Journal of Food Protection, Vol. 62, No. 8, 1999, Pages 857–860

Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Plastic Kitchen Cutting Boards by Electrolyzed Oxidizing Water

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MS 98-347: Received 23 December 1998/Accepted 2 May 1999

ABSTRACT

One milliliter of culture containing a five-strain mixture of *Escherichia coli* O157:H7 (~10^{10} CFU) was inoculated on a 100-cm^2 area marked on unscarred cutting boards. Following inoculation, the boards were air-dried under a laminar flow hood for 1 h, immersed in 2 liters of electrolyzed oxidizing water or sterile deionized water at 23°C or 35°C for 10 or 20 min; 45°C for 5 or 10 min; or 55°C for 5 min. After each temperature–time combination, the surviving population of the pathogen on cutting boards and in soaking water was determined. Soaking of inoculated cutting boards in electrolyzed oxidizing water reduced *E. coli* O157:H7 populations by ≥5.0 log CFU/100 cm^2 on cutting boards. However, immersion of cutting boards in deionized water decreased the pathogen count only by 1.0 to 1.5 log CFU/100 cm^2. Treatment of cutting boards inoculated with *Listeria monocytogenes* in electrolyzed oxidizing water at selected temperature–time combinations (23°C for 20 min, 35°C for 10 min, and 45°C for 10 min) substantially reduced the populations of *L. monocytogenes* in comparison to the counts recovered from the boards immersed in deionized water. *E. coli* O157:H7 and *L. monocytogenes* were not detected in electrolyzed oxidizing water after soaking treatment, whereas the pathogens survived in the deionized water used for soaking the cutting boards. This study revealed that immersion of kitchen cutting boards in electrolyzed oxidizing water could be used as an effective method for inactivating foodborne pathogens on smooth, plastic cutting boards.

Escherichia coli O157:H7 and *Listeria monocytogenes* are foodborne pathogens of major concern in the United States *(2, 5).* A potential source of foodborne pathogens is cross-contamination from food preparation surfaces, kitchen cutting boards, and knives *(14).* Improper handling of foods in homes is one of the major factors contributing to foodborne illness *(4, 7).* Effective methods to reduce or inactivate pathogens on kitchen cutting boards can help to reduce the incidence of foodborne disease outbreaks. Methods for killing foodborne pathogens on food contact surfaces and appliances are necessary for establishing critical control points at homes, restaurants, and other food service units.

Electrolyzed oxidizing water (EO water) is the product of a new concept developed in Japan. Research carried out in Japan revealed that addition of deionized water containing sodium chloride (0.2%) to an electrolysis chamber, where the anode and cathode electrodes are separated by a diaphragm, and subjecting the water to electrolysis, 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 less, an oxidation–reduction potential (ORP) of >1,100 mV, and a free chlorine concentration of 10 to 80 ppm *(1, 12).* Previously, we also found that EO water was highly effective in killing *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* in pure cultures. The objective of this study was to evaluate the efficacy of EO water for reducing or eliminating *E. coli* O157:H7 and *L. monocytogenes* on unscarred, plastic kitchen cutting boards.

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MATERIALS AND METHODS

Bacterial culture. A five-strain mixture of *E. coli* O157:H7 and a five-strain mixture *of L. monocytogenes* were used for the study. The five strains of *E. coli* O157:H7 were E06 (milk isolate), E08 (meat isolate), E10 (meat isolate), E16 (meat isolate), and E22 (calf feces isolate). The five strains of *L. monocytogenes* included LM ATCC 19117 (sheep), LM 101 (salami), LM 109 (pepperoni), LM 116 (cheese), and LM 201 (milk). Each strain was cultured individually in 100 ml of sterile tryptic soy broth (Difco, Detroit, Mich.) at 37°C for 24 h with agitation (150 rpm). Following incubation, the bacteria (100 ml) were sedimented by centrifugation (4,000 × *g* for 30 min) in separate tubes and washed and resuspended in 10 ml of sterile 0.1% peptone water. The five strains of each pathogen (10 ml each) were combined and 50 ml of the suspension was used as the source of the inoculum. The bacterial population of the five-strain mixture of each pathogen was also determined by surface plating 0.1-ml portions of appropriate dilutions (0.1% peptone water) of the suspension on duplicate tryptic soy agar (Difco) plates and incubating the plates at 37°C for 24 h.

EO water. EO water was generated using the Hoshizaki EO water generator (ROX 20TA, Hoshizaki Electric Company Ltd., Japan). The current passing through the EO water generator and voltage between the electrodes were set at 19.8 A and 10 V, respectively. A 12% solution of sodium chloride and deionized wa-

ter from the laboratory supply line were simultaneously pumped into the equipment. The display indicator was activated and observed until the machine stabilized. The EO water was collected from the appropriate outlet in sterile 5-liter beakers for the microbial study. Samples for determination of pH, ORP, and free chlorine were also collected simultaneously. For the studies involving EO water at temperatures (35°C, 45°C, and 55°C) other than ambient (23°C), an external water pump (model FPOF360AC, Flotec, Inc., Delavan, Wis.) matching the capacity of the pump installed in the EO water generator was used to pump deionized water preheated to the desired temperature from a water bath (model 25, Precision Scientific, Chicago, Ill.) into the machine.

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

Kitchen cutting board. New (unscarred), plastic (polypropylene) cutting boards (WalMart Stores, Inc., Bentonville, Ark.) were used for the study. The boards were wrapped in aluminum foil and sterilized by heating in an autoclave. On each cutting board, a volume of 1 ml of bacterial culture (approximately 10^{10} CFU of *E. coli* O157:H7) was applied and spread evenly on a 100-cm^2 area marked on the cutting board by a sterile, bent glass rod. Following inoculation, the cutting boards were dried under a laminar flow hood for 1 h at room temperature (23°C). Immediately after drying, two cutting boards were separately kept immersed in 2,000 ml each of EO water at a temperature of 23°C for 10 or 20 min, 35°C for 10 or 20 min, 45°C for 5 or 10 min, or 55°C for 5 min (treatment). Simultaneously, two inoculated cutting boards were separately immersed in 2,000 ml each of sterile deionized water (control) under the same conditions and durations described above. Immediately after immersion in the treatment or control water, the surviving population of the pathogen on each cutting board and in the soaking solution was determined. The population of bacteria was also determined on a separate inoculated cutting board not subjected to immersion in deionized or EO water to check the efficiency of inoculation (baseline reading).

The experiment for each temperature–time combination was replicated at least three times using duplicate samples for treatments and controls. The most effective and practical temperature–time combinations from the above study were selected and they were evaluated for inactivating *L. monocytogenes* on cutting boards using the same protocols described above.

Bacteriological analyses. After immersion in the treatment or control water for the specified time and temperature, each cutting board was aseptically removed using sterile tongs and water was allowed to drain completely from the board. The inoculated 100-cm^2 surface area on each board was swabbed twice with two separate sterile cotton swabs from top to bottom and from right to left. The swabs were washed in 9 ml of sterile 0.1% peptone water. One milliliter of the washing peptone water was then serially (1:10) diluted with 9 ml of sterile 0.1% peptone water and 0.1-ml portions from appropriate dilutions were surface plated on duplicate tryptic soy agar plates. The colonies of bacteria were counted after incubating the plates at 37°C for 24 h. The population of *E. coli* O157:H7 or *L. monocytogenes* in EO water or deionized water after soaking the cutting boards was also determined by plating 0.1-ml portions from appropriate samples on duplicate tryptic soy agar plates.

A volume of 1 ml of the treatment or control water was also transferred to separate 250-ml Erlenmeyer flasks containing 100 ml of sterile tryptic soy broth and incubated at 37°C for 24 h. Following enrichment in tryptic soy broth, the culture was streaked on sorbitol MacConkey agar (Oxoid Division, Unipath Co., Ogdensburg, N.Y.) containing 0.1% 4-methylumbelliferyl-β-D-glucuronide (Oxoid) (for *E. coli* O157:H7) and Oxford agar (Gene-Trak, Framingham, Mass.) (for *L. monocytogenes*), and the plates were incubated at 37°C for 24 h. Representative sorbitol-negative and 4-methylumbelliferyl-β-D-glucuronide-negative colonies from the sorbitol MacConkey agar plates were then confirmed using the *E. coli* O157:H7 latex agglutination assay (Remel microbiology products, Lenexa, Kans.). The colonies of *L. monocytogenes* on Oxford agar were confirmed biochemically by API diagnostic kit (Biomerieux, Hazelwood, Mo.).

Statistical analysis. For each treatment, the data from the independent replicate trials were pooled and the mean value and standard deviation were determined *(13).*

RESULTS AND DISCUSSION

The mean pH, ORP, and free chlorine concentration of EO water at various temperatures are presented in Tables 1 and 2. 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.

Inactivation studies of *E. coli* O157:H7 on cutting boards by EO water was temperature and time dependent (Table 1). The mean population of *E. coli* O157:H7 recovered from the cutting boards following 1 h of drying after inoculation was 8.12 log CFU/100 cm^2. Soaking of inoculated cutting boards in EO water at 23°C for 10 or 20 min decreased *E. coli* O157:H7 populations by approximately 5.0 log CFU and 6.0 log CFU/100 cm^2, respectively (Table 1). However, control cutting boards immersed in sterile deionized water at 23°C for 10 or 20 min yielded a mean population of 7.30 log CFU and 6.60 log CFU/100 cm^2, respectively. At 35°C, *E. coli* O157:H7 counts on the cutting boards were reduced to <1.0 log CFU/100 cm^2 (detected by an enrichment procedure only) after 10 min of soaking, whereas soaking of boards for 20 min decreased the pathogen populations to undetectable levels (as determined by direct plating and enrichment procedures) (Table 1). However, the bacterial count on the control cutting boards soaked in deionized water was reduced only by 1.1 log and 1.2 log CFU/100 cm^2 after 10 min and 20 min of soaking at 35°C, respectively. Exposure of inoculated cutting boards to EO water at 45°C for 5 min resulted in greater than a 5.0-log CFU/100 cm^2 reduction in *E. coli* O157:H7 counts, whereas 10 min of soaking decreased the pathogen populations to undetectable levels (Table 1). At 55°C, immersion of cutting boards for 5 min reduced *E. coli* O157:H7 levels to <1.0 log CFU/100 cm^2. However, an average count of 7.0 log CFU/100 cm^2 was recovered from the cutting boards immersed in deionized water at the same temperature and duration.

Soaking of cutting boards in EO water at higher temperatures decreased the exposure time needed to achieve the same reduction in bacterial counts obtained with longer duration at lower temperatures. However, increasing the temperature (from 23°C to 55°C) of deionized water used

TABLE 1. *Inactivation of* E. coli *O157:H7 on kitchen cutting boards by EO water at different temperatures*

	Bacterial count (mean ± standard deviation)							
	Cutting board (log_{10} CFU/100 cm^2)			Soaking water (log_{10} CFU/ml)		EO water properties[c]		
Treatment	Cells applied[a]	EO water	DH_2O[b]	EO water	DH_2O	pH	ORP (mV)	Chlorine (ppm)
23°C for 10 min	8.26 ± 0.42	3.17 ± 0.34	7.31 ± 0.42	0[e]	3.75 ± 0.31	2.50 ± 0.10	1,163 ± 7	87 ± 11
23°C for 20 min	8.53 ± 0.90	2.60 ± 0.22	6.63 ± 0.03	0	3.46 ± 0.37	2.56 ± 0.01	1,165 ± 1	80 ± 10
35°C for 10 min	7.94 ± 0.05	<1.0[d]	6.81 ± 0.29	0	3.73 ± 0.20	2.58 ± 0.02	1,161 ± 1	87 ± 10
35°C for 20 min	7.98 ± 0.10	0	6.77 ± 0.20	0	3.90 ± 0.51	2.56 ± 0.00	1,162 ± 2	90 ± 7
45°C for 5 min	8.14 ± 0.13	2.43 ± 0.12	7.38 ± 0.38	0	4.00 ± 0.05	2.46 ± 0.01	1,154 ± 1	87 ± 8
45°C for 10 min	8.01 ± 0.10	0	7.03 ± 0.02	0	4.15 ± 0.28	2.51 ± 0.06	1,157 ± 3	93 ± 5
55°C for 5 min	8.07 ± 0.07	<1.0	6.98 ± 0.08	0	4.04 ± 0.20	2.29 ± 0.06	1,147 ± 5	45 ± 8

[a] Number of *E. coli* O157:H7 recovered from cutting boards following 1 h of drying after inoculation.
[b] DH_2O, sterile deionized water.
[c] EO water properties were measured before the treatments.
[d] <1.0, Positive by an enrichment procedure and no detectable survivors by a direct plating procedure.
[e] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

for soaking the control cutting boards did not have any effect on the bacterial counts recovered from the boards. Irrespective of the temperature of exposure, soaking the cutting boards in deionized water resulted in only a 1.0- to 1.5-log CFU/100 cm^2 reduction in bacterial counts. An average count of 3.80 log CFU of the pathogen/ml was recovered from the deionized water (used for soaking the cutting boards), whereas no *E. coli* O157:H7 could be detected in EO water.

From the above results, 23°C for 20 min, 35°C for 10 min, and 45°C for 10 min were selected for inactivation studies with *L. monocytogenes* on cutting boards. As observed in the studies with *E. coli* O157:H7, treatment of cutting boards with EO water at all temperatures substantially reduced the populations of *L. monocytogenes* in comparison with the counts recovered from the boards immersed in deionized water (Table 2). However, none of temperature–time combinations resulted in more than a 5.0-log CFU/100 cm^2 reduction in *L. monocytogenes* counts. This in part may be due to the lower levels of chlorine observed in the EO water used for the studies with *L. monocytogenes*. The mean levels of free chlorine in the EO water used for the experiments with *L. monocytogenes* were 72 ppm at 23°C, 66 ppm at 35°C, and 52 ppm at 45°C as opposed to the chlorine levels of 84 ppm, 88 ppm, and 90 ppm, respectively, in the EO water used for *E. coli* O157:H7. Although the EO water generator was operated at the same level of setting for free chlorine level for experiments with *E. coli* O157:H7 and *L. monocytogenes*, the machine consistently yielded lower levels of chlorine in the water used for studies with *L. monocytogenes.* As observed with *E. coli* O157:H7, a mean population of 3.80 log CFU of *L. monocytogenes*/ml was detected in the deionized water used for soaking the cutting boards, whereas the pathogen was undetected in the soaking EO water.

The ORP of a solution is an indicator of the ability of the solution to oxidize or reduce, with positive and higher ORP values correlated with greater oxidizing strength *(6, 10, 11).* Aerobic microorganisms require for growth an optimum ORP of +200 to +800 mV, whereas an optimum range of −200 to −400 mV is favored for growth of anaerobic microorganisms *(6).* Because the ORP of EO water in this study was greater than 1,100 mV, the ORP of EO water played a critical role in combination with low pH and

TABLE 2. *Inactivation of* L. monocytogenes *on kitchen cutting boards by EO water at different temperatures*

	Bacterial count (mean ± standard deviation)							
	Cutting board (log_{10} CFU/100 cm^2)			Soaking water (log_{10} CFU/100 cm^2)		EO water properties[c]		
Treatment	Cells applied[a]	EO water	DH_2O[b]	EO water	DH_2O	pH	ORP (mV)	Chlorine (ppm)
23°C for 20 min	8.46 ± 0.18	3.63 ± 0.10	7.88 ± 0.19	0[d]	4.18 ± 0.45	2.50 ± 0.08	1,156 ± 2	72 ± 12
35°C for 10 min	8.89 ± 0.98	3.55 ± 0.03	7.96 ± 0.20	0	3.70 ± 0.08	2.38 ± 0.02	1,156 ± 2	66 ± 7
45°C for 10 min	8.15 ± 0.20	3.36 ± 0.39	7.86 ± 0.15	0	3.63 ± 0.41	2.33 ± 0.02	1,150 ± 3	52 ± 4

[a] Number of *L. monocytogenes* recovered from cutting boards following 1 h of drying after inoculation.
[b] DH_2O, sterile deionized water.
[c] EO water properties were measured before the treatments.
[d] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

free chlorine in killing microorganisms. Leyer and Johnson *(9)* reported that acid-adapted cells of *Salmonella* Typhimurium were more sensitive to inactivation by hypochlorous acid than nonadapted cells due to increased outer membrane sensitivity to hypochlorous acid. The low pH in EO water may sensitize the outer membrane of bacterial cells to the entry of hypochlorous acid into bacterial cells. Experiments are underway in our laboratory to determine the individual role of pH, ORP, and free chlorine of EO water in killing bacteria.

Because free chlorine is an important antibacterial element in EO water, studies were done to determine the survival of *E. coli* O157:H7 and *L. monocytogenes* in deionized water containing free chlorine concentrations (70 to 80 ppm) similar to those present in EO water. The reductions in bacterial populations observed were similar to those obtained with EO water. However, the effect of chlorine (hypochlorous acid) on bacterial pathogens is reported to be minimal especially in the presence of food or organic materials that are frequently present on kitchen cutting boards after use *(3, 8).* Experiments to evaluate the effect of organic materials on the antimicrobial components of EO water are under study in our laboratory.

Results of this study revealed that immersion of kitchen cutting boards in EO water could be used as an effective method for inactivating foodborne pathogens on smooth, plastic cutting boards. The treatment of cutting boards in deionized water decreased the pathogens only by 1.0 to 1.5 log CFU/100 cm^2. Further, *E. coli* O157:H7 and *L. monocytogenes* were not detected in EO water, whereas the pathogens survived in the deionized water used for soaking the cutting boards, thus representing a potential source of cross-contamination or recontamination in case the same water is used for soaking or washing cutting boards. Used plastic cutting boards are often scarred by knife edges and the scarred surface may protect bacteria from cleaning and sanitation procedures. Therefore, the efficacy of EO water for inactivating foodborne pathogens on used (scarred) cutting boards needs to be investigated.

REFERENCES

1. Anonymous. 1997. Principle of formation of electrolytic water. Hoshizaki Electric Co. Ltd., Sakae, Toyoake, Aichi, Japan.
2. Beuchat, L. R. 1995. Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59:204–216.
3. Beuchat, L. R., B. V. Nail, B. B. Adler, and M. R. S. Clavero. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. J. Food Prot. 61:1305–1311.
4. Bryan, F. L. 1988. Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. J. Food Prot. 51:663–673.
5. Doyle, M. P., T. Zhao, J. Meng, and S. Zhao. 1997. *Escherichia coli* O157:H7, p. 175–178. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers. American Society for Microbiology, Washington, D.C.
6. Jay, J. M. 1996. Modern food microbiology, 5th ed., p. 48–49. Aspen Publishers, Frederick, Md.
7. Knabel, S. J. 1995. Foodborne illness: role of home food handling practices. Food Technol. 49:119–131.
8. Kotula, K. L., A. W. Kotula, B. E. Rose, C. J. Pierson, and M. Camp. 1997. Reduction of aqueous chlorine by organic material. J. Food Prot. 60: 276–282.
9. Leyer, G. J., and E. A. Johnson. 1997. Acid adaptation sensitizes *Salmonella typhimurium* to hypochlorous acid. Appl. Environ. Microbiol. 63:461–467.
10. McPherson, L. L. 1993. Understanding ORP's in the disinfection process. Water Eng. Manage. November:29–31.
11. Robbs, P. G., J. A. Bartz., J. K. Brecht, and S. A. Sargent. 1995. Oxidation–reduction potential of chlorine solutions and their toxicity to *Erwinia caratovora* subsp. *caratovora* and *Geotrichum candidum.* Plant Dis. 79:158–162.
12. Shimizu, Y., and T. Hurusawa. 1992. Antiviral, antibacterial, and antifungal actions of electrolyzed oxidizing water through electrolysis. Dental J. 37:1055–1062.
13. Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw Hill, New York.
14. Zhao, P., T. Zhao, M. P. Doyle, J. R. Rubino, and J. Meng. 1998. Development of a model for evaluation of microbial cross-contamination in the kitchen. J. Food Prot. 61:960–963.