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Effectiveness of Electrolyzed Acidic Water in Killing *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* on the Surfaces of Tomatoes

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ABSTRACT

A study was conducted to evaluate the efficacy of electrolyzed acidic water, 200-ppm chlorine water, and sterile distilled water in killing *Escherichia coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* on the surfaces of spot-inoculated tomatoes. Inoculated tomatoes were sprayed with electrolyzed acidic water, 200-ppm chlorine water, and sterile distilled water (control) and rubbed by hand for 40 s. Populations of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* in the rinse water and in the peptone wash solution were determined. Treatment with 200-ppm chlorine water and electrolyzed acidic water resulted in 4.87- and 7.85-$\log_{10}$ reductions, respectively, in *Escherichia coli* O157:H7 counts and 4.69- and 7.46-$\log_{10}$ reductions, respectively, in *Salmonella* counts. Treatment with 200-ppm chlorine water and electrolyzed acidic water reduced the number of *L. monocytogenes* by 4.76 and 7.54 $\log_{10}$ CFU per tomato, respectively. This study's findings suggest that electrolyzed acidic water could be useful in controlling pathogenic microorganisms on fresh produce.

Fresh fruits and vegetables are an essential part of the diets of people around the world. Nutritionists emphasize the importance of fruits and vegetables in a healthy diet, and researchers have recommended the consumption of at least five servings per day *(16).* Possibly as a result of these efforts, over the past decade there has been an increase in the consumption of fresh fruits and vegetables, concurrent with increased global distribution, which has made more varieties of produce available year-round *(5).* An increased number of microbial infections associated with the consumption of fresh fruits and vegetables also have been documented in recent years. Enterohemorrhagic *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* are foodborne pathogens of major public health concern worldwide. A variety of foods, including poultry, eggs, meat, milk, fruits, and vegetables, have been implicated as vehicles for one or more of these pathogens in outbreaks of foodborne illness *(2, 6, 8).* Effective methods of reducing or eliminating pathogens in food are important for the successful implementation of hazard analysis critical control point programs by the food industry and for the establishment of critical control points in restaurants, homes, and other food service units. Raw agricultural produce is washed with water in the industry; however, washing alone does not render a product completely free of pathogens. Although many chemicals that are generally recognized as safe, including organic acids, have antimicrobial activity against foodborne pathogens, none, when used individually at concentrations acceptable in foods, can eliminate large populations of pathogens. The treatment of fruits and vegetables with water containing sanitizers, including chlorine, may reduce but not eliminate pathogens on the surfaces of produce *(2, 29).* Hence, there is a need for, and interest in, the development of practical and effective antimicrobial treatments for the inactivation of pathogenic microorganisms on foods.

Electrolyzed acidic water is the product of a new concept developed in Japan. Research carried out in Japan revealed that electrolysis of deionized water containing a low concentration of sodium chloride (0.1%) in an electrolysis chamber where anode and cathode electrodes were separated by a diaphragm imparted strong bactericidal and virucidal properties to the water collected from the anode. Water from the anode normally has a pH of $\leq$2.7, an oxidation reduction potential (ORP) of $>$1,100 mV, and a free-chlorine concentration of 10 to 80 ppm *(23).* Electrolyzed acidic water has been used experimentally in Japan by medical and dental professionals for treating wounds or disinfecting medical equipment. The objective of this study was to evaluate the efficacy of electrolyzed acidic water in killing *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* on fresh produce with a view to its potential application to foods and food contact surfaces as an antimicrobial treatment.

MATERIALS AND METHODS

Test strains. The strains studied and their sources were as follows. Enterohemorrhagic *E. coli* O157:H7 strains CR-3, MN-28, MY-29, and DT-66 were isolated from bovine feces. *Salmonella* Enteritidis strains SE-1, SE-3, and SE-4 (from chicken feces)

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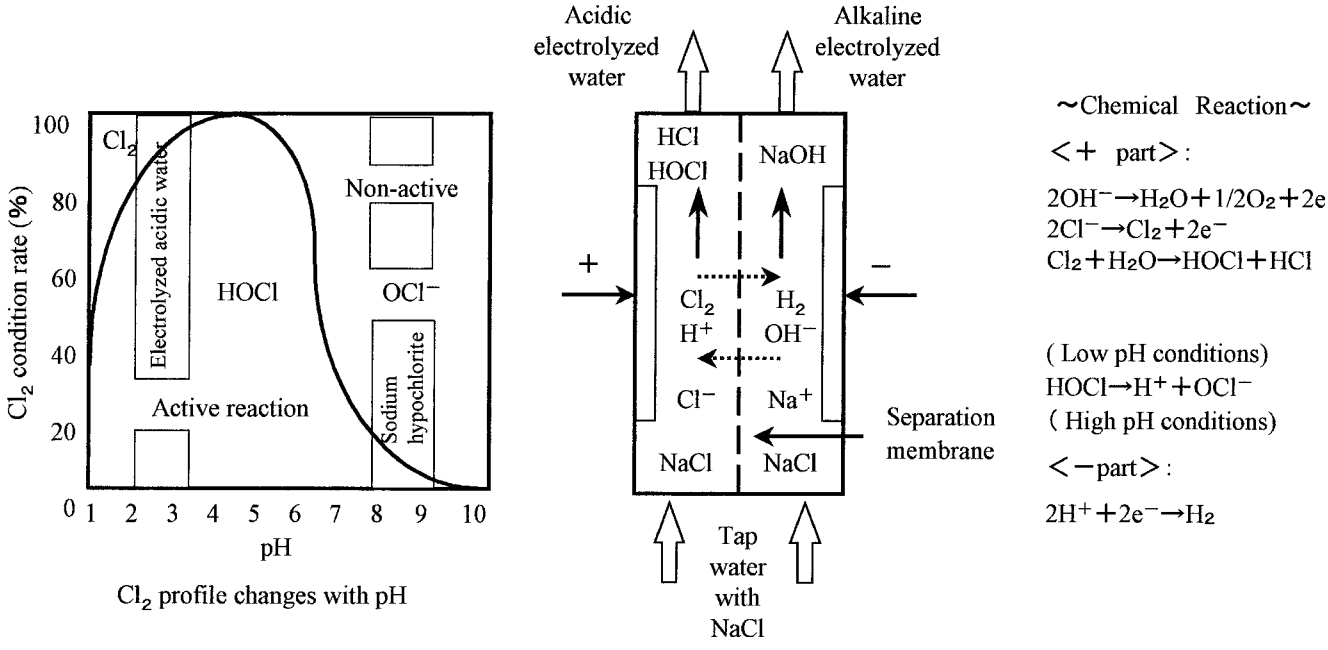

FIGURE 1. *Principle of EO water.*

and SE-2 (from bovine feces) were provided by the Laboratory of Zoonosis, National Institute of Animal Health, Tsukuba, Japan. *Salmonella* strain IFO-3313 (unknown) was obtained from the Institute for Fermentation, Osaka, Japan. *L. monocytogenes* strains ATCC 43256 (from Mexican-style cheese) and ATCC 49594 (derived from *L. monocytogenes* strain Scott A) (American Type Culture Collection, Manassas, Va.) and JCM 7676 (from roast beef), JCM 7672 (from salami sausage), and JCM 7671 (from lax ham) (Japan Collection of Microorganisms) were used in this study.

To minimize the growth of microorganisms naturally present on tomatoes, all test strains of *E. coli* O157:H7 and *Salmonella* were adapted for growth in tryptic soy broth (TSB, pH 7.3; Nissui Seiyaku, Tokyo, Japan) supplemented with nalidixic acid (50 μg/ml). Although some gram-positive microorganisms are less affected by nalidixic acid, *L. monocytogenes* strains were grown in tryptose phosphate broth (pH 7.0; Difco) containing 50 μg of nalidixic acid per ml before their use as an inoculum. Plating on media containing nalidixic acid greatly minimized interference with colony development by naturally occurring microorganisms, thus facilitating the detection of the test pathogen on recovery media.

EO water. Electrolyzed oxidizing (EO) water was generated with a model ROX-20TA EO water generator (Hoshizaki Electric Company Ltd., Toyoake, Aichi, Japan). The current passing through the EO water generator and the voltage between the electrodes were set at 19.8 A and 10 V, respectively. A 12% solution of sodium chloride (Sigma Chemical Co., St. Louis, Mo.) and deionized water from the laboratory supply line were simultaneously pumped into the equipment. The display indicator was activated and observed until the machine stabilized at a reading of 19.8 A. The EO water was collected from the appropriate outlet in sterile containers and was used within 2 to 3 h for the microbial study. Samples to be used for the determination of pH, ORP, and free-chlorine concentration were also collected at the same time. The pH of the tested solution was measured with a pH meter (D-22, Horiba, Kyoto, Japan). The ORP was measured with an ORP meter (HM-60V, TOA Electronics Ltd., Tokyo, Japan). The initial concentration of available chlorine in the test solution was quantified by spectrophotometric analysis at 530 nm with the use of *N,N*-diethyl-*p*-phenylen-diamine *(26).*

The theoretical sequence of chemical reactions involved in the production of EO water is shown in Figure 1. During electrolysis, sodium chloride dissolved in deionized water in the electrolysis chamber dissociates into negatively charged chloride (Cl) and hydroxy (OH) ions and positively charged sodium (Na^+) and hydrogen (H^+) ions. The chloride and hydroxy ions are adsorbed to the anode, with each ion releasing an electron to become a radical. The chloric and hydroxy radicals combine, forming hypochlorous acid (HOCl), which separates from the anode. Two chloric radicals can also combine to produce chlorine gas. In the cathode section, each positively charged sodium ion receives an electron and becomes metallic sodium. The metallic sodium combines with water molecules, forming sodium hydroxide and hydrogen gas. A bipolar membrane separating the electrodes enhances the electrolysis of water to produce strong acidic and alkali waters from the anode and cathode, respectively. Electrolyzed acidic water at pH 2.7 contains available chlorine as a form of hypochlorous acid (HOCl), which is more effective in disinfection than hypochlorite (ClO^-) (Fig. 1).

Produce evaluation. Produce selected for evaluation consisted of red ripe tomatoes (90 ± 20 g each) to which no oil or wax had been applied. Tomatoes (*Lycopersicum esculentum* Mill.) var. Momotaro, used in each experiment, were purchased from a local supermarket and stored at room temperature (22 ± 2°C) for a maximum of 2 days before they were used in experiments.

Preparation of inocula. Each strain of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* was cultured in TSB (10 ml) supplemented with 50 μg of nalidixic acid per ml at 37°C. Cultures were transferred to TSB by loop at three successive 24-h intervals immediately before they were used as inocula. Cells of each strain were collected by centrifugation (3,000 × *g,* 10 min, 20°C) and resuspended in 5 ml of phosphate-buffered saline (PBS, pH 7.2) solution. The inoculum was maintained at 22 ± 2°C and applied to tomatoes within 1 h of preparation.

Procedure for inoculation. Tomatoes (22 ± 2°C) were placed stem-end-down in a biosafety cabinet. Within a 3-cm-diameter circle on the top of the tomato, 100 μl of a suspension of cells in PBS was applied with a micropipettor, with care taken to avoid placing inoculum on the blossom scar. To prevent the inoculum from running off the sides of the tomatoes and to facilitate drying, small, approximately equal numbers of cells were applied to 10 to 12 spots. The total numbers of *E. coli* O157: H7, *Salmonella,* and *L. monocytogenes* cells applied to the tomatoes were determined by surface plating the serially diluted inoculum in 0.1% peptone on appropriate enumeration media. Inoculated tomatoes were stored at 22 ± 2°C for 30 min before they were used for various treatments.

Survivability study. The survivability study involved the determination of numbers of pathogens that could be recovered from inoculated tomatoes after inoculation and drying at 22 ± 2°C for 30 min. *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* were evaluated separately. Three replicate trials for each pathogen were performed, with three or four tomatoes being used for each trial. Thirty minutes postinoculation, each inoculated tomato in a biosafety cabinet was placed in a sealable Pyxon-20 (ELMEX Co. Ltd., Tokyo, Japan) bag containing 20 ml of EO water, 200-ppm chlorine water, or sterile distilled water and thoroughly rubbed by hand for 20 s. Sterile distilled water (200 ml) was then added to the bag, and the tomato was rinsed with vigorous agitation for 20 s (rinse step). The tomato was transferred to a clean bag, 20 ml of sterile 0.1% peptone water was added, and the tomato was thoroughly rubbed by hand for 40 s (residual wash step). Populations of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* in rinse water and peptone wash water were determined as described below. The tomato was removed from the bag, and the area of skin (ca. 10 g) originally inoculated with the pathogen was excised with a sterile scalpel. Care was taken to remove tomato tissue (pulp) no more than 0.1 cm below the skin surface. The excised tomato skin-pulp was then combined with 20 ml of sterile 0.1% peptone in a new quart bag and macerated between the fingers until the pulp was removed from the skin. The homogenate (macerate) was then analyzed to determine the level (CFU/ml) of the test pathogen. Excised tomato skin-pulp not macerated in sterile 0.1% peptone was also analyzed to determine populations of test pathogens.

Chlorine water treatment studies. The chlorine solution was prepared by adding sodium hypochlorite (Wako Chemical, Japan) solution to distilled water (vol/vol). The effectiveness of chlorine and electrolyzed acidic water in killing *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* separately applied to the surfaces of tomatoes was determined according to the basic protocol developed by Beuchat et al. *(3).*

Microbiological analysis. Single- and mixed-strain suspensions of each pathogen in PBS were serially diluted in 0.1% sterile peptone water. Duplicate 0.1-ml quantities of appropriately diluted suspensions of *E. coli* O157:H7 and *Salmonella* were surface plated on tryptic soy agar (TSA) and TSA supplemented with 0.1% pyruvic acid and 50 μg of nalidixic acid per ml (TSAPN). In addition, diluted *E. coli* O157:H7 suspensions were surface plated onto sorbitol MacConkey agar (Nissui) supplemented with cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter) (CT-selective supplement, Oxoid) (CT-SMAC) and CT-SMAC containing 50 μg of nalidixic acid per ml (CT-SMACN); samples containing *Salmonella* were plated on bismuth sulfite agar (BSA; Difco) and BSA supplemented with 50 μg of nalidixic acid per ml (BSAN). Diluted suspensions (0.1 ml) of *L. monocytogenes* were surface plated in duplicate on tryptose phosphate agar (TPA), TPA supplemented with 0.1% pyruvic acid and 50 μg of nalidixic acid per ml (TPAPN), and modified Oxford medium (Oxoid) supplemented with 50 μg of nalidixic acid per ml (MOXN). This medium contains 55.5 g of *Listeria* selective agar base (Oxoid CM 856, Unipath-Oxoid US) per liter of deionized water, 0.01 g of colistin methanesulfate (Sigma) per liter, 0.02 g of ceftazadime pentahydrate (Glaxo Group Research Ltd., Ware, Hertfordshire, UK) per liter, 50 μg of nalidixic acid per ml, and 5.0 g of agar (Difco) per liter. All ingredients except ceftazadime pentahydrate and nalidixic acid were combined and sterilized by heating at 121°C for 15 min. Ceftazadime solution and nalidixic acid were added to the molten agar before the medium was poured into petri plates.

Inoculated enumeration media were incubated at 37°C for 24 to 28 h before presumptive colonies of each pathogen were counted. At least five presumptive colonies of *E. coli* O157:H7 were confirmed with the *E. coli* O157 direct immunoassay test kit (Universal Health Watch, Columbia, Md.). *Salmonella* confirmation was carried out by testing reactions on triple sugar iron (Nissui) slants. Randomly picked presumptive colonies of *L. monocytogenes* were confirmed with API Listeria diagnostic kits.

Peptone wash water and phosphate-buffered macerate of skin-pulp from tomatoes prepared in the survivability study were analyzed for populations of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes.* Serially diluted peptone wash water (0.1 ml) from tomatoes inoculated with *E. coli* O157:H7 were surface plated in duplicate on TSAPN and CT-SMACN, samples from tomatoes inoculated with *Salmonella* were plated on TSAPN and BSAN, and samples inoculated with *L. monocytogenes* were plated on TPAPN and MOXN. Quadruplicate 0.25-ml samples of skin-pulp homogenate were also plated on appropriate recovery media. Plates were incubated at 37°C for 18 to 24 h before presumptive colonies of pathogens were counted and confirmed as described above.

Populations of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* in rinse water (200 ml) and peptone wash water (20 ml) after the rinsing or washing of tomatoes treated with chlorine (200 ppm), electrolyzed acidic water, or sterile water (control) were determined. Undiluted samples (0.25 ml in quadruplicate and 0.1 ml in duplicate) and samples (0.1 ml in duplicate) serially diluted in sterile 0.1% peptone were surface plated on TSAPN and CT-SMACN for the recovery of *E. coli* O157:H7, on TSAPN or BSAN for the recovery of *Salmonella,* and on MOXN for the recovery of *L. monocytogenes.* Quantities (100 ml) of *E. coli* broth (Nissui), selenite cystine broth (Nissui), and *Listeria* enrichment broth (Oxoid), each supplemented with 50 μg of nalidixic acid per ml, were then combined with the 20-ml quantities of peptone wash solution in the bags containing the treated, rinsed, washed tomatoes that had been inoculated with *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes,* respectively, and incubated for 24 to 26 h at 37°C. Cultures were streaked on appropriate recovery media for presumptive colonies, and confirmation of randomly selected colonies was carried out as described above.

Sensory evaluation. The quality of treated and untreated uninoculated tomatoes was evaluated by panelists (10 judges) selected from the National Food Research Institute who were experienced with sensory panels. The evaluation was based on a five-point hedonic scale (1, unacceptable; 2, limited quality for consumption; 3, medium; 4, good; 5, very good). The sensory characteristics of appearance, color, and taste were assessed for raw tomatoes after 6 h of treatment at room temperature. For each of the quality attributes, three of each treated and untreated to-

TABLE 1. *Physicochemical properties of tested solutions*[a]

Solution	pH	ORP (mV)	ACC (ppm)
Distilled water	7.1 ± 0.15	355 ± 7.0	—
AcEW	2.6 ± 0.1	1,140 ± 7.0	30.3 ± 3.1
NaOCl	9.3 ± 0.2	638 ± 18	198.5 ± 5.8

[a] Values are means ± standard deviations ($n = 5$). ORP, oxidation reduction potential; ACC, available chlorine concentration; AcEW, electrolyzed acidic water; NaOCl, sodium hypochlorite.

matoes were examined and/or tested by the judges. Rejection of a sample was based on a score of <2.5, which was the quality criterion used for both treated and untreated tomatoes.

Statistical analyses. All trials were replicated three times. Reported plate count data represented the mean values obtained for three individual trials, with each of these values being obtained from duplicate samples. Sensory evaluation tables represented mean values ± standard deviations obtained from three individual trails. Significant differences in plate count data were established by the least significant difference at the 5% level of significance.

RESULTS

The physicochemical properties of the tested solutions are shown in Table 1. The mean pHs of acidic electrolyzed water, chlorine water, and distilled water were 2.6, 9.3, and 7.1, respectively. The ORP values for acidic electrolyzed water, chlorine water, and distilled water were 1,140, 638, and 355 mV, respectively. The available chlorine concentrations of acidic electrolyzed water and chlorine water were 30.3 and 198.5 ppm, respectively. No free chlorine was detected in distilled water.

The results of studies undertaken to determine the efficacy of treatments with 200-ppm chlorine water and electrolyzed acidic water in killing *E. coli* O157:H7 on tomatoes are reported in Table 2. The numbers of *E. coli* O157:H7 cells applied, as calculated by plating of the inoculum on CT-SMACN and TSAPN, were 7.63 and 7.85 $\log_{10}$ CFU per tomato, respectively. The pHs of water rinses from tomatoes treated with chlorine and electrolyzed acidic water were 7.0 and 6.22, respectively, whereas the pHs of peptone washes were 7.2 and 6.8, respectively. Since the water rinse was diluted in 0.1% peptone before application to CT-SMACN and TSAPN, the residual effects of sanitizers or pH on *E. coli* O157:H7 would be minimal or nonexistent. Note that an additional 20 s elapsed from the time the tomato was rubbed to the time cells were plated or diluted in 0.1% peptone. Therefore, rubbing for 40 s represented approximately 1 min of exposure to sanitizers. The number of *E. coli* O157:H7 cells in rinse water represents the number removed from the control and treated tomatoes, and thus the number available to cross-contaminate other tomatoes or food preparation surfaces in food service or home use situations. The number of *E. coli* O157:H7 cells in peptone wash solutions represents the population remaining on the surfaces of control and treated tomatoes. The number of *E. coli* O157:H7 cells in macerated skin-pulp represents the population firmly attached to the skins of control and treated tomatoes. Overall, CT-SMACN and TSAPN were equally suitable for *E. coli* O157:H7 colony development regardless of the exposure of cells to sanitizers. Washing with water (control) resulted in a reduction of 2.10 $\log_{10}$ CFU per tomato; further significant reductions of 4.31 and 7.63 $\log_{10}$ CFU per tomato were achieved by treatment with 200-ppm chlorine water and electrolyzed acidic water, respectively, as detected on CT-SMACN. However, even with these large reductions, the pathogen was detected by enrichment of treated tomatoes.

Shown in Table 3 are the results of studies undertaken to determine the efficacy of 200-ppm chlorine water and electrolyzed acidic water in killing *Salmonella* on tomatoes. The numbers of *Salmonella* cells applied, as calculated by plating of the inoculum on BSAN and TSAPN, were 7.36 and 7.46 $\log_{10}$ CFU per tomato, respectively. TSAPN and BSAN were equally suitable for colony development, regardless of the exposure of cells to sanitizers. Washing with water (control) resulted in a reduction of 2.11 $\log_{10}$ CFU

TABLE 2. *Populations of* Escherichia coli *O157:H7 recovered from treated and untreated tomatoes*

Recovery medium	Treatment	Population (log CFU/tomato) recovered from[a]: Water rinse	Peptone wash	Skin-pulp homogenates	Reduction[b]	Enrichment[c]
CT-SMACN	None	ND	6.04	2.13	−0.54	9
	Water	3.27	1.23	1.03	2.10	9
	Chlorine	1.78	0.87	0.67	4.31	9
	EO water	<1.0[d]	<1.0[d]	<1.0[d]	7.63	2
TSAPN	None	ND	5.64	2.37	−0.16	
	Water	3.19	2.16	0.72	1.78	
	Chlorine	1.69	0.73	0.56	4.87	
	EO water	<1.0[d]	<1.0[d]	<1.0[d]	7.85	

[a] Mean value for three replicate experiments ($P \leq 0.05$). ND, not determined.

[b] Populations (log CFU per tomato) recovered from rinse water, peptone wash, and macerated skin-pulp combined and then subtracted from the population applied to the tomatoes. Populations applied to tomatoes were 7.63 and 7.85 $\log_{10}$ CFU per tomato as detected on CT-SMACN and TSAPN, respectively.

[c] Number of tomatoes (three tomatoes per replicate) on which *E. coli* O157:H7 was detected by enrichment.

[d] No colonies were observed during the incubation period.

TABLE 3. *Populations of* Salmonella *recovered from treated and untreated tomatoes*

Recovery medium	Treatment	Population (log CFU/tomato) recovered from[a]: Water rinse	Peptone wash	Skin-pulp homogenates	Reduction[b]	Enrichment[c]
BSAN	None	ND	4.95	2.79	−0.38	9
	Water	2.77	1.36	1.12	2.11	9
	Chlorine	1.63	0.76	0.43	4.54	9
	EO water	<1.0[d]	<1.0[d]	<1.0[d]	7.36	3
TSAPN	None	ND	5.12	2.23	−0.31	
	Water	2.96	1.57	1.03	1.90	
	Chlorine	1.37	0.83	0.57	4.69	
	EO water	<1.0[d]	<1.0[d]	<1.0[d]	7.46	

[a] Mean value for three replicate experiments ($P \leq 0.05$). ND, not detected.
[b] Populations (log CFU per tomato) recovered from rinse water, peptone wash, and macerated skin-pulp combined and then subtracted from the population applied to the tomatoes. Populations applied to tomatoes were 7.36 and 7.46 $\log_{10}$ CFU per tomato as detected on BSAN and TSAPN, respectively.
[c] Number of tomatoes (three tomatoes per replicate) on which *Salmonella* was detected by enrichment.
[d] No colonies were observed during the incubation period.

per tomato; further significant reductions of 4.54 and 7.36 $\log_{10}$ CFU per tomato were achieved by treatment with 200-ppm chlorine water and electrolyzed acidic water, respectively, as detected on BSAN. However, even with these large reductions, the pathogen was detected by enrichment of treated tomatoes.

Results of experiments undertaken to determine the efficacy of sanitizers in killing *L. monocytogenes* on the surfaces of tomatoes are summarized in Table 4. The numbers of *L. monocytogenes* cells applied, as calculated by plating of the inoculum on MOXN and TPAPN, were 7.54 and 7.59 $\log_{10}$ CFU per tomato, respectively. Overall, TPAPN was more suitable for *L. monocytogenes* colony development than MOXN was, regardless of the treatment of inoculated tomatoes with sanitizers. This finding indicates that some of the cells were injured and unable to resuscitate in the presence of selective chemicals in MOXN. This phenomenon was particularly evident when numbers of *L. monocytogenes* recovered from peptone wash samples on MOXN and TPAPN were compared. Treatment with water resulted in a reduction of 2.14 $\log_{10}$ CFU per tomato; treatment with 200-ppm chlorine water and electrolyzed acidic water significantly reduced populations by 4.76 and 7.54 $\log_{10}$ CFU per tomato, respectively.

DISCUSSION

Test strains of pathogens from different food sources were chosen for evaluation. The method evaluated involved four- or five-strain mixtures of each pathogen, which is consistent with currently accepted practices for use in studying the survival and growth of pathogens in food *(9, 10, 12, 28)* and with Scientific Advisory Panel recommendations *(11)*. The use of multiple-strain inocula likewise represents a conservative strategy, since the mixture may provide a more representative challenge than a single strain for the sanitization of products. Thus, data should more accurately

TABLE 4. *Populations of* Listeria monocytogenes *recovered from treated and untreated tomatoes*

Recovery medium	Treatment	Population (log CFU/tomato) recovered from[a]: Water rinse	Peptone wash	Skin-pulp homogenates	Reduction[b]	Enrichment[c]
MOXN	None	ND	5.34	2.35	−0.15	9
	Water	3.09	1.33	0.98	2.14	9
	Chlorine	1.49	0.93	0.36	4.76	9
	EO water	<1.0[d]	<1.0[d]	<1.0[d]	7.54	3
TPAPN	None	ND	5.41	2.53	−0.35	
	Water	3.23	1.49	1.12	1.75	
	Chlorine	1.54	1.07	0.47	4.51	
	EO water	<1.0[d]	<1.0[d]	<1.0[d]	7.59	

[a] Mean value for three replicate experiments ($P \leq 0.05$). ND, not detected.
[b] Populations (log CFU per tomato) recovered from rinse water, peptone wash, and macerated skin-pulp combined and then subtracted from the population applied to the tomatoes. Populations applied to tomatoes were 7.54 and 7.59 $\log_{10}$ CFU per tomato as detected on MOXN and TPAPN, respectively.
[c] Number of tomatoes (three tomatoes per replicate) on which *L. monocytogenes* was detected by enrichment.
[d] No colonies were observed during the incubation period.

predict physiological sensitivities of pathogenic strains occasionally present on produce and should also provide a conservative estimate of risk to public health. All strains examined were adapted to grow in the presence of 50 μg of nalidixic acid per ml, one of several markers used to evaluate the survival of bacterial pathogens in food products with potentially large numbers of interfering background microflora. Antibiotic-resistant markers have been widely used in studies to determine the fate of pathogens in nonsterile foods, including fresh produce *(22),* meats *(7),* and milk *(25).* With the use of spot inoculation, a specified number of cells can be applied to the surface of produce, and the decrease in the number of viable cells during drying or under other pretreatment conditions can be more accurately assessed. Spot inoculation also facilitates the application of a large population of the test pathogen.

We used a short holding time in this experiment because a longer holding time at room temperature reduces populations to levels appropriate for use in sanitizer efficacy studies. For example, the population of *E. coli* O157:H7 decreased from 6.88 to 3.85 $\log_{10}$ CFU per tomato within 2 h at 22 ± 2°C *(3).* Therefore, it may be difficult to use tomatoes containing large numbers of *E. coli* O157:H7 cells to test the efficacy of sanitizers. The existence of a large proportion of desiccation-stressed and injured cells throughout the ≥24-h drying period would likely be demonstrated as a decrease in tolerance to sanitizers, thereby potentially giving an overestimation of lethal activity. However, *Salmonella* and *L. monocytogenes* survived in adequate numbers for tests of susceptibility to the lethal activity of sanitizers.

The antagonistic effects of chlorine and low pH on microorganisms are well documented. Although organic acids (with low pHs) and hypochlorite solutions (with free chlorine) have been used widely in treatments for killing foodborne bacteria in the food industry, systems involving high ORP values (1,000 mV) have not commonly been used. The ORP of a solution is an indicator of its ability to oxidize or reduce, with positive and higher ORP values being correlated with greater oxidizing strengths *(15, 20, 21).* An ORP of +200 to +800 mV is optimal for the growth of aerobic microorganisms, whereas an optimum range of 200 to 400 mV is favorable for the growth of anaerobic microorganisms *(15).* Since the ORP of EO water in this study was >1,100 mV, ORP likely played an influential role (in combination with low pH and free chlorine) in killing microorganisms *(1).* It is hypothesized that the low pH in EO water sanitizes the outer membranes of bacterial cells, thereby enabling hypochlorous acid to enter the bacterial cells more efficiently *(27).* Moreover, Nakagawara et al. *(24)* showed that the microbicidal activity of electrolyzed acidic water depends primarily on the chemical equilibrium of Cl_2, HOCl, and ClO^-. These investigators also showed that the microbicidal activity of electrolyzed acidic water is quantitatively correlated with the concentration of hypochlorous acid that exists in the solution.

There have been reports on the antimicrobial and antiviral activities of electrolyzed acidic water produced by the electrolysis of an aqueous sodium chloride solution with an instrument in which the anode and the cathode are separated by a membrane to form two compartments *(13, 17).* The application of this technique to areas other than chlorine production, such as agriculture, water treatment, and food sanitation, is new. Recently, electrolyzed acidic water has been reported to be effective as a disinfectant for fruits and vegetables *(14, 18, 19).*

TABLE 5. *Sensory evaluation of treated and untreated tomatoes at room temperature*

Parameter	Score for tomatoes[a]	
	Treated with EO water	Untreated
Taste	4.6 ± 0.25 A	4.6 ± 0.25 A
Color	4.8 ± 0.20 AB	4.8 ± 0.24 A
Appearance	4.5 ± 0.22 ABC	4.6 ± 0.20 AB

[a] Mean ± standard deviation ($n = 10$). Means with different letters in the same row are significantly different ($P \leq 0.05$). The values of the hedonic scale were as follows: 1, unacceptable; 2, limited quality for consumption; 3, medium; 4, good; 5, very good. The rejection criterion was a score of 2.5.

The effects of electrolyzed acidic water on the three pathogens were evaluated at ambient temperature in the interest of developing home use antibacterial treatments for unprocessed fresh produce. Although chlorine is highly effective in killing pathogenic microorganisms in simple aqueous systems, its antibacterial effects on microorganisms on foods are minimal, especially in the presence of organic materials that convert chlorine into inactive forms *(4).*

No significant influence on the appearance, taste, or color of tomatoes was observed after treatment with electrolyzed acidic water (Table 5). These results, in combination with those of the efficacy studies, suggest that electrolyzed acidic water could be applied to control *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* on the surfaces of tomatoes.

The results of this study reveal that electrolyzed acidic water is highly effective in killing *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* on the surfaces of tomatoes, indicating the potential for its application for the decontamination of fresh produce contact surfaces. An advantage of electrolyzed acidic water is that it can be produced with tap water, with no added chemicals other than sodium chloride. A large-scale series of experiments using this method must be carried out to determine the reproducibility of the results obtained in the studies reported here.

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Erratum

In the article "Effectiveness of Electrolyzed Acidic Water in Killing *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* on the Surfaces of Tomatoes," *Journal of Food Protection* 66(4):542–548, in Tables 2, 3, and 4, log values of microbial populations were added without first taking the antilog to calculate the reduction values, and thus the residual microbial populations are overstated in control, water, and 200-ppm chlorine water.

The actual reduction values in the control, water, and 200-ppm chlorine cases in Table 2 should be read 1.59, 4.35, and 5.75 log CFU/tomato, respectively, in CT-SMACN medium and 2.21, 4.62, and 6.08 log CFU/tomato, respectively, in TSAPN medium. In Table 3 those values are 2.40, 4.56, and 5.65 log CFU/tomato, respectively, in BSAN medium, and 2.34, 4.48, and 5.93 log CFU/tomato, respectively, in TSAPN medium. In Table 4, the values will be 2.20, 4.44, and 5.92 log CFU/tomato, respectively, in MOXN medium, and 2.18, 4.35, and 5.90 log CFU/tomato, respectively, in TPAPN medium. However, this error does not occur in electrolyzed oxidizing (EO) water data, because no colonies were recovered from rinse water, peptone wash, and skin-pulp homogenates.

TABLE 2. *Populations of* E. coli *O157:H7 recovered from treated and untreated tomatoes*

Recovery medium	Treatment	Population (log CFU/tomato) recovered from[a]: Water rinse	Peptone wash	Skin-pulp homogenates	Reduction[b]	Enrichment[c]
CT-SMACN	None	ND	6.04	2.13	1.57	9
	Water	3.27	1.23	1.03	4.35	9
	Chlorine	1.78	0.87	0.67	5.75	9
	EO water	<1.0	<1.0	<1.0	7.63	2
TSAPN	None	ND	5.64	2.37	2.21	
	Water	3.19	2.16	0.72	4.62	
	Chlorine	1.69	0.73	0.56	6.08	
	EO water	<1.0	<1.0	<1.0	7.85	

[a] Mean values of three replicate experiments ($P \leq 0.05$). ND, not determined; <1.0, no colonies were observed during the incubation period.
[b] Populations (log CFU/tomato) recovered in rinse water, peptone wash, and macerated skin-pulp combined and then subtracted from the population applied to the tomatoes. Populations applied to tomatoes were 7.63 and 7.85 log CFU/tomato as detected on CT-SMACN and TSAPN, respectively.
[c] Number of tomatoes (three tomatoes per replicate) on which *E. coli* O157:H7 was detected by enrichment.

TABLE 3. *Populations of* Salmonella *recovered from treated and untreated tomatoes*

Recovery medium	Treatment	Population (log CFU/tomato) recovered from[a]: Water rinse	Peptone wash	Skin-pulp homogenates	Reduction[b]	Enrichment[c]
BSAN	None	ND	4.95	2.79	2.40	9
	Water	2.77	1.36	1.12	4.56	9
	Chlorine	1.63	0.76	0.43	5.65	9
	EO water	<1.0	<1.0	<1.0	7.36	3
TSAPN	None	ND	5.12	2.23	2.34	
	Water	2.96	1.57	1.03	4.48	
	Chlorine	1.37	0.83	0.57	5.93	
	EO water	<1.0	<1.0	<1.0	7.46	

[a] Mean values of three replicate experiments ($P \leq 0.05$). ND, not determined; <1.0, no colonies were observed during the incubation period.
[b] Populations (log CFU/tomato) recovered in rinse water, peptone wash, and macerated skin-pulp combined and then subtracted from the population applied to the tomatoes. Populations applied to tomatoes were 7.36 and 7.46 log CFU/tomato as detected on BSAN and TSAPN, respectively.
[c] Number of tomatoes (three tomatoes per replicate) on which *Salmonella* was detected by enrichment.

TABLE 4. *Populations of* Listeria monocytogenes *recovered from treated and untreated tomatoes*

Recovery medium	Treatment	Population (log CFU/tomato) recovered from[a]: Water rinse	Peptone wash	Skin-pulp homogenates	Reduction[b]	Enrichment[c]
MOXN	None	ND	5.34	2.35	2.20	9
	Water	3.09	1.33	0.98	4.44	9
	Chlorine	1.49	0.93	0.36	5.92	9
	EO water	<1.0	<1.0	<1.0	7.54	3
TPAPN	None	ND	5.41	2.53	2.18	
	Water	3.23	1.49	1.12	4.35	
	Chlorine	1.54	1.07	0.47	5.90	
	EO water	<1.0	<1.0	<1.0	7.59	

[a] Mean values of three replicate experiments ($P \leq 0.05$). ND, not determined; <1.0, no colonies were observed during the incubation period.

[b] Populations (log CFU/tomato) recovered in rinse water, peptone wash, and macerated skin-pulp combined and then subtracted from the population applied to the tomatoes. Populations applied to tomatoes were 7.54 and 7.59 log CFU/tomato as detected on MOXN and TPAPN, respectively.

[c] Number of tomatoes (three tomatoes per replicate) on which *Listeria monocytogenes* was detected by enrichment.