times at 24-h intervals before being used as an inoculum. Cells from 24- to 26-h static cultures incubated at 37 ± 2C were sedimented twice by centrifugation (2,000× g, 22C, 15 min) and pellets were resuspended in 10 mL of sterile 0.1% peptone water (pH 7.1). For each pathogen, equal portions (2 mL each) from each of the five strains were combined to make the inoculum. The inoculum (10 mL) was *c*. 10^8 cfu/ml. The concentration of this stock suspension was confirmed by making serial dilutions in sterile 0.1% peptone water and surface plating 0.1 mL portions on sorbitol MacConkey Agar and counting plates after 24 h of incubation at 37 ± 2C (Lukasik *et al*. 2000). Seven milliliters of the 10-mL inoculum were then added to a sterile 20-L plastic container containing 7 L of sterile 0.1% peptone water (pH 7.1, 24 ± 2C) and shaken gently at 120 rpm for 4 min to yield approximately 10^5 cfu/mL.

Preparation of Treatment Solutions

The following chemical treatments were evaluated for their efficacy in inactivating *E. coli* O157:H7 on strawberries and broccoli. EO waters (ROX-20TA EO water generator, Hoshizaki Electric Inc., Toyoake, Aichi, Japan) was produced at settings of 8.0 ± 0.4 A and 14.0 ± 0.4 A. Chlorinated water was produced by diluting $Ca(OCl)_2$ (certified for biological work, Fisher Scientific Co., Fair Lawn, NJ) to a residual chlorine concentration similar to the level produced in EO water at 14.0 ± 0.4 A. Deionized water was used as the control. Due to greater numbers of vegetative folds and crevices on broccoli, EO water was generated at a higher amperage (14.0 ± 0.4 and 20.0 ± 0.4 A) and chlorinated water with residual chlorine concentrations similar to EO water produced at 20.0 ± 0.4 A were used for the broccoli study. Detailed descriptions of EO water generation can be found in Kim *et al*. (2000b). To investigate the effects of treatment temperatures in killing microorganisms on strawberries and broccoli, treatment solutions were prepared in 2-L beakers at 24 ± 2C and 4 ± 2C. To achieve a 4C temperature, the treatment solutions were covered with aluminum foil and stored for 5 h at 2 ± 2C, before the treatment solutions were applied.

Residual chlorine concentrations were measured by an iodometric method using a digital titrator (Model16900, Hach Company, Loveland, CO). Treatment solution pH and ORP were measured in triplicate using a pH meter (ACCUMET, model 50, Denver Instrument Company, Denver, CO) with a pH and an ORP electrode.

Preparation of Produce

Strawberries (*Fragaria Xananassa*, Naturipe™, CA) at the same ripening stage (three-quarter to full red) and broccoli (*Brassica oleracea L. var Italica*) used in the evaluation were purchased from a local grocery store in Griffin, GA. They were stored for 12 h at 4 ± 2C in their original plastic box until experiments were conducted. Fruits were graded for uniformity of size and damaged fruits were removed. Strawberries with weights ranging from 25 to 30 g were used for this study. Broccoli was selected based on uniform head size and floret color without any visible decay. Broccoli florets (including 2 cm of the stems) were cut into segments with a diameter of *c*. 4 cm. Each of the broccoli florets had final weights of between 15 and 20 g. Sets of 16 strawberries were prepared at room temperature (24 ± 2C) with two strawberries for each of the eight treatment groups. Two samples were also used to determinate initial bacterial populations. For the broccoli study, each set of 16 broccoli florets was prepared at room temperature (24 ± 2C) with two broccoli florets for each of the eight treatment groups. Two samples were also used to determine initial bacterial populations. Inactivation of microorganisms on strawberries and broccoli was also tested by subjecting samples to treatment solutions described above in conjunction with ultrasonication (Model FS60, 44–48 kHz, Fischer Scientific Co., Fair Lawn, NJ).

Procedure for Inoculating Strawberries and Broccoli

Strawberries and broccoli were completely submerged into 7 L of sterile 0.1% peptone water containing 10^5 cfu/mL of a five-strain mixture of *E. coli* O157:H7 and gently shaken on a platform shaker (Model CIO, New Brunswick Scientific Inc., Edison, NJ) at 120 rpm for 4 min to ensure an even distribution of the organism. Samples were separated from the cell suspension by pouring the mixture over a double layer of sterile cheesecloth supported by a wire screen under a laminar flow hood. The strawberries and broccoli were then allowed to dry for 20 min and 60 min, respectively, under the hood at 24 ± 2C. To allow for attachment of *E. coli* O157:H7, inoculated strawberries and broccoli were stored in a cooler at 5 ± 2C for 24 h before being subjected to treatments.

Procedure for Treating Strawberries and Broccoli

Treatment of inoculated strawberries or broccoli was performed by immersing an individual whole sample in a sterile Nasco Whirl-pack® bag (18 oz) containing 125 mL of the treatment solution at either 24 ± 2C or 4 ± 2C for strawberries and 90 mL for broccoli, either with or without ultrasonication. On termination of the treatment, the treatment solution was then decanted and 125 mL of sterile neutralizing buffer for strawberries and 90 mL for broccoli was added before stomaching (Model 80, Seward, London, U.K.) samples for 1 min at medium speed for strawberries and for 6 min at high speed for broccoli to neutralize residual chlorine on the sample. Strawberries or broccoli treated with deionized water serving as controls were also placed into either 125 mL or 90 mL of sterile neutralizing buffer solution, respectively, and stomached as described above. Three independent replicate trials were conducted for each treatment solution (a total of six samples per treatment solution).

Bacterial Analysis

Strawberry or broccoli homogenate was serially (1:10) diluted in sterile 0.1% peptone water and surface-plated in duplicate (0.1 mL) on sorbitol MacConkey Agar. Presumptive *E. coli* O157:H7 colonies were counted after incubation at 37 ± 2C for 24 h with a colony counter (model Leica, Quebec, Darkfield). Selected colonies were confirmed by *E. coli* O157 latex agglutination test (Unipath-Oxoid, Colombia, MD). One milliliter of each homogenate was then inoculated into 10 mL of tryptic soy broth for enrichment to test for the presence of low numbers of survivors that could not be detected by direct plating.

Data Analysis

Data were analyzed using the general linear model procedures. Comparisons of means were performed using Duncan's multiple range tests (SAS 2000). All the statistical analyses were conducted at $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Properties of Treatment Solutions Used for the Strawberry Study

The mean pH, ORP and residual chlorine concentrations of treatment solutions at various temperatures are presented in Table 1. The pH and ORP of sterile deionized water at 4C was 4.9 and 299.4 mV, respectively, whereas at 24C, it was 5.4 and 294.6 mV. No residual chlorine was detected in deionized water.

Chlorinated water at 4 and 24C, had residual chlorine concentrations of 53.9 and 53.8 mg/L, respectively, closely matching the chlorine level of EO water generated at 14 A (55.5 mg/L at 4C and 53.6 mg/L at 24C). Chlorinated water, however, had a higher residual chlorine concentration than EO water produced at 8 A (23.9 and 22.3 mg/L, at 4 and 24C, respectively). The pH of the two EO waters ranged from 2.6 to 2.8 and was much lower than the pH of chlorinated water (8.2 to 8.4). The ORP of the two EO waters (1046.5 to 1067.6 mV) were much higher than the ORP of chlorinated water (668.8 to 715.1 mV).

Efficacy of Different Treatment Solutions for Inactivation of *E. coli* O157:H7 on Strawberries

Results of inactivation studies of *E. coli* O157:H7 on strawberries was temperature and time dependent. Additional ultrasonication treatment also affected the treatment efficiency. No detectable *E. coli* O157:H7 was found on the strawberries purchased from the supplier. The population of *E. coli*

TABLE 1.
PROPERTIES (PH, ORP AND RESIDUAL CHLORINE) OF SANITIZERS USED FOR BACTERIAL INACTIVATION STUDY ON STRAWBERRIES

Treatment	Temperature (C)	pH	ORP (mV)	Residual chlorine (mg/L)
Deionized water	4	4.9 ± 0.4*	299.4 ± 8.7*	0.0 ± 0.0†
	24	5.4 ± 1.4	294.6 ± 18.4	0.0 ± 0.0
EO water 8 A	4	2.8 ± 0.1	1,058.0 ± 6.7	23.9 ± 0.7
	24	2.7 ± 0.1	1,046.5 ± 3.6	22.3 ± 1.0
EO water 14 A	4	2.7 ± 0.1	1,067.6 ± 1.6	55.5 ± 1.1
	24	2.6 ± 0.1	1,059.5 ± 1.2	53.6 ± 0.9
Chlorinated water	4	8.2 ± 0.4	715.1 ± 39.7	53.9 ± 1.5
	24	8.4 ± 0.4	668.8 ± 42.8	53.8 ± 1.4

* Data are means ± standard deviation of six samples.
† Data are means ± standard deviation of eighteen samples.
ORP, oxidation-reduction potential.

O157:H7 recovered from the strawberry following inoculation was 3.43 ± 0.05 log cfu/g (mean ± standard deviation [SD]).

Increasing soaking times from 1 to 5 min reduced populations of the pathogen by 0.16 to 0.83 log cfu/g regardless of treatment solution or ultrasonication (Table 2). The greatest reduction due to the increase in soaking time was obtained with EO water generated at 8 A accompanied by ultrasonication at 4C, which produced 0.83 and 1.66 log cfu/g pathogen reductions when treated for 1 and 5 min, respectively.

Similar results were reported by Brackett (1987) who found that dipping vegetables in 100 mg/L chlorine for 10 s reduced bacterial concentrations by 2 log cfu/g and dipping in water alone reduced counts by 1 log cfu/g. Yu *et al*. (2001) also reported that dipping inoculated strawberries in 100 mg/L chlorine for 1 min reduced the pathogen population by 1.3 log cfu/g and dipping in water alone reduced counts by 0.8 log cfu/g. Gulati

TABLE 2.
EFFICACY OF SANITIZERS FOR INACTIVATION OF *ESCHERICHIA COLI* O157:H7 ON STRAWBERRIES

Treatment	Soak time (min)	Additional treatment	Bacteria reduction* (log cfu/g of strawberry)	
		Ultrasonication	Temperature (C)	
			4	24
Deionized water	1	No	0.67 ± 0.03 ab	0.35 ± 0.06 b
	5	No	0.92 ± 0.04 bc	0.62 ± 0.10 c
EO water 8 A	1	No	1.05 ± 0.01 cd	0.60 ± 0.03 c
	5	No	1.28 ± 0.03 e	0.76 ± 0.11 de
EO water 14 A	1	No	1.17 ± 0.04 de	0.76 ± 0.03 de
	5	No	1.58 ± 0.06 f	1.32 ± 0.03 g
Chlorinated water	1	No	1.24 ± 0.03 e	1.05 ± 0.03 f
	5	No	1.51 ± 0.12 f	1.43 ± 0.04 h
Deionized water	1	Yes	0.8 ± 0.06 a	0.22 ± 0.04 a
	5	Yes	1.15 ± 0.04 de	0.8 ± 0.04 e
EO water 8 A	1	Yes	0.83 ± 0.03 ab	0.68 ± 0.05 cd
	5	Yes	1.66 ± 0.12 fg	0.84 ± 0.02 e
EO water 14 A	1	Yes	1.49 ± 0.14 f	1.42 ± 0.02 h
	5	Yes	1.89 ± 0.10 h	1.62 ± 0.06 I
Chlorinated water	1	Yes	1.14 ± 0.24 de	0.86 ± 0.11 e
	5	Yes	1.79 ± 0.15 gh	1.14 ± 0.09 f

* The initial bacterial population recovered on strawberry was 3.43 ± 0.05 log cfu/g (mean ± standard deviation [SD]) and the bacterial suspension where strawberries were submerged was 4.96 ± 0.14 log cfu/g. Variations were from inoculum quantities and sample fruit weight. Values in the same column that are followed by the same letter are not significantly different ($P > 0.05$).
Data are means ± SD of six samples. All the samples were positive by enrichment.

et al. (2001) found sodium hypochlorite up to 400 mg/L achieved no additional reduction of calicivirus on strawberry than water wash. Udompijitkul *et al*. (2007) soaked strawberries in EO water (53 mg/L) and sodium hypochlorite (199 mg/L) for 5 min and found no additional reduction on *E. coli* O157:H7 than the distilled water treatment and were only 0.5 to 0.7 log unit better than the 0 min treatments. Lukasik *et al*. (2003) soaked strawberries in 43C sodium hypochlorite solution (50 mg/L) for 2 min and achieved 95.7% reductions on *E. coli* O157:H7. Combining EO water and ultrasonication treatments as reported in the current study achieved 1.9 log cfu/g pathogen reductions in strawberries and is higher than the 1 to 1.3 log cfu/g reductions reported in the literature (Yu *et al*. 2001; Koseki *et al*. 2004).

Moreover, an increase in treatment temperature influenced the survival of the pathogen as illustrated in Table 2. Decreasing the treatment temperature reduced the bacterial population by 0.35 log cfu/g regardless of treatment solution, time and ultrasonication. The greatest difference in bacterial reduction based on differences in treatment temperature occurred with 8 A EO water treatment for 5 min accompanied by ultrasonication, which produced 0.84 and 1.66 log cfu/g reductions at 24 and 4C, respectively.

Significant ($P < 0.05$) differences in pathogen populations occurred based on ultrasonication and reduced the pathogen population by an average of additional 0.16 log cfu/g. EO water generated at 14 A for 1 min at 24C had the greatest additional bacterial reduction due to ultrasonication (0.76 and 1.42 log cfu/g reductions without and with ultrasonication, respectively; Table 2).

Treating strawberries with EO water produced at 14 A for 5 min in conjunction with ultrasonication at 4C achieved the greatest reductions in *E. coli* O157:H7 (1.89 log cfu/g). There was no significant ($P > 0.05$) difference in the efficacy between chlorinated water and EO water produced at 14 A in killing *E. coli* O157:H7 for 5 min at 4C with or without the application of ultrasonication. EO water produced at 14 A was more effective in killing *E. coli* O157:H7 than EO water generated at 8 A for the same treatment time and conditions. Average reductions in populations of *E. coli* (calculated by pooling different treatment times, temperatures and ultrasonication treatments together, Table 2) were 1.48 and 0.97 log cfu/g with EO water produced at 14 and 8 A, respectively.

Properties of Sanitizers Used for the Broccoli Study

The mean pH, ORP and residual chlorine concentrations of sanitizers used for the study of broccoli are presented in Table 3. The pH and ORP of sterile deionized water at 4C were 4.7 and 337.3 mV, respectively, whereas at

TABLE 3.
PROPERTIES (PH, ORP AND RESIDUAL CHLORINE) OF SANITIZERS USED FOR BACTERIAL INACTIVATION STUDY ON BROCCOLI

Treatment	Temperature (C)	pH	ORP (mV)	Residual chlorine (mg/L)
Deionized water	4	4.7 ± 0.3*	337.3 ± 48.3*	0.0 ± 0.0†
	24	4.5 ± 0.3	334.4 ± 47.8	0.0 ± 0.0
EO water 14 A	4	2.7 ± 0.1	1,115.9 ± 35.3	54.8 ± 1.6
	24	2.5 ± 0.1	1,108.6 ± 37.8	54.1 ± 1.4
EO water 20 A	4	2.6 ± 0.1	1,126.0 ± 34.2	98.0 ± 1.8
	24	2.4 ± 0.1	1,116.9 ± 41.1	97.8 ± 1.7
Chlorinated water	4	9.0 ± 0.3	716.3 ± 46.2	100.0 ± 1.6
	24	8.8 ± 0.3	675.2 ± 37.6	99.3 ± 1.5

* Data are means ± standard deviation of six samples.
† Data are means ± standard deviation of 18 samples.

24C they were 4.5 and 334.4 mV. No residual chlorine was detected in deionized water.

Chlorinated water at 4 and 24C, with residual chlorine of 100.0 and 99.3 mg/L, respectively, closely matched the chlorine level of EO water generated at 20 A (98.0 mg/L at 4C and 97.8 mg/L at 24C). Residual chlorine concentrations of EO water produced at 14 A were 54.8 and 54.1 mg/L at 4 and 24C, respectively. The pH of the two EO waters ranged from 2.4 to 2.7, which was much lower than the pH of chlorinated water (8.8 to 9.0). The ORP of the two EO waters (1108.6 to 1126.0 mV) were also much higher than the ORP of chlorinated water (675.2 to 716.3 mV).

Efficacy of Different Treatment Solutions for Inactivation of *E. coli* O157:H7 on Broccoli

Inactivation efficacy of *E. coli* O157:H7 on broccoli was temperature, time and ultrasonication dependent (Table 4). No detectable *E. coli* O157:H7 was found on the broccoli purchased from the supplier. The population of *E. coli* O157:H7 recovered from the broccoli following inoculation was 4.18 ± 0.10 log cfu/g (mean ± SD).

Dipping broccoli in EO water or chlorinated water did not completely eliminate *E. coli* O157:H7, although it significantly ($P < 0.05$) reduced the *E. coli* O157:H7 counts compared with inoculated, undipped broccoli. Increasing soaking time from 1 min to 5 min reduced the bacterial population by an average of an additional 0.33 log cfu/g (Table 4). Longer treatment times (5 min) always achieved significantly ($P < 0.05$) higher reductions in pathogen populations than shorter treatment times (1 min) at 4C except when deionized

TABLE 4.
EFFICACY OF SANITIZERS FOR INACTIVATION OF *ESCHERICHIA COLI* O157:H7 ON BROCCOLI

Treatment	Soak time (min)	Additional treatment	Bacteria reduction* (log cfu/g of broccoli)	
		Ultrasonication	Temperature (C)	
			4	24
Deionized water	1	No	0.47 ± 0.05 a	0.43 ± 0.06 a
	5	No	0.71 ± 0.16 b	0.58 ± 0.05 a
EO water 14 A	1	No	1.36 ± 0.06 cd	1.28 ± 0.03 bc
	5	No	1.62 ± 0.06 ef	1.57 ± 0.09 bcde
EO water 20 A	1	No	1.51 ± 0.15 de	1.49 ± 0.10 bcde
	5	No	1.78 ± 0.06 fg	1.73 ± 0.24 cde
Chlorinated water	1	No	1.26 ± 0.14 c	1.24 ± 0.07 bc
	5	No	1.6 ± 0.23 ef	1.48 ± 0.20 bcde
Deionized water	1	Yes	0.46 ± 0.05 a	0.55 ± 0.14 a
	5	Yes	0.68 ± 0.23 ab	0.61 ± 0.17 a
EO water 14 A	1	Yes	1.24 ± 0.16 c	1.19 ± 0.15 b
	5	Yes	1.93 ± 0.02 g	1.84 ± 0.07 de
EO water 20 A	1	Yes	1.65 ± 0.16 ef	1.41 ± 0.15 bcd
	5	Yes	2.18 ± 0.18 h	1.99 ± 0.17 e
Chlorinated water	1	Yes	1.5 ± 0.07 de	1.87 ± 0.80 de
	5	Yes	1.82 ± 0.08 fg	1.96 ± 0.54 e

* The initial bacterial population recovered on broccoli was 4.18 ± 0.10 log cfu/g (mean ± standard deviation [SD]) and the bacterial suspension where broccoli were submerged was 4.91 ± 0.07 log cfu/g. Variations were from inoculum quantities and sample fruit weight. Values in the same column that are followed by the same letter are not significantly different ($P > 0.05$).
Data are means ± SD of six samples.

water was used in conjunction with ultrasonication. The greatest difference in pathogen populations based on soaking time was attended with 14 A EO water treated with ultrasonication at 4C which achieved 1.24 and 1.93 log cfu/g reductions for 1 and 5 min, respectively.

Behrsing *et al*. (2000) found that dipping inoculated broccoli in a hypochlorite solution reduced *E. coli* O157:H7 cells by approximately 1.7–2.5 log cfu/g depending on the time and concentration of free chlorine. Results obtained from the current study achieved similar reductions as reported by Behrsing *et al*. (2000). Generally, water washes only decreased initial microbial counts by 1 log cfu/g on fresh produce (Adams *et al*. 1989; Izumi 1999). It should be noted that the reductions seen in these experiments occurred under what can be considered ideal conditions where a fresh dipping solution was prepared for each sample. Hence, the reduction in viable *E. coli* O157:H7 cell numbers observed here or reported in the literature may be higher than those achieved under commercial conditions.

Moreover, the greatest temperature effect on bacterial reduction was obtained from treatments with chlorinated water for 1 min in conjunction with ultrasonication, which resulted in population reductions of 1.5 and 1.87 log cfu/g at 4 and 24C, respectively (Table 4). Significant ($P < 0.05$) differences in *E. coli* populations occurred between the treatments with and without ultrasonication (Table 4). Ultrasonication reduced the pathogen population by an average of 0.17 log cfu/g over non-ultrasonication treatments. Treatments with EO water generated at 20 A for 5 min at 4C achieved the greatest bacterial reduction due to ultrasonication resulting in 1.78 and 2.18 log cfu/g reductions, without and with ultrasonication, respectively.

Treating broccoli with EO water produced at 20 A for 5 min in conjunction with ultrasonication at 4C achieved the highest reductions of *E. coli* O157:H7 (2.2 log cfu/g). EO water produced at 20 A achieved an average 1.72 log cfu/g reduction of *E. coli* O157:H7 and is significantly ($P < 0.05$) higher than the average reduction of 1.59 log cfu/g achieved by chlorinated water (Table 4). The averaged reductions for chlorinated water and EO water produced at 14 A were 1.50 and 1.59 log cfu/g, respectively (Table 4). EO water generated at 14 A also had a lower efficiency in reducing the pathogen population than EO water produced at 20 A (1.50 versus 1.72 log cfu/g, respectively). On average, broccoli immersed in sterile deionized water reduced the bacterial population by 0.60 log cfu/g, which was less than the other sanitizer treatments (Table 4).

Comparison of the Efficacy of Sanitizers on the Two Types of Produce

The population of *E. coli* O157:H7 on inoculated broccoli (4.18 ± 0.10 log cfu/g) was greater than on strawberries (3.43 ± 0.05 log cfu/g). EO water treatments with water generated at the highest amperage (14 and 20 A for the strawberry and broccoli studies, respectively) for 5 min with ultrasonication at 4C resulted in the greatest reduction in populations of both types of produce (1.89 and 2.18 log cfu/g reductions for the strawberry and broccoli study, respectively). For both studies, increasing soaking time reduced the bacterial population on produce (an additional 0.30 log cfu/g reductions for strawberries and 0.33 log cfu/g for broccoli). Ultrasonication also achieved additional reductions in bacterial populations for both types of produce and resulted in an average additional 0.16 log cfu/g reduction on strawberries and a 0.17 log cfu/g reduction on broccoli. Decreasing the treatment solution temperature reduced the bacterial populations on strawberries by an average of 0.35 log cfu/g, whereas decreasing the treatment solution temperature for broccoli had no effect on bacterial reduction.

In both studies, it was observed that EO water could be more than or as efficient as chlorinated water in reducing bacterial counts due to acidic pH and presence of other oxidants in EO water including hydrogen peroxide, hydroxyl radical and hypochlorous acid (Shimizu and Huruzawa 1992; Park *et al.* 2001). The ORP of a solution is an indicator of the solution's oxidizing or reducing capacity, where positive and higher ORP values are correlated with greater oxidizing strength (McPherson 1993; Robbs *et al.* 1995; Jay 1996). Aerobic microorganisms require ORP levels of +200 to +800 mV for survival (Jay 1996). Because the ORP of EO water in this study was greater than 1000 mV, it played a critical role, in combination with low pH and free chlorine, in killing *E. coli* (Kim *et al.* 2000a). The EO water containing 55 and 100 mg/L chlorine had the strongest microbicidal effect for the strawberry and broccoli study, respectively.

Longer exposure times or higher residual chlorine concentrations for EO water should achieve additional microbial reductions. Zhang and Farber (1996), however, reported that the numbers of bacterial cells decreased only marginally with increased exposure time (from 1 to 10 min) on inoculated fresh-cut lettuce, regardless of the chlorine concentration. Brackett (1987) added that the action of chlorine against *L. monocytogenes* occurs primarily during the first 30 s of exposure. Low reductions observed in this study may be due to surface properties of the strawberry and broccoli which may have played a role in repelling water (Morris and Nguyen-The 1996; Behrsing *et al.* 2000; Yu *et al.* 2001). Sanitizer efficacy may have been reduced because of the rough surface of strawberries or broccoli and the presence of numerous surface-borne achenes, which provide sites for bacteria to attach and become less accessible to sanitizing solutions.

A significant ($P < 0.05$) difference was observed in the level of bacterial inactivation at the two different temperatures for the strawberry study, but not in the broccoli study (Tables 2 and 4). Behrsing *et al.* (2000) also found no significant differences in bacterial inactivation on broccoli treated with chlorinated water at temperatures between 4 and 25C. Moreover, several scientists reported that lowering the temperature and the pH of the chlorine solution was more effective in killing *L. monocytogenes* (El-Kest and Marth 1988) and resulted in less stability of available chlorine (Hoffman *et al.* 1981), respectively. However, another research done by Boyette *et al.* (1993) indicated that chlorine efficiency *in vitro* increases with temperature up to the point just prior to vaporization implying that *in vivo* effects may differ from those *in vitro*. Thus, Behrsing *et al.* (2000) states that the effectiveness of chlorine in killing pathogens at different temperatures is complex and involves a number of factors.

The effectiveness of the treatments was greater with ultrasonication and achieved an average of 0.3 log cfu/g greater reduction than without ultrasonication. Lillard (1993) indicated that ultrasonication detached bacteria from poultry skin and reduced counts by 1 to 1.5 log cfu/g by making the bacteria more susceptible to the chlorine. Kim *et al*. (2003) found that application of EO water in conjunction with ultrasonication enhanced the bactericidal effectiveness of EO water on alfalfa sprouts by 80%. Shukla (1992) also reported that when combined with a sanitizer, ultrasonic energy destroyed microorganisms by the physical forces of cavitation, which is the mechanical effect responsible for the destruction of bacterial cells.

CONCLUSIONS

Electrolyzed water produced at 14 A was as effective as chlorinated water containing 55 and 100 mg/L residual chlorine for strawberries and broccoli, respectively. EO water could be used to complement a production system that maintains cleanliness at all stages of production and postharvest handling, eliminating the need for chlorinated water, which may require of handling potentially dangerous concentrations to make dilutions needed for the applications. However, it cannot be assumed that EO water will completely eliminate pathogens and ensure that the produce is free of bacteria, which may be harmful to humans if consumed raw. Hence, the application of EO water technology in combination with other intervention methods in inactivating food-borne pathogens on fresh produce is recommended.

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REFERENCES

ADAMS, M.R., HARTLEY, A.D. and COX, L.J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiol. *59*, 1999–2006.

AYEBAH, B. and HUNG, Y.-C. 2005. Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. J. Food Process Eng. *28*, 247–264.

AYEBAH, B., HUNG, Y.-C. and FRANK, J.F. 2005. Enhancing the bactericidal effect of electrolyzed water on *Listeria monocytogenes* biofilms formed on stainless steel. J. Food Prot. *68*, 1375–1380.

AYEBAH, B., HUNG, Y.-C., KIM, C. and FRANK, J.F. 2006. Efficacy of electrolyzed water in the inactivation of planktonic and biofilm *Listeria monocytogenes* in the presence of organic matter. J. Food Prot. *69*, 2143–2150.

BARI, M.I., SABINA, Y., ISOBE, S., UEMURA, T. and ISSHIKI, K. 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.

BEHRSING, J., WINKLER, S., FRANZ, P. and PREMIER, R. 2000. Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. Postharvest Biol. Technol. *19*, 187–192.

BEUCHAT, L.R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Prot. *59*, 204–216.

BEUCHAT, L.R. 1999. Survival of enterohemorragic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. J. Food Prot. *62*, 845–849.

BOYETTE, M.D., RITCHIE, D.F., CARBALLO, S.J., BLANKENSHIP, S.M. and SANDERS, D.C. 1993. Chlorination and postharvest disease control. Horttechnology *3*, 395–400.

BRACKETT, R.E. 1987. Antimicrobial effect of chlorine on *Listeria monocytogenes* by chlorine. J. Food Prot. *50*, 999–1003, 1008.

BRACKETT, R.E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. Postharvest Biol. Technol. *15*, 305–311.

BRANDL, M.T. 2006. Fitness of human enteric pathogens on plants and implications for food safety. Annu. Rev. Phytopathol. *44*, 367–392.

DELAQUIS, P.J., FUKUMOTO, L.R., TOIVONEN, P.M.A. and CLIFF, M.A. 2004. Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. Postharvest Biol. Technol. *31*, 81–91.

EL-KEST, S.E. and MARTH, E.H. 1988. Inactivation of *Listeria monocytogenes* by chlorine. J. Food Prot. *51*, 520–524.

FAIN, A.R. 1996. A review of the microbiological safety of fresh salads. Dairy Food Environ. Sanit. *16*, 146–149.

GULATI, B.R., ALLWOOD, P.B., HEDBERG, C.W. and GOYAL, A.M. 2001. Efficacy of commonly used disinfectants for the inactivation of calicivirus on strawberry, lettuce, and a food-contact surface. J. Food Prot. *64*, 1430–1434.

HOFFMAN, P.N., DEATH, J.E. and COATES, D. 1981. The stability of sodium hypochlorite solutions. In *Disinfectants: Their Use and Evaluation of Effectiveness* (C.H. Collins, M.C. Allwood, S.F. Bloomfield and A. Fox, eds.) The Society for Applied Bacteriology Technical Series No. 16 pp. 77–83, Academic Press, London.

HUANG, Y.-R., HSIEH, H.-S., LIN, S.-Y., LIN, S.-J., HUNG, Y.-C. and HWANG, D.-F. 2005. Application of electrolyzed oxidizing water on the reduction of bacterial contamination for seafood. Food Control. *17*, 987–993.

HUANG, Y.-R., SHIAU, C.-Y., HUNG, Y.-C. and HWANG, D.-F. 2006. Change of hygienic quality and freshness in tuna treated with electrolyzed oxidized water and carbon monoxide gas during refrigerated and frozen storage. J. Food Sci. *71*, M127–M133.

IZUMI, H. 1999. Electrolyzed water as a disinfectant for fresh–cut vegetables. J. Food Sci. *64*, 536–539.

JAY, J.M. 1996. *Modern Food Microbiology*, 5th Ed., pp. 48–49, Chapman & Hall, New York.

JOHNSTON, L.M., JAYKUS, L.A., MOL, D., ANCISO, J., MORA, B. and MOE, C.L. 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. Int. J. Food Microbiol. *112*, 83–95.

KIM, C., HUNG, Y.-C. and BRACKETT, R.E. 2000a. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. Int. J. Food Microbiol. *61*, 199–207.

KIM, C., HUNG, Y.-C. and BRACKETT, R.E. 2000b. Roles of oxidation-reduction potential (ORP) in electrolyzed oxidizing (EO) water and chemically modified water for the inactivation of food-related pathogens. J. Food Prot. *63*, 19–24.

KIM, C., HUNG, Y.-C., BRACKETT, R.E. and FRANK, J.F. 2001. Inactivation of *Listeria monocytogenes* biofilms by electrolyzed oxidizing water. J. Food Proc. Pres. *25*, 91–100.

KIM, C., HUNG, Y.-C., BRACKETT, R.E. and LIN, C.-Y. 2003. Efficacy of electrolyzed water in inactivating *Salmonella* on alfalfa seeds and sprouts. J. Food Prot. *66*, 208–214.

KIM, C., HUNG, Y.-C. and RUSSELL, S.M. 2005. Efficacy of electrolyzed (EO) water in the prevention and removal of fecal material attachment and its microbicidal effectiveness during simulated industrial poultry processing. Poult. Sci. *84*, 1778–1784.

KOSEKI, S., YOSHIDA, Y., KAMITANI, Y. and ITOH, K. 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.

KOSEKI, S., YOSHIDA, Y., ISOBE, S. and ITOH, K. 2004. Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. J. Food Prot. *67*, 1247–1251.

LILLARD, H.S. 1993. Bactericidal effect of chlorine on attached *Salmonella* with and without sonification. J. Food Prot. *56*(8), 716–717.

LITTLE, C.L., MONSEY, H.A., NICHOLS, G.L. and DE LOUVOIS, J. 1997. The microbial quality of refrigerated salads and crudites. PHLS Microbiol. Digest *14*, 142–146.

LUKASIK, J., BRADLEY, M.L., SCHOTT, T.M., HSU, W.-Y., FARRAH, S.R. and TAMPLIN, M.L. 2000. Elution, detection, and quantification of polio I, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157:H7 from seeded strawberries and tomatoes. J. Food Prot. *64*, 292–297.

LUKASIK, J., BRADLEY, M.L., SCHOTT, T.M., DEA, M., KOO, A., HSU, W.-Y., BARTZ, J.A. and FARRAH, S.R. 2003. Reduction of poliovirus 1, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. J. Food Prot. *66*, 188–193.

MCPHERSON, L.L. 1993. Understanding ORPs in the disinfection process. Water Eng. Manage. *40*(11), 29–31.

MEAD, P.S., SLUTSKER, L., DIETZ, V., MCCAIG, L.F., BRESEE, J.S., SHAPIRO, C., GRIFFIN, P.M. and TAUXE, R.V. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. *5*, 607–625.

MORRIS, C.E. and NGUYEN-THE, C. 1996. The role of plant surface bacteria in the hygienic and market quality of minimally processed vegetables. In *Aerial Plant Surface Microbiology* (C.E. Morris, P.C. Nicot and C. Nguyen-The, eds.) pp. 191–208, Plenum Press, New York.

NAGAWARA, S., GOTO, T., NARA, M., OZAWA, Y., HOTTA, K. and ARATA, Y. 1998. Spectroscopic characterization and the pH dependence of bactericidal activity of the aqueous chlorine solution. Anal. Sci. *14*, 691–698.

NGUYEN-THE, C. and CARLIN, F. 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci. Nutr. *34*, 371–401.

NUNES, M.C.N. and EDMOND, J.P. 1999. Chlorinated water treatments affects post harvest quality of green bell peppers. J. Food Qual. *22*, 353–361.

ONGENG, D., DEVLIEGHERE, F., DEBEVERE, J., COOSEMANS, J. and RYCKEBOER, J. 2006. The efficacy of electrolyzed oxidizing water for inactivating spoilage microorganisms in process water and on minimally processed vegetables. Int. J. Food Microbiol. *109*, 187–197.

PARK, C.M., HUNG, Y.-C., DOYLE, M.P., EZEIKE, G.O.I. and KIM, C. 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. J. Food Sci. *66*, 1368–1372.

PARK, H., HUNG, Y.-C. and BRACKETT, R.E. 2002a. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. Int. J. Food Microbiol. *72*, 77–83.

PARK, H., HUNG, Y.-C. and KIM, C. 2002b. Effectiveness of electrolyzed water as a sanitizer for treating different surfaces. J. Food Prot. *65*, 1276–1280.

PARK, C.-M., HUNG, Y.-C., LIN, C.-S. and BRACKETT, R.E. 2005. Efficacy of electrolyzed water in inactivating *Salmonella enteritidis* and *Listeria monocytogenes* on shell eggs. J. Food Prot. *68*, 986–990.

PETERSON, R.V. and PITT, W.G. 1999. The effect of frequency and power density on the ultrasonically enhanced killing of biofilm-sequestred *Escherichia coli*. Colloids Surfaces B: Biointerfaces *17*, 219–227.

RICHARDSON, S.D., THRUSTON, J.R., CAUGHRAN, A.D., COLETTE, T.V., PATTERSON, T.W., PATTERSON, K.S. and LYKINS, B.W., Jr 1998. Chemical by-product of chlorine and alternative disinfectants. Food Technol. *52*(4), 58–61.

ROBBS, P.G., BARTZ, J.A., BRECHT, J.K. and SARGENT, S.A. 1995. Oxidation–reduction potential of chlorine solutions and their toxicity to *Erwinia caratovora* subsp. *Caratovora* and *Geotrichum candidum*. Plant Dis. *79*, 158–162.

SAPERS, G.M. and SIMONS, G.F. 1998. Hydrogen peroxide disinfections of minimally processed fruits and vegetables. Food Technol. *52*, 48–52.

SAS. 2000. *Statistical Analysis System. User's Guide, Versions 8*, SAS Institute, Inc., Cary, NC.

SEYMOUR, I.J., BURFOOT, D., SMITH, R.L., COX, L.A. and LOCKWOOD, A. 2002. Ultrasound decontamination of minimally processed fruits and vegetables. Int. J. Food Sci. Technol. *37*, 547–557.

SHIMIZU, Y. and HURUZAWA, T. 1992. Antiviral, antibacterial, and antifungial actions of electrolyzed oxidizing water through electrolysis. Dent. J. *37*, 1055–1062.

SHUKLA, T.P. 1992. Microwave ultrasonics in food processing. Cereal Foods World *37*, 332–333.

SU, Y.-C., LIU, C. and HUNG, Y.-C. 2007. Electrolyzed water: Principles and applications. In *New Biocides Development: The Combined Approach of Chemistry and Microbiology*. American Chemical Society, Washington, DC.

UDOMPIJITKUL, P., DAESCHEL, M.A. and ZHAO, Y. 2007. Antimicrobial effect of electrolyzed oxidizing water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fresh strawberries (*Fragaria x ananassa*). J. Food Sci. *72*, M397–M406.

XU, L. 1999. Use of ozone to improve the safety of fresh fruits and vegetables. Food Technol. *53*, 58–62.

YU, K., NEWMAN, M.C., ARCHBOLD, D.D. and HAMILTON-KEMP, T.R. 2001. Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. J. Food Prot. *64*, 1334–1340.

ZHANG, S. and FARBER, J.M. 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microbiol. *13*, 311–321.