Efficacy of Electrolyzed Oxidizing Water for Inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*

KUMAR S. VENKITANARAYANAN,[1] GABRIEL O. EZEIKE,[2] YEN-CON HUNG,[2]
AND MICHAEL P. DOYLE[2]*

*Department of Animal Science, University of Connecticut, Storrs, Connecticut 06269,[1]
and Center for Food Safety and Quality Enhancement, College of Agricultural and
Environmental Sciences, University of Georgia, Griffin, Georgia 30223-1797[2]*

The efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* was evaluated. A five-strain mixture of *E. coli* O157:H7, *S. enteritidis*, or *L. monocytogenes* of approximately 10^8 CFU/ml was inoculated in 9 ml of electrolyzed oxidizing water (treatment) or 9 ml of sterile, deionized water (control) and incubated at 4 or 23°C for 0, 5, 10, and 15 min; at 35°C for 0, 2, 4, and 6 min; or at 45°C for 0, 1, 3, and 5 min. The surviving population of each pathogen at each sampling time was determined on tryptic soy agar. At 4 or 23°C, an exposure time of 5 min reduced the populations of all three pathogens in the treatment samples by approximately 7 log CFU/ml, with complete inactivation by 10 min of exposure. A reduction of ≥7 log CFU/ml in the levels of the three pathogens occurred in the treatment samples incubated for 1 min at 45°C or for 2 min at 35°C. The bacterial counts of all three pathogens in control samples remained the same throughout the incubation at all four temperatures. Results indicate that electrolyzed oxidizing water may be a useful disinfectant, but appropriate applications need to be validated.

Enterohemorrhagic *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* are food-borne pathogens of major public health concern in the United States. A variety of foods, including poultry, eggs, meat, milk, fruits, and vegetables, have been implicated as vehicles of one or more of these pathogens in outbreaks of food-borne illness (2, 4, 5). The Pathogen Reduction program of the U.S. Department of Agriculture Food Safety and Inspection Service recommends antimicrobial treatments as a method for reducing or inactivating pathogenic bacteria in foods (13). Effective methods of reducing or eliminating pathogens in foods are important to the successful implementation of Hazard Analysis and Critical Control Point (HACCP) programs by the food industry and for the establishment of critical control points in restaurants, homes, and other food service units. Washing of raw agricultural produce with water is practiced in the industry; however, washing alone does not render the product completely free from pathogens. Although many chemicals generally recognized as safe (GRAS), including organic acids, possess antimicrobial activity against food-borne pathogens, none can eliminate high populations of pathogens when they are used individually at concentrations acceptable in foods. Treatments of fruits and vegetables with water containing sanitizers, including chlorine, may reduce but not eliminate pathogens on the surface of produce (2, 14). Hence, there is a need for, and interest in, developing practical and effective antimicrobial treatments for the inactivation of pathogenic microorganisms on foods.

Electrolyzed oxidizing water (EO 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 (EO water). Water from the anode normally has a pH of 2.7 or lower, an oxidation-reduction potential (ORP) greater than 1,100 mV, and a free-chlorine concentration of 10 to 80 ppm (10). EO water has been experimentally used 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 EO water for killing *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* with a view to its potential application to foods and food contact surfaces as an antimicrobial treatment.

Bacterial culture and media. Five strains each of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* were used for the study. The five strains of *E. coli* O157:H7 (with origins in parentheses following strain designations) were E06 (milk), E08 (meat), E10 (meat), E16 (meat), and E22 (calf feces). The *S. enteritidis* isolates included SE180 (human), SE457 (egg), SE565 (salad), SE294 (egg), and SE1697 (human). The five strains of *L. monocytogenes* were LM ATCC 19117 (sheep), LM101 (salami), LM109 (pepperoni), LM116 (cheese), and LM201 (milk). The *E. coli* O157:H7 and *L. monocytogenes* strains, but not ATCC 19117, were isolated by one of the authors, whereas the *S. enteritidis* isolates were obtained from the Centers for Disease Control and Prevention, Atlanta, Ga. The strains of each pathogen were cultured separately in 100 ml of sterile tryptic soy broth (TSB) (Difco Laboratories, Detroit, Mich.) in 250-ml Erlenmeyer flasks at 37°C for 24 h with agitation (150 rpm). Following incubation, 10 ml of each culture was sedimented by centrifugation (4,000 × *g* for 20 min), washed, and resuspended in 10 ml of 0.1% peptone water (pH 7.1). The optical density of the suspension was determined and adjusted with 0.1% peptone water to 0.5 at 640 nm (representing approximately 10^9 CFU/ml). The bacterial population in each culture was confirmed by plating 0.1-ml portions of appropriately di-

* Corresponding author. Mailing address: Center for Food Safety and Quality Enhancement, College of Agricultural and Environmental Sciences, University of Georgia, 1109 Experiment St., Griffin, GA 30223-1797. Phone: (770) 228-7284. Fax: (770) 229-3216. E-mail: mdoyle @cfsqe.griffin.peachnet.edu.

TABLE 1. Inactivation of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* by EO water at 4 or 23°C

Bacterial species	Temp (°C)	Surviving bacterial population (mean log CFU/ml) after exposure for:				EO water property		
		0 min	5 min	10 min	15 min	pH	ORP (mV)	Free chlorine (ppm)
E. coli O157:H7	4	7.98 ± 0.04	<1.0[a]	0[b]	0[b]			
Control		7.98 ± 0.04	7.99 ± 0.07	7.96 ± 0.06	7.99 ± 0.04	2.36 ± 0.03	1,153 ± 3	86.3 ± 5.4
S. enteritidis		7.74 ± 0.08	1.06 ± 0.15	0[b]	0[b]			
Control		7.74 ± 0.08	7.68 ± 0.09	7.61 ± 0.11	7.60 ± 0.12	2.48 ± 0.03	1,153 ± 2	83.5 ± 7.8
L. monocytogenes		7.91 ± 0.05	1.34 ± 0.37	0[b]	0[b]			
Control		7.91 ± 0.05	7.88 ± 0.06	7.87 ± 0.06	7.91 ± 0.03	2.63 ± 0.03	1,160 ± 4	43.0 ± 4.6
E. coli O157:H7	23	8.04 ± 0.07	<1.0[a]	0[b]	0[b]			
Control		8.04 ± 0.07	7.97 ± 0.03	7.99 ± 0.07	7.76 ± 0.42	2.37 ± 0.01	1,155 ± 1	82.3 ± 2.2
S. enteritidis		7.76 ± 0.08	<1.0[a]	0[b]	0[b]			
Control		7.76 ± 0.08	7.65 ± 0.09	7.73 ± 0.08	7.69 ± 0.10	2.45 ± 0.12	1,151 ± 1	82.0 ± 5.8
L. monocytogenes		7.89 ± 0.10	1.25 ± 0.33	0[b]	0[b]			
Control		7.89 ± 0.10	7.83 ± 0.06	7.85 ± 0.04	7.85 ± 0.07	2.63 ± 0.04	1,158 ± 5	48.5 ± 4.1

[a] Positive by enrichment.
[b] Negative by enrichment and no detectable survivors by a direct plating procedure.

luted culture on tryptic soy agar (TSA) (Difco Laboratories) plates and incubating the plates at 37°C for 48 h. For each pathogen, equal portions from each of the five strains were combined, and 1 ml of the suspension was used as the inoculum (10^9 CFU).

EO water. 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 5 min for the microbial study. Samples for determination of the pH, ORP, and free-chlorine concentration also were collected simultaneously.

Sample inoculation and treatments. A volume of 9 ml of EO water (treatment) or sterile deionized water (control) was transferred to separate, sterile screw-cap tubes, and the caps were tightly closed. The tubes were placed in a water bath in order to prewarm the water samples to the desired temperature. To each tube containing 9 ml of EO water or deionized water, 1 ml (equivalent to 10^9 CFU) of the five-strain mixture of *E. coli* O157:H7, *S. enteritidis*, or *L. monocytogenes* was added, and the samples were incubated in a water bath (Pharmacia LKB, Piscataway, N.J.) at 4°C for 0, 5, 10, and 15 min; at 23°C for 0, 5, 10, and 15 min; at 35°C for 0, 2, 4, and 6 min; and at 45°C for 0, 1, 3, and 5 min. Following each incubation, the number of viable cells in each sample was determined by plating 0.1-ml portions directly or after serial (1:10) dilutions in 0.1% peptone water on duplicate TSA plates. Colonies of the inoculated pathogen were enumerated on TSA plates after

incubation at 37°C for 48 h. A volume of 1 ml of the inoculated solution (treatment or control) after exposure to each temperature-time combination was also transferred to separate 250-ml Erlenmeyer flasks containing 100 ml of sterile TSB and incubated at 37°C for 24 h. Following enrichment in TSB, the culture was streaked on either sorbitol MacConkey agar no. 3 (Oxoid Division, Unipath Co., Ogdensburg, N.Y.) (for *E. coli* O157:H7), xylose lysine deoxycholate agar (Gene-Trak, Framingham, Mass.) (for *S. enteritidis*), or Oxford agar (Gene-Trak) (for *L. monocytogenes*), and the plates were incubated at 37°C for 24 h. Representative colonies of *E. coli* O157:H7 and *S. enteritidis* from the respective plates were confirmed by the *E. coli* O157:H7 latex agglutination assay (Remel Microbiology Products, Lenexa, Kans.) and the *Salmonella* latex test (Oxoid), respectively. The colonies of *L. monocytogenes* on Oxford agar were confirmed by the API-20E diagnostic test kit (Biomerieux, Hazelwood, Mo.). At least duplicate samples of treatments and controls were assayed at each sampling time, and the entire study with each pathogen was replicated three times.

The pH and ORP of the EO water were measured in duplicate samples immediately after sampling by using pH and ORP electrodes (model 50, ACCUMET meter; Denver Instrument Company, Denver, Colo.). The free-chlorine concentration was determined by an iodometric method using a digital titrator (model 16900; Hach Company, Loveland, Colo.). The assay was verified periodically by using a 100 ± 0.05 ppm chlorine standard solution (Orion Research Inc., Beverly, Mass.).

Statistical analysis. For each treatment, the data from the independent replicate trials were pooled and the mean value and standard deviation were determined (11).

The mean pH, ORP, and free-chlorine concentration of EO water at the different temperatures used for treatment are presented in Tables 1 through 3. The mean pH and ORP of sterile deionized water were 7.1 ± 0.15 and 355 ± 7.0 mV, respectively. No free chlorine was detected in deionized water.

TABLE 2. Inactivation of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* by EO water at 35°C

Bacterial species	Surviving bacterial population (mean log CFU/ml) after exposure for:				EO water property		
	0 min	2 min	4 min	6 min	pH	ORP (mV)	Free chlorine (ppm)
E. coli O157:H7	7.97 ± 0.03	0[b]	0[b]	0[b]	2.38 ± 0.00	1,154 ± 1	84.3 ± 4.6
Control	7.97 ± 0.03	7.94 ± 0.04	7.96 ± 0.03	7.94 ± 0.04			
S. enteritidis	7.68 ± 0.14	<1.0[a]	0[b]	0[b]	2.44 ± 0.04	1,153 ± 1	79.8 ± 3.3
Control	7.68 ± 0.14	7.63 ± 0.06	7.59 ± 0.11	7.64 ± 0.11			
L. monocytogenes	7.91 ± 0.10	0[b]	0[b]	0[b]	2.48 ± 0.05	1,159 ± 4	73.3 ± 1.8
Control	7.91 ± 0.10	7.88 ± 0.11	7.86 ± 0.08	7.81 ± 0.12			

[a] Positive by enrichment.
[b] Negative by enrichment and no detectable survivors by a direct plating procedure.

EO water had major antibacterial activity at 4 and 23°C on the five-strain mixtures of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* (Table 1). At time zero, both treatment and control samples for all three pathogens had approximate mean bacterial counts of 8.0 log CFU/ml. At 5 min of exposure at 4°C, the *E. coli* O157:H7 count in the treatment samples was reduced to less than 1.0 log CFU/ml (detected only by enrichment in TSB for 24 h), whereas the populations of *S. enteritidis* and *L. monocytogenes* were slightly greater than 1.0 log CFU/ ml. All three pathogens decreased to undetectable levels (as determined by both plating and enrichment procedures) after 10 min of exposure to EO water at 4°C. However, no differences in bacterial counts were observed in the control samples throughout the study. At 5 min of exposure at 23°C, the populations of *E. coli* O157:H7 and *S. enteritidis* in the treatment samples decreased to less than 1.0 log CFU/ml, whereas the *L. monocytogenes* count was reduced to 1.25 log CFU/ml. In agreement with the results obtained at 4°C, all three pathogens were undetectable after 10 min of contact with EO water at 23°C.

E. coli O157:H7, *S. enteritidis*, and *L. monocytogenes* were more rapidly inactivated by EO water at 35 or 45°C (Tables 2 and 3) than at 4 or 23°C. At 35°C, the populations of *E. coli* O157:H7 and *L. monocytogenes* in the treated samples decreased to undetectable levels within 2 min of exposure to EO water, whereas *S. enteritidis* was detected only by enrichment of the treated sample in TSB. After 1 min of exposure to EO

water at 45°C, *E. coli* O157:H7 was killed completely (a reduction of approximately 8.0 log CFU/ml), whereas the populations of *S. enteritidis* and *L. monocytogenes* were reduced by approximately 7.0 log CFU/ml. The bacterial counts of all three pathogens in control samples remained the same throughout the study at both 35 and 45°C.

The theoretical sequence of chemical reactions involved in the production of EO water can be summarized as follows (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 (e^-) 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.

The antagonistic effects of chlorine and low pH on microorganisms are well documented. Although organic acids (with low pH) and hypochlorite solution (with free chlorine) have been used widely in treatments for killing food-borne bacteria

TABLE 3. Inactivation of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* by EO water at 45°C

Bacterial species	Surviving bacterial population (mean log CFU/ml) after exposure for:				EO water property		
	0 min	1 min	3 min	5 min	pH	ORP (mV)	Free chlorine (ppm)
E. coli O157:H7	7.96 ± 0.03	0[b]	0[b]	0[b]	2.39 ± 0.02	1,153 ± 4	85.8 ± 2.7
Control	7.96 ± 0.03	7.89 ± 0.03	7.87 ± 0.03	7.86 ± 0.11			
S. enteritidis	7.70 ± 0.12	1.13 ± 0.33	0[b]	0[b]	2.44 ± 0.03	1,155 ± 1	79.33 ± 3.0
Control	7.70 ± 0.12	7.63 ± 0.12	7.67 ± 0.15	7.61 ± 0.14			
L. monocytogenes	7.91 ± 0.10	<1.0[a]	0[b]	0[b]	2.48 ± 0.05	1,159 ± 4	73.3 ± 1.8
Control	7.91 ± 0.10	7.88 ± 0.10	7.88 ± 0.08	7.83 ± 0.12			

[a] Positive by enrichment.
[b] Negative by enrichment and no detectable survivors by a direct plating procedure.

in the food industry, systems involving high ORP values, greater than 1,000 mV, have not normally been used. The ORP of a solution is an indicator of its ability to oxidize or reduce, with positive and higher ORP values correlated to greater oxidizing strength (6, 8, 9). An ORP of +200 to +800 mV is optimal for growth of aerobic microorganisms, whereas an optimum range of −200 to −400 mV is favored for growth of anaerobic microorganisms (6). Since the ORP of EO water in this study was greater than 1,100 mV, the ORP likely played an influential role, in combination with low pH and free chlorine, in killing microorganisms. A possible explanation for the high ORP of EO water is the oxygen released by the rupture of the weak and unstable bond between hydroxy and chloric radicals (1). It is hypothesized that the low pH in EO water sensitizes the outer membranes of bacterial cells, thereby enabling hypochlorous acid to enter the bacterial cells more efficiently. Acid-adapted cells of *Salmonella typhimurium* were reported to be more sensitive to inactivation by hypochlorous acid than nonadapted cells, due to increased outer membrane sensitivity to hypochlorous acid in acid-adapted cells (7). Experiments to identify the contributions of the different components of EO water to its antimicrobial activity are under way in our laboratory.

The effects of EO water on the three pathogens were evaluated at low and moderate temperatures in the interest of developing potential antibacterial dip treatments for unprocessed agricultural foods. No differences in the inactivation rates of the three pathogens were observed between treatment at 4°C and treatment at 23°C. However at 35 and 45°C, much higher rates of inactivation were observed for all three pathogens.

Since chlorine is one of the antimicrobial components of EO water, we evaluated the survival of *E. coli* O157:H7 and *L. monocytogenes* in sterile deionized water containing a free-chlorine concentration of 70 to 80 ppm, which was similar to that present in EO water. Results revealed reductions in the bacterial counts of both pathogens similar to those observed with EO water, indicating that the concentration of free chlorine present in EO water is sufficient to bring about the reductions in bacterial counts achieved by EO water. Although chlorine is highly effective in killing pathogenic microorganisms in simple aqueous systems, its antibacterial effect on microorganisms on foods is minimal, especially in the presence of organic materials which convert chlorine into inactive forms (3). For example, treatment of fresh produce with 200 ppm chlorine results in a reduction in the *L. monocytogenes* count of less than 2 log CFU/g (15). Studies comparing the efficacies of chlorinated water and EO water for inactivating *E. coli* O157:H7 on apples are in progress in our laboratory.

Results revealed that EO water is highly effective in killing *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes*, indicating its potential application for decontamination of food and food contact surfaces. An advantage of EO water is that it can be produced with tap water, with no added chemicals other than sodium chloride.

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