Journal of Food Protection, Vol. 68, No. 5, 2005, Pages 986–990

Efficacy of Electrolyzed Water in Inactivating *Salmonella* Enteritidis and *Listeria monocytogenes* on Shell Eggs

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MS 04-43: Received 9 February 2004/Accepted 18 September 2004

ABSTRACT

The efficacy of acidic electrolyzed (EO) water produced at three levels of total available chlorine (16, 41, and 77 mg/liter) and chlorinated water with 45 and 200 mg/liter of residual chlorine was investigated for inactivating *Salmonella* Enteritidis and *Listeria monocytogenes* on shell eggs. An increasing reduction in *Listeria* population was observed with increasing chlorine concentration from 16 to 77 mg/liter and treatment time from 1 to 5 min, resulting in a maximal reduction of 3.70 log CFU per shell egg compared with a deionized water wash for 5 min. There was no significant difference in antibacterial activities against *Salmonella* and *Listeria* at the same treatment time between 45 mg/liter of chlorinated water and 14-A acidic EO water treatment ($P \geq 0.05$). Chlorinated water (200 mg/liter) wash for 3 and 5 min was the most effective treatment; it reduced mean populations of *Listeria* and *Salmonella* on inoculated eggs by 4.89 and 3.83 log CFU/shell egg, respectively. However, reductions (log CFU/shell egg) of *Listeria* (4.39) and *Salmonella* (3.66) by 1-min alkaline EO water treatment followed by another 1 min of 14-A acidic EO water (41 mg/liter chlorine) treatment had a similar reduction to the 1-min 200 mg/liter chlorinated water treatment for *Listeria* (4.01) and *Salmonella* (3.81). This study demonstrated that a combination of alkaline and acidic EO water wash is equivalent to 200 mg/liter of chlorinated water wash for reducing populations of *Salmonella* Enteritidis and *L. monocytogenes* on shell eggs.

Consumption of shell eggs contaminated with *Salmonella* or foods made from contaminated eggs have frequently been linked to foodborne disease outbreaks *(6, 37).* The exterior egg surface may be contaminated with pathogens, primarily from infected hen's feces but secondarily by contaminated environments of egg production facilities *(11, 14).* In a survey of egg farms, ca. 7.8% of eggshells were contaminated with *Salmonella* before washing and ca. 1.1% of washed eggshells were contaminated with the bacterium *(14).* When hens were artificially infected with *Salmonella*, 6.3 to 9.5% of their eggs were contaminated *(7). Salmonella* on the shell egg surface can also penetrate into eggshell membranes and contaminate internal contents of eggs *(27, 33).* In general, prevalence of salmonellae in shell eggs has been reported at about 1% of tested samples *(14, 31).*

Domestic fowl and birds harboring *L. monocytogenes* have been suggested as potential vehicles to transmit *Listeria* to human beings *(12). L. monocytogenes* has also been isolated from egg washing water *(24)* and commercially broken raw liquid whole egg *(25).* The survivability of *Listeria* on the surface of shell eggs and processed egg products *(4, 5)* is a potential health concern.

Disinfecting shell eggs is a fundamental and common practice to eliminate or reduce populations of pathogens on the surface of shell eggs. When the presence or the level of pathogens is controlled on the surface of shell eggs, egg products (e.g., liquid or powdered egg) requiring further processing such as pasteurization, drying, or high pressure *(31, 32)* will attain better microbiological quality.

In selecting an appropriate disinfectant to clean shell eggs, several factors such as effectiveness of the sanitizer to eliminate target organisms from the shell's surface, safety to workers and environments, and economic feasibility are considered *(37).* A variety of disinfectants have been examined for reducing the populations of pathogens on shell eggs. Although chlorine is among the most frequently used chemical disinfectant in shell egg washing, hot water *(26, 36),* UV irradiation *(2, 9, 20),* peroxidase-catalyzed compound *(21, 22, 23),* iodine-based sanitizer *(19),* quaternary ammonium and sodium carbonate *(41),* hydrogen peroxide *(28),* zinc sulfate solution and formaldehyde fumigation *(3),* formalin, sodium hydroxide, commercial benzyltrialkonium compound, and commercial *p*-chloro-*m*-xylenol *(10)* have been evaluated.

Electrolyzed (EO) water has been reported to have a strong microbicidal effect against most foodborne pathogens *(16).* EO water is produced through electrolysis of a dilute salt (0.1% NaCl) solution to produce two types of EO water. An electrolyzed basic solution (alkaline EO water) is a strong reducing agent, which destroys free radicals in biological systems. An electrolyzed strong acid solution (acidic EO water), which possesses high oxidation potential in combination with hypochlorous acid, is bactericidal. Ezeike and Hung *(8)* found EO water properties were significantly affected by salt concentration, voltage, and flow rate. In general, the higher the salt concentration and volt-

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age for EO water generation, the higher the oxidation potential and total available chlorine in EO water. Some advantages of using EO water are that the EO water treatment is effective for microbial inactivation, the apparatus is easy to operate, and it is relatively inexpensive. In addition, it is less damaging to the environment because the disinfectant is produced using only water with sodium chloride; thus there is no need for handling potentially dangerous chemicals. A final advantage is the properties of EO water can be generated and controlled at point of use.

EO water inactivates foodborne pathogens in vitro *(40, 16)* and is an effective treatment for disinfecting kitchen cutting boards *(39)* and several fresh-cut vegetables *(15)*. EO water also reduced *Escherichia coli* and *L. monocytogenes* 2.41 and 2.65 log CFU per lettuce with 3 min of treatment, respectively *(29)*. Recently, Park et al. *(30)* reported that EO water reduced the *Campylobacter jejuni* population by about 3 log CFU/g on chicken and that no viable cells were recovered from the washing solution after the treatment. Kim et al. *(18)* found that application of EO water in conjunction with ultrasonication enhanced the bactericidal effectiveness of EO water on alfalfa sprouts by 80%. In addition, EO water was effective in eliminating *L. monocytogenes* biofilms on stainless steel *(17)*. The adherent cell population on the stainless steel coupons was reduced by about 9 log cycles after 300 s of EO water treatment. Russell *(34)* applied EO water using an electrostatic spray system and found that EO water could effectively eliminate pathogenic and indicator bacteria from hatching eggs. However, applying EO water through electrostatic spray system resulted in an 85 to 90% reduction of total available chlorine and hence reduced the efficacy of EO water *(13)*. The objective of this study was to evaluate the efficacy of EO water as an immersion treatment for removing *Salmonella* Enteritidis and *L. monocytogenes* on shell egg surface.

MATERIALS AND METHODS

Bacterial strains used. A five-strain mixture of *Listeria monocytogenes* (ATCC 19117, sheep isolate; 109, pepperoni isolate; 201, milk isolate; 315, salami isolate; 116, cheese isolate) and a five-strain mixture of *Salmonella* Enteritidis (H3353, 4638, 4639, 4717, 4267, all isolates from egg-associated outbreaks) were used in all experiments. Each bacterial strain was cultured in 10 ml of tryptic soy broth (Becton Dickinson, Sparks, Md.) at 37°C for 24 h. To make bacterial suspension for shell egg inoculation, a loopful of each bacterium was transferred into 100 ml of tryptic soy broth and cultured at 37°C for 24 h with agitation (100 rpm). Three successive 24-h interval transfers were made prior to use as an inoculum for shell eggs. The five strains of each pathogen (100 ml each) were combined and used as the source of inoculum. To determine the population of each bacterial dip (*Salmonella* as ca. 9.48 log CFU/ml and *Listeria* as ca. 9.60 log CFU/ml), 1 ml of bacterial suspension was serially diluted in sterile 0.1% peptone water, and appropriate diluents were surface plated on tryptic soy agar (TSA; Difco) and incubated at 37°C for 24 h.

Shell eggs used. Freshly laid clean shell eggs (65 ± 2 g) without any treatment were provided from Cal-Maine Foods, Inc. (Shady Dale, Ga.). Shell eggs were stored at 4°C for no more than 1 week before washing treatment.

TABLE 1. *Properties of acidic EO, alkaline EO, and chlorinated water*

Water type	Amperage	pH	Oxidation reduction potential (mV)	Total available chlorine (mg/liter)
Acidic EO	8.0 ± 0.2	2.7	1,089	16
	14.0 ± 0.2	2.5	1,117	41
	19.0 ± 0.2	2.5	1,121	77
Chlorinated	—	9.3	606	45
	—	9.4	598	200
Alkaline EO	19.0 ± 0.2	11.2	−940	0

Preparation of acidic EO, chlorinated, and alkaline EO water. Acidic and alkaline EO water (200 ml) were produced using a Hoshizaki EO water generator (model ROX 20TA, Hoshizaki Electric Co. Ltd., Toyoake, Aichi, Japan). The currents passing through the EO water generator were 8.0 ± 0.2, 14.0 ± 0.2, or 19 ± 0.2 amperes, and voltage between the electrodes was 10 volts. At each amperage (A) setting, 200 ml of acidic EO water was collected in a sterile 500-ml bottle from the anode outlet. Alkaline EO water was collected from the cathode outlet at the 19-A setting only. Chlorinated water (45 and 200 mg/liter of residual chlorine) was prepared by addition of appropriate amount of sodium hypochlorite (Aldrich, Milwaukee, Wis.) in sterile deionized water. The total available chlorine concentration was determined with a chlorine test kit (24620-00, Hach Co., Ames, Iowa). The pH and oxidation-reduction potential were measured in duplicate samples using pH and oxidation-reduction potential electrodes (Accumet, model 50, Denver Instrument, Denver, Colo.). All treatment solutions (22°C) were used within 1.5 h after production. The properties of different treatment solutions used for washing shell eggs are shown in Table 1.

Inoculation of shell eggs with each bacterial inoculum. Five 24-h cultures (100 ml each) of each bacterial pathogen were combined in a sterile stomacher bag. Shell eggs were brought to room temperature first before being inoculated with *Salmonella* Enteritidis or *L. monocytogenes* by dipping six or seven eggs in each bacterial suspension for 2 min. Inoculated eggs were manually placed in a sterile paper egg container (using 75% alcohol spray) and dried for 30 min at 37°C.

Washing shell eggs. To investigate the efficacy of washing treatment in removing pathogens on shell eggs, shell eggs individually inoculated with *Salmonella* Enteritidis or *L. monocytogenes* were placed in a sterile stomacher bag (model Seward 400, London, UK). Individual shell eggs were then submerged in 200 ml of sterile deionized (control), 8-, 14-, or 19-A acidic EO, chlorinated, or alkaline EO water at room temperature for 1, 3, or 5 min while shaking at 100 rpm. After treatment, individual shell eggs were then aseptically transferred into a sterile stomacher bag and combined with 50 ml of sterile neutralizing buffer (Difco). Eggs were gently rubbed by hand for 1 min, and the neutralizing buffer solution was then subjected to bacterial assay.

To examine the combined effects of alkaline and acidic EO water, an egg inoculated with each pathogen was prewashed in deionized (control) or alkaline EO water (200 ml) for 1 min with shaking (100 rpm) and then aseptically transferred into another sterile stomacher bag. The alkaline EO washed shell egg was then followed by a postwashing treatment using 200 ml of deionized (control), alkaline EO, or 8-, 14-, or 19-A acidic EO water for

TABLE 2. *Efficacy of different treatments in reducing* L. monocytogenes *and* Salmonella *Enteritidis on shell eggs*

Treatment	Time (min)	Populations (log CFU/shell egg) recovered[a]	
		L. monocytogenes	*Salmonella* Enteritidis
Unwashed	—	7.03 A	5.61 A
Deionized water	1	6.78 A	5.18 AB
	3	6.74 A	5.00 B
	5	6.73 A	4.76 B
Alkaline EO water	1	6.44 AB	4.13 C
	3	6.42 AB	3.45 DE
	5	6.09 B	2.48 GHI
8-A Acidic EO water	1	4.80 C	3.59 CD
	3	4.41 CDE	2.99 DEFGH
	5	4.13 DEF	2.86 EFGH
14-A Acidic EO water	1	4.48 CD	3.25 DEF
	3	3.78 EFG	2.63 FGHI
	5	3.56 FGH	2.11 IJ
19-A Acidic EO water	1	3.73 FG	3.11 DEFG
	3	3.28 GH	2.40 HIJ
	5	3.03 H	2.13 IJ
Cl-45[b]	1	4.41 CDE	3.34 DE
	3	4.02 DEF	2.17 IJ
	5	3.19 GH	1.82 J
Cl-200[b]	1	3.02 H	1.80 J
	3	2.14 L	1.80 J
	5	2.13 L	1.76 J

[a] Values within the same column not followed by the same letter are significantly different ($P \leq 0.05$). Detection limit was 50 CFU per shell egg.

[b] Chlorinated water containing 45 (Cl-45) or 200 (Cl-200) mg/liter of total available chlorine.

another 1 min with shaking (100 rpm). The postwashed shell egg was rinsed with 50 ml of neutralizing buffer (Difco) in a new sterile bag and the neutralizing buffer solution was then used for bacterial assay.

Bacterial assay. Neutralizing buffer solution used after egg rinsing was surface plated in quadruplicate (0.25 ml) and serially (1:10) diluted in sterile 0.1% peptone water and plated in duplicate (0.1 ml) on appropriate enumeration agar media. *L. monocytogenes* was enumerated on modified Oxford Listeria agar with selective supplements (Gene-Trak, Framingham, Mass.) and incubated at 37°C for 48 h. Selected presumptive colonies on modified Oxford Listeria agar were further confirmed by the API 20E diagnostic kit (bioMérieux Vitek, Inc., Hazelwood, Mo.).

Salmonella in neutralizing buffer solution was enumerated using a xylose lysine deoxycholate agar (Becton Dickinson) and incubated at 37°C for 24 h. Selected presumptive colonies on xylose lysine deoxycholate agar were confirmed by latex agglutination test (Unipath-Oxoid, Columbia, Md.) and API 20E diagnostic kits (bioMérieux Vitek, Inc.). *Salmonella* and *Listeria* were not detected in any of the 4 uninoculated shell eggs.

For aerobic plate counts (APC), 1 ml of the neutralizing buffer solution from uninoculated shell eggs was serially diluted in 0.1% sterile peptone water and appropriate diluents (0.1 ml) were surface plated on TSA (Difco) and incubated at 30°C for 48 h before counting.

Statistical analysis. Three replicate experiments were performed for each bacterium studied. Data were analyzed using the general linear model of Statistical System *(35).* Duncan's multiple range test was used to separate means using a level of significance of $P \leq 0.05$.

RESULTS AND DISCUSSION

The effect of washing treatments is presented in Table 2. In general, the mean population of *Listeria* (7.03 log CFU per shell egg) on inoculated eggs was higher than the mean population of *Salmonella* (5.61 log CFU per shell egg). Alkaline water wash significantly reduced *Salmonella* population as compared with the deionized water wash. Increasing treatment time significantly reduced the *Salmonella* populations on treated shell eggs (from 1.05 log CFU per shell egg reduction at 1 min to 2.28 log CFU per shell egg at 5 min of treatment). The efficacy of alkaline wash for 5 min was similar to all acidic EO water washes for 5 min and chlorinated water wash (45 mg/liter) for 3 min. However, alkaline water was not effective in removing *Listeria* on shell eggs except for the 5-min treatment, which achieved a 0.64 log CFU per shell egg greater reduction than the 5-min deionized water wash (Table 2). Similar results were obtained from the combination study in which washing shell eggs twice in alkaline EO water (1 min each) achieved more than 2 log CFU per shell egg reduction on *Salmonella*, whereas less than 0.5 log CFU per shell egg reduction was achieved for *Listeria* (Table 3) when compared with deionized water wash. A double wash with alkaline EO water was not effective in reducing *Listeria* population but significantly reduced *Salmonella* population compared with deionized water double wash. A major component in alkaline EO water is dilute sodium hydroxide (0.013%, wt/wt) with pH 11.5 *(1).* Results presented in Table 3 agree with those of Frank and Wright *(10)* who ob-

TABLE 3. *Efficacy of EO water treatments for inactivating* L. monocytogenes *and* Salmonella *Enteritidis on shell eggs*

Treatment		Populations (log CFU/shell egg) recovered[a]	
Prewash (1 min)	Postwash (1 min)	*L. monocytogenes*	*Salmonella* Enteritidis
Unwashed	Unwashed	6.92 A	5.49 A
Deionized water	Deionized water	6.49 AB	5.48 A
Alkaline EO water	Alkaline EO water	6.04 B	3.45 B
Alkaline EO water	8-A Acidic EO water	3.94 C	1.81 C
Alkaline EO water	14-A Acidic EO water	2.93 D	1.86 C
Alkaline EO water	19-A Acidic EO water	2.53 D	1.83 C

[a] Values not followed by the same letter are significantly different ($P \leq 0.05$). Detection limit was 50 CFU per shell egg.

served that inoculated *Salmonella* on eggshell pieces could be removed using 0.5% of NaOH solution for 5 min.

Immersing shell eggs in 8-A acidic EO water for 1 min was effective in reducing *Listeria* population by 1.98 log CFU per shell egg more than deionized water wash for 1 min (Table 2). Results presented in Table 2 also indicate that the reduction of *Listeria* on shell eggs increased with increasing residual chlorine concentration and treatment time resulting in a maximal reduction of 3.70 log CFU per shell egg when compared with deionized water wash for 5 min. Chlorinated water treatment (45 mg/liter) achieved a similar reduction of *Listeria* compared with 14-A acidic EO (41 mg/liter) water (3.54 and 3.17 log CFU per shell egg reduction for 5-min treatment, respectively). Chlorinated water (200 mg/liter) was the most effective treatment for each treatment time and achieved 4.6 log CFU per shell egg reduction after a 5-min treatment. Similar results were found for *Salmonella*, in which reduction increased with acidic EO water treatment time and amperage setting (Table 2).

Two hundred milligrams per liter of chlorine is the maximum concentration allowed to be used in shell egg wash *(38)*. In this study, 200 mg/liter of chlorinated water was also the most effective treatment in reducing the populations of *Listeria* and *Salmonella* (4.6 and 3.03 log CFU per shell egg, respectively for 5-min treatment). Kuo et al. *(22)* recovered no viable salmonellae after chlorinated water treatment (200 mg/liter) for 1 min. In the current study, only 50% of the inoculated shell eggs treated with a chlorinated (200 mg/liter) water wash were negative for *Salmonella* (data not shown).

For the combination treatment, alkaline EO water was used to prewash inoculated shell eggs before acidic EO water (Table 3). Compared with a single acidic EO water wash for 1 min (Table 2), a second wash of 8-, 14-, or 19-A acidic EO water for 1 min following a 1-min alkaline EO water prewash further reduced populations of *Salmonella* by 2.62, 2.03, and 1.72 log CFU per shell egg and *Listeria* by 0.57, 1.26, and 0.91 log CFU per shell egg, respectively. Alkaline EO water treatment alone for 1 min reduced populations of *Listeria* and *Salmonella* by only 0.34 and 1.05 log CFU per shell egg, respectively (Table 2). These results demonstrate a synergistic effect of alkaline EO and enhancing bactericidal activity of acidic EO water on shell eggs. The reductions (log CFU per shell egg) of *Salmonella* (3.62) and *Listeria* (3.56) on shell eggs by the combination treatment of alkaline and 14-A EO (41 mg/liter) water are similar to the 200 mg/liter chlorinated water treatment on *Salmonella* (3.38) and *Listeria* (3.76). This indicates a potential for combining acidic EO water and alkaline EO water to replace high concentration of chlorinated water for shell egg washing.

The efficacy of EO water for removing indigenous microbial flora from the surface of shell eggs is presented in Table 4. Kuo et al. *(20)* observed populations of molds ranging from 3.3 to 3.7 log CFU per shell egg and APC ranging from 4.7 to 5.0 log CFU per shell egg. In the current study, the population of APC on untreated eggshell surface was 5.32 log CFU per shell egg. Deionized or alkaline EO water wash for 1 or 3 min significantly ($P \leq 0.05$) reduced initial APC ranging by 0.82 to 1.39 log CFU per shell egg. Treatment of acidic EO or chlorinated water for 1 or 3 min was effective in further significantly ($P \leq 0.05$) reducing APC by 1.31 to 3.00 log CFU per shell egg when compared with the unwashed eggs. At the same treatment time (1 or 3 min), bactericidal activity of three different levels of acidic EO water was not significantly different. The efficacy of 8-A acidic EO water (16 mg/liter) in reducing APC was not significantly different from that of 200 mg/liter of chlorinated water. Acidic EO water produced at 14 A (41 mg/liter) achieved an additional 1.2 log CFU per shell egg reduction than 45 mg/liter chlorinated water for 1-min treatment (Table 4). These results also support the notion that EO water has potential for replacing chlorinated water in washing shell eggs.

TABLE 4. *Reduction of indigenous aerobic microflora of shell egg surface by treatment of eggs with deionized, alkaline EO, and acidic EO water wash for 1 or 3 min*

Treatment	Time	Population (log CFU/egg) recovered[a]
Unwashed	—	5.32 A
Deionized water	1	4.50 B
	3	3.98 BC
Alkaline EO water	1	4.22 B
	3	3.93 BC
8-A Acidic EO water	1	3.42 CD
	3	2.94 DEF
14-A Acidic EO water	1	2.81 DEF
	3	2.60 EF
19-A Acidic EO water	1	2.69 DEF
	3	2.32 F
Cl-45[b]	1	4.01 BC
	3	3.17 DE
Cl-200[b]	1	3.15 DE
	3	2.61 EF

[a] Values not followed by the same letter are significantly different ($P \leq 0.05$).

[b] Chlorinated water containing 45 (Cl-45) or 200 (Cl-200) mg/liter of total available chlorine.

In conclusion, this study revealed that a combination of alkaline and acidic EO water wash has potential to replace 200 mg/liter of chlorinated water wash in reducing pathogens and indigenous microbial flora on shell eggs.

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