Efficacy of Electrolyzed Oxidizing Water for the Microbial Safety and Quality of Eggs

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ABSTRACT During commercial processing, eggs are washed in an alkaline detergent and then rinsed with chlorine to reduce dirt, debris, and microorganism levels. The alkaline and acidic fractions of electrolyzed oxidizing (EO) water have the ability to fit into the 2-step commercial egg washing process easily if proven to be effective. Therefore, the efficacy of EO water to decontaminate *Salmonella* Enteritidis and *Escherichia coli* K12 on artificially inoculated shell eggs was investigated. For the in vitro study, eggs were soaked in alkaline EO water followed by soaking in acidic EO water at various temperatures and times. Treated eggs showed a reduction in population between ≥ 0.6 to ≥2.6 $\log_{10}$ cfu/g of shell for *S.* Enteritidis and ≥0.9 and ≥2.6 $\log_{10}$ for *E. coli* K12. Log_{10} reductions of 1.7 and 2.0 for *S.* Enteritidis and *E. coli* K12, respectively, were observed for typical commercial detergent-sanitizer treatments, whereas $\log_{10}$ reductions of ≥2.1 and ≥2.3 for *S.* Enteritidis and *E. coli* K12, respectively, were achieved using the EO water treatment. For the pilot-scale study, both fractions of EO water were compared with the detergent-sanitizer treatment using *E. coli* K12. Log_{10} reductions of ≥ 2.98 and ≥ 2.91 were found using the EO water treatment and the detergent-sanitizer treatment, respectively. The effects of 2 treatments on egg quality were investigated. EO water and the detergent-sanitizer treatments did not significantly affect albumen height or eggshell strength; however, there were significant affects on cuticle presence. These results indicate that EO water has the potential to be used as a sanitizing agent for the egg washing process.

(*Key words*: electrolyzed oxidizing water, decontamination, *Salmonella*, *Escherichia coli*, washing)

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INTRODUCTION

Americans consume an average of 234 eggs annually, and in the year 2002 the egg industry produced 73 billion table eggs (American Egg Board, 2003). The risk of a *Salmonella* outbreak from consuming contaminated eggs is a societal and governmental concern. *Salmonella* infection, or salmonellosis, usually causes severe gastroenteritis, which is characterized by diarrhea, headache, nausea, abdominal pain, and vomiting. Over 75% of the reported salmonellosis cases are caused by contaminated eggs [Centers for Disease Control and Prevention (CDC), 2000]. There are approximately 2,300 serotypes of *Salmonella* that have been identified, but the serotype *Salmonella* Enteritidis has been linked to over 20% of salmonellosis outbreaks (CDC, 2002). In 1999, there were 1,080 reported cases of salmonellosis caused by *Salmonella* Enteritidis (CDC, 2000).

During commercial processing, eggs are washed in an alkaline detergent and then rinsed with a chlorine solution to reduce dirt, debris, and microbial load. The minimum alkaline wash water temperature should be 32°C and at least 6°C warmer than the egg. The chlorine rinse should be slightly warmer than the alkaline wash water and have at least 50 ppm of available chlorine but no more than 200 ppm (Agricultural Marketing Service, 2001). High levels of chlorine can be detrimental to the quality of the egg by washing away the cuticle surrounding the egg. The cuticle helps protect the pores of the egg from potential contaminants.

Previous research has shown that wash water temperature and pH are important in egg washing. Brant and Starr (1962) showed that a greater number of eggs became spoiled after being washed in 20°C water than eggs washed in 40 or 60°C water. When eggs were immersed in 65°C water for 3 min, a 90% reduction in spoilage microorganisms was observed (Knowles, 1956). Teo et al. (1996) found a synergistic effect between high temperature and high pH. At a temperature of 45°C and a pH of

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Abbreviation Key: CDC = Centers for Disease Control and Prevention; EO = electrolyzed oxidizing; ORP = oxidation reduction potential; PERC = Pennsylvania State University Poultry Education and Research Center; TSBYE = tryptic soy broth supplemented with 0.6% yeast extract; TSAYE = tryptic soy agar supplemented with 0.6% yeast extract.

7, there was no destruction of *S.* Enteritidis or *E. coli* O157:H7, but when the temperature was raised to 55°C, $\log_{10}$ reductions of 2.75 and 7.00 were achieved for *E. coli* O157:H7 and *S.* Enteritidis, respectively. At a water pH of 10.0 and temperature of 45°C, $\log_{10}$ reductions of 4.35 and 3.70 were observed for *E. coli* O157:H7 and *S.* Enteritidis, respectively. Holley and Proulx (1986) showed that using wash water with a pH greater than 10 and a temperature greater than 38°C, bacteria in the wash water would not be able to grow or survive for long periods of time. Catalano and Knabel (1994) showed that at least a 4 $\log_{10}$ reduction of *S.* Enteritidis was observed when the egg wash water had a temperature of at least 37.7°C and a pH of at least 11.

Electrolyzed oxidizing water is a novel sanitizing solution that is generated by combining electrolysis and membrane separation to produce 2 solutions from a weak salt water solution. Acidic EO water has a pH of 2.6, an oxidation-reduction potential (ORP) of 1,150 mV and 50 to 80 ppm of free chlorine. Alkaline EO water has a pH of 11.4 and an ORP of –795 mV. Several studies have demonstrated the effectiveness of EO water for the inactivation of pathogenic microorganisms in suspension solutions (Venkitanarayanan et al., 1999a; Kim et al., 2000), in foods (Koseki et al., 2001; Al-Haq et al., 2002; Fabrizio et al., 2002; Park et al., 2002; Bari et al., 2003; Russell, 2003; Sharma and Demirci, 2003), and on solid surfaces (Venkitanarayanan et al., 1999b; Walker et al., 2003a,b).

The EO water has the potential to fit into the 2-step washing process for commercial table eggs. The high pH of alkaline EO water may make it a substitute for the high pH detergents traditionally used to remove soil. Also the low pH, high ORP, and presence of available chlorine may make acidic EO water an effective sanitizer for eggs. Therefore, this study was undertaken to compare EO water treatment with a commercial detergent-sanitizer treatment, both in vitro and using a pilot-scale egg washer, and to evaluate the effects of EO water treatment on egg quality.

MATERIALS AND METHODS

Eggs

Nest-run eggs from Hy-line W36 White Leghorn hens (aged 22 wk) were obtained from the Pennsylvania State University Poultry Education and Research Center (PERC). Eggs were held at room temperature for 24 h before an experiment to ensure that thermal cracks would not occur and to avoid the creation of a large pressure differential during treatment. At the time of all experiments, eggs were 2 d old.

[2]Difco, Detroit, MI.
[3]Fisher Scientific Co., Fair Lawn, NJ.
[4]Sigma-Aldrich, St. Louis, MO.
[5]Model 38BL52 Waring Products, New Hartford, CT.

Microorganisms

Salmonella Enteritidis phage type 8 was obtained from the *Salmonella* Center at the University of Pennsylvania. The culture was maintained on tryptic soy agar[2] supplemented with 0.6% yeast extract[2] (TSAYE). *Escherichia coli* K12 was obtained from the *E. coli* Research Center at the Pennsylvania State University. This culture was also maintained on TSAYE.

Preparation of Resistant Strains

To suppress any naturally occurring microorganisms that may be present, antibiotic-resistant cultures were prepared as described by Catalano and Knabel (1994). *Salmonella* Enteritidis phage type 8 or *E. coli* K12 was grown in 100 mL of trypic soy broth[2] supplemented with 0.6 % yeast extract (TSBYE) for 24 h at 37°C and then centrifuged for 30 min at 3,300 × *g* and 10°C. The supernatant was discarded, and a portion of the pellet was spread with a loop on TSAYE plates containing 100 μg/mL of nalidixic acid[3] and incubated for 24 h at 37°C. Colonies were picked and grown in 100 mL of TSBYE with 100 μg/mL of nalidixic acid for 24 h at 37°C and then centrifuged for 30 min at 3,300 × *g* and 10°C. The supernatant was discarded, and the pellet was spread on TSAYE plates with 100 μg/mL of nalidixic acid and streptomycin sulfate.[4] Colonies (spontaneous mutants) were then streaked onto TSAYE plates with both antibiotics and isolated colonies were maintained on TSAYE with 100 μg/mL of nalidixic acid and streptomycin sulfate. The nalidixic acid and streptomycin sulfate resistant strains were named as *S.* Enteritidis PT8NSR (phage type 8 nalidixic acid and streptomycin sulfate resistant) and *E. coli* K12NSR (K12 nalidixic acid and streptomycin sulfate resistant).

Preparation of Manure Slurry and Inoculation

To mimic the horizontal contamination in egg processing facilities, shell eggs were contaminated with a manure slurry inoculated with approximately 10^6 cfu/mL of *S. Enteritidis PT8NSR or E. coli* K12NSR. Manure from Hy-line W36 White Leghorn hens was obtained from PERC. A 10% manure slurry was prepared by homogenizing 200 g of chicken manure with 2 L of 0.1% peptone water[2] in a Waring blender[5] on low speed for 1 min. The manure slurry was autoclaved at 121°C for 60 min for sterilization. A 200 mL of a 24-h culture of *S.* Enteritidis PT8NSR or *E. coli* K12NSR grown in TSBYE with 100 μg/mL of nalidixic acid and streptomycin sulfate was centrifuged for 30 min at 3,300 × *g* and 10°C. The supernatant was discarded, and the pellet was mixed with 100 mL of the presterilized manure slurry and then added to the rest of the presterilized manure slurry to yield 10^5 to 10^6 cfu/mL of manure slurry. Each egg was soaked in the prepared manure slurry for 10 min and then dried for 2 h in a laminar flow hood.

In Vitro Study

Preparation of Electrolyzed Oxidizing Water. Electrolyzed oxidizing water was produced using an EO water generator (model ROX 20TA).[6] A 12% salt solution and softened tap water were continuously pumped into the EO water generator set at 19 A and 10 V. The generator was run for 15 min before EO water was collected so that the system could equilibrate; then the water was dispensed at 1.5 L/min. The alkaline EO water had a pH of 11.4 and an ORP of –795 mV. The acidic EO water had a pH of 2.7, an ORP of 1150 mV, and free chlorine level of 70 to 80 ppm. The pH and ORP of the solutions were checked using a pH/ORP meter,[7] and the free chlorine content of the acidic EO water was tested by titration with an N,N-diethyl-p-phenylenediamine-ferrous ethylene diammonium sulfate (DPD-FEAS) test kit.[8] The EO water was heated using a hot plate and then placed in preheated water bath at the desired temperature. After heating, paper test strips[9] were used to verify that the chlorine content of the acidic EO water was above 50 ppm.

Design of Experiments. To minimize the number of trials needed to evaluate the efficacy of EO water, the Box Behnken technique was used to select treatment time and temperature combinations (Myers and Montgomery, 1995). A temperature range of 40 to 50°C and a time range of 1 to 5 min were used for the design. The design was generated using MINITAB Statistical Software[10] and consisted of 15 runs (Table 1).

Treatment of Shell Eggs with EO Water. Alkaline and acidic EO water were heated to the desired temperature (between 40 and 50°C) selected using the response surface design, and then 500 mL of alkaline or acidic EO water was transferred to 1,000-mL beakers and placed in a preheated water bath. The artificially inoculated egg was first placed in the alkaline EO water for the selected time (1 to 5 min) and then taken out using sterile tongs and transferred to the acidic EO water for the selected time (1 to 5 min). After the treatment, the egg was immediately removed and placed in a sterile plastic bag containing 25 mL of buffered peptone water[2] and shaken vigorously for 1 min. Three eggs were used for each run in the response surface table; all of the runs (15) in the table were replicated twice, and averages were used for analysis.

Effect of Alkaline or Acidic EO Water Alone. The effect of alkaline or acidic EO water individually on microbial reduction was investigated. Eggs were contaminated as described earlier. Each egg was placed in a 1,000-mL beaker with 500 mL of acidic or alkaline EO water at 45°C for 3 min. Two replicates of 3 eggs were performed for each type of EO water and for both bacteria.

TABLE 1. *Salmonella* Enteritidis PT8NSR reduction after treatment with electrolyzed oxidizing (EO) water[1]

Treatment temperature (°C)	Alkaline EO water treatment time (min)	Acidic EO water treatment time (min)	Average $\log_{10}$ reduction per gram of shell membrane[2,3]
40	3	1	0.63
40	1	3	2.20[4]
40	5	3	2.00[4]
40	3	5	1.40
45	1	1	0.71
45	3	3	2.15[4]
45	3	3	2.66[4]
45	1	5	2.15
45	5	1	1.84
45	5	5	2.35[4]
45	3	3	2.28[4]
50	3	5	2.41[4]
50	1	3	2.30[4]
50	5	3	2.13[4]
50	3	1	2.09[4]

[1]$\log_{10}$ reduction is stated as the $\log_{10}$ reduction compared with contaminated but untreated samples (average initial count of 5.2 $\log_{10}$).

[2]Minimum $\log_{10}$ reductions out of 6 replicates (3 eggs × 2 replications).

[3]Reductions are greater than or equal to stated reductions. There is no significant difference between samples ($P > 0.05$).

[4]Treatments yielded no colonies on the plates. $\log_{10}$ reductions were obtained by subtracting the minimum detection limit from the initial $\log_{10}$ population.

Comparison of EO Treatment and a Commercial Detergent-Sanitizer Treatment. To compare EO water treatment with commercial treatments, Diversey egg detergent[11] was prepared by adding 1.9 g/L of detergent to tap water to reach a pH of 10.5. A 100 ppm free chlorine solution sanitizer was prepared from sodium hypochlorite.[12] This treatment was called the detergent-sanitizer treatment. Similar to EO water treatment, eggs were inoculated with *S.* Enteritidis PT8NSR or *E. coli* K12NSR as described earlier. The eggs were first soaked in the detergent solution at 45°C for 3 min and then soaked in the chlorine solution for 3 min at 45°C. For the EO water treatment, eggs were soaked in alkaline EO water at 45°C for 3 min followed by soaking in acidic EO water for 3 min at 45°C. The eggs were immediately removed and placed in a plastic bag with 25 mL of buffered peptone water. Three eggs were used for each evaluation, and the whole experiment was replicated twice.

Pilot-Scale Study

Preparation of Washing Solutions. The EO water was generated using a model ROX15SA EO water generator.[6] A Seymour egg washer[13] was used for this study. The wash tank was filled with 260 L of alkaline EO water and heated to 45°C. A stainless steel wash sink with a 3,000-W stainless steel immersion heater was used to heat and hold the acidic EO water. The sink was filled with 19 L of acidic EO water and heated to 50°C, which was 5°C higher than the alkaline EO water.

Diversey egg detergent was used at pH 10.5. However, tap water was used during the preparation instead of

[6]Hoshizaki Electric Co. Ltd., Sakae, Toyoake, Aichi, Japan.
[7]Model 445, Corning, Inc. Big Flats, NY.
[8]Hach, Inc., Loveland, CO.
[9]Advantec MHS, Inc., Dublin, CA.
[10]Version 13, MINITAB, State College, PA.
[11]Johnson Diversey, Sturtavent, WI.
[12]The Chlorox Company, Oakland, CA.
[13]Seymour Foods Inc., Topeka, KS.

deionized water due to a larger volume requirement. The alkaline detergent water was heated to 45°C. The sanitizer was a 100 ppm free chlorine solution that was held and heated in the stainless steel tank to 50°C. The heated acidic EO water and the sanitizer were pumped from the sink to the egg washer for spraying.

Washing Procedure. Eggs, artificially inoculated with *E. coli* K12NSR, were placed on the rollers of the egg washer using sterile gloves and exposed to alkaline EO water or detergent and constant brushing for 3 min and then sprayed with acidic EO water or chlorine sanitizer that had an exposure time of 1 min and 23 s. After the treatment, the egg was immediately removed and placed in a sterile plastic bag containing 25 mL of buffered peptone water and shaken vigorously for 1 min. Thirty eggs were used for each run. The whole experiment was replicated 3 times.

Microbial Analysis

A treated or untreated egg in a plastic bag with 25 mL of buffered peptone water was shaken vigorously for 1 min. The egg was then removed from the bag, and the buffered peptone water was serially diluted in buffered peptone water and spiral-plated in duplicate on TSAYE with 100 μg/mL of nalidixic acid and streptomycin sulfate by using the Autoplate 4000.[14] The plates were incubated at 37°C for 24 h and enumerated using Q-count.[15] The weight of the shell was also determined so that colony-forming units could be determined per gram of eggshell + membrane. After treatment the egg was cracked, and the shell and membrane were rinsed with deionized water and allowed to dry overnight at room temperature and then weighed. The log reduction was calculated using the following formula:

$$\log_{10} \text{reduction} = \log_{10} \text{cfu/g of untreated eggshell/membrane} - \log_{10} \text{cfu/g of treated eggshell/membrane} - \log_{10} 9.0 \times 10^2 \text{ cfu/g eggshell/membrane}$$

The minimum detection limit of 9.0×10^2 cfu/g of shell and membrane was subtracted from the calculated $\log_{10}$ reduction when plates with zero colonies were obtained.

Enrichments were performed for samples demonstrating zero plate counts. For *S.* Enteritidis PT8NSR enrichment 1 mL of buffered peptone rinsing solution was transferred to 9 mL of TSBYE with 100 μg/mL of nalidixic acid and streptomycin sulfate or TT Broth Base Hajna.[2] TT Broth Base Hajna was used as a selective enrichment for *Salmonella* and TSBYE was used as a general enrichment. The TSBYE broth was incubated at 37°C for 24 h, and the TT Broth Base Hajna was incubated at 45°C for 48 h. A loopful of each enrichment solution was then streaked onto xylose lysine desoxycholate[2] (XLD) agar and incubated for 24 h at 37°C. Colonies were confirmed using *Salmonella* O Antiserum A-1 latex agglutination test.[16]

For *E. coli* K12NSR enrichment, 1 mL of buffered peptone rinsing solution was transferred to 9 mL of TSBYE with 100 μg/mL of nalidixic acid and streptomycin sulfate and MacConkey[2] broth. Again TSBYE was used as a general enrichment, and MacConkey broth was used as a selective enrichment. The inoculated tubes were incubated for 24 h at 37°C. A loop of TSBYE broth was streaked onto TSAYE agar and incubated for 24 h at 37°C. A change in color, from purple to yellow, in MacConkey broth or growth on TSAYE agar indicated the presence of *E. coli* K12NSR.

Effect of Washing Treatment on Egg Quality

To evaluate the effect of egg washing with EO water and compare it with the commercial detergent-sanitizer treatment, albumen height, presence of cuticle, and eggshell strength were measured for untreated eggs and treated eggs after pilot-scale washing.

Measurement of Albumen Height

Albumen height was used as a measure of overall egg quality, because higher albumen height is an indicator of a better quality egg (Stadelman and Cotterill, 1995). Eggs were cracked onto a flat glass surface, and a micrometer was used to measure the height of the albumen in millimeters. Thirty eggs were analyzed for each treatment.

Presence of Cuticle

To determine whether the cuticle was intact after treatment, MST Cuticle Blue,[17] a cuticle sensitive dye, was used (Board and Halls, 1973). Each egg was immersed in the dye solution for 1 min and then rinsed with tap water for 2 to 3 s. The eggs were then allowed to dry, and the color was monitored.

To quantify the color of the cuticle stain a Minolta Chromo Meter CR 200[18] colorimeter was used to measure the L*a*b color space (CIELAB). The CIELAB color space uses the following parameters: L* indicates lightness; a* and b* are chromaticity coordinates. Value –a* indicates a green color, +a* a red color, –b* a blue color, and +b* a yellow color. Three randomly selected spots on the side of each egg were analyzed and then averaged to get an overall measurement. Three replicates of 10 eggs were used for each treatment.

Measurement of Eggshell Strength

To determine whether the EO water treatment had any effect on eggshell quality, the failure force using quasistatic compression was used (Fajardo et al., 1996). An

[14]Spiral Biotech, Norwood, MA.
[15]Version 2.1, Spiral Biotech, Norwood, MA.
[16]Remel Microbiology Products, Lenex, KS.
[17]MS Technologies, Memphis, TN.
[18]Minolta, Ramsey, NJ.

Instron Universal Testing Machine[19] was used with 2 flat plates. The eggs were compressed at 1.27 cm/min. The force and deformation data were recorded using Labview 5.0[20] at 40 readings/s. Thirty eggs were analyzed at polar and equatorial orientations.

Statistical Analysis

The MINITAB statistical software was used to produce and analyze the response surface design. A one-way ANOVA with a 95% confidence level was used to compare the differences in untreated, EO water treated, and commercial detergent-sanitizer treated eggs. To discern any differences in the treatments, a Tukey's comparison was performed at the 95% confidence level.

RESULTS AND DISCUSSION

Response Surface Design model for S. Enteritidis PT8NSR

The response surface model was used to illustrate the response of *S.* Enteritidis PT8NSR inactivation based on 3 factors: treatment temperature, alkaline EO water treatment time, and acidic EO water treatment time. To estimate the experimental variability, the response surface design contained 3 replicates of the center point of the model: 45°C, 3 min, and 3 min (Table 1). When the center points were analyzed a variance of 0.1 for *S.* Enteritidis PT8NSR obtained with a *P*-value of 0.25. The data were observed by examining trends through regression.

Table 1 shows the minimum $\log_{10}$ reduction for *S.* Enteritidis PT8NSR, which is between ≥0.63 $\log_{10}$ and ≥2.60 $\log_{10}$. The effects of treatment temperature and treatment times in alkaline and acidic EO water were not significant. The majority of plates for treatments at 50°C yielded no growth indicating complete inactivation; however, both enrichment procedures demonstrated positive growth. This result clearly demonstrates that the plating procedure could not detect the low populations because the detection limit was 9.0×10^2 cfu/g of shell and membrane (an average weight of 5.6 g was used for eggshell and membrane).

The response surface design was analyzed using a regression analysis, which enabled the trends and significant factors of the model to be illustrated. A full quadratic model was used. Based on the *P*-values in the regression there were no significant factors at a 95% confidence level. The R^2 for the model was 0.88. There was no significant difference in the responses of the response surface design. Based on this, it can be inferred that the model is a poor fit due to the insignificant differences in treatment responses under the tested conditions.

[19]Model 4444, Instron Corporation, Canton, MA.
[20]National Instruments, Austin, TX.

TABLE 2. *Escherichia coli* K12NSR reduction after treatment with EO water[1]

Treatment temperature (°C)	Alkaline EO water treatment time (min)	Acidic EO water treatment time (min)	Average $\log_{10}$ reduction per gram of shell membrane[2,3]
40	3	1	2.02
40	1	3	1.55
40	5	3	1.44[4]
40	3	5	1.71
45	1	1	0.97
45	3	3	2.63[4]
45	3	3	2.52[4]
45	1	5	1.32[4]
45	5	1	1.30
45	5	5	1.76[4]
45	3	3	1.47[4]
50	3	5	1.44
50	1	3	2.25[4]
50	5	3	1.03[4]
50	3	1	1.50

[1]$\log_{10}$ reduction is stated as the $\log_{10}$ reduction compared with contaminated but untreated samples (average initial count of 5.2 $\log_{10}$).
[2]Minimum $\log_{10}$ reductions out of 6 replicates (3 eggs × 2 replications).
[3]Reductions are greater than or equal to stated reductions. There is no significant difference between samples ($P > 0.05$).
[4]Treatments yielded no colonies on the plates. $\log_{10}$ reductions were obtained by subtracting the minimum detection limit from the initial $\log_{10}$ population.

Response Surface Design Model for E. coli K12NSR

The results of the surface response design for *E. coli* K12NSR are shown in Table 2. The average $\log_{10}$ reductions were between ≥0.97 and ≥2.63. As in the case of *S.* Enteritidis PT8NSR, there is not much of a trend in the data. Treatment temperature and treatment times in alkaline and acidic EO waters did not seem to affect the reduction. The majority of plates for treatments at 50°C yielded no growth indicating complete inactivation; however, both enrichment procedures indicated positive growth.

A regression was performed on the data, and it was determined that there were no significant factors in the model with an R^2 of 0.52. As with the model for *S.* Enteritidis PT8NSR there was no significant difference between the responses, and again it can be concluded that the model was weak and could not be used with confidence.

Determination of EO Water Treatment Parameters

Even though there were no significant differences in the responses of both models a treatment combination needed to be selected for subsequent experiments. A treatment of 45°C, 3 min alkaline EO water treatment, and 3 min acidic EO water treatment was selected. A 45°C temperature was selected because it is in the range of temperatures commonly used in industry. At this treatment combination, there was also a high correlation between *S.* Enteritidis PT8NSR and *E. coli* K12NSR with an R^2 of 0.92.

TABLE 3. Log_{10} reductions[1] per gram of eggshell and membrane for in vitro treatments

	Salmonella Enteritidis PT8NSR[4]	*Escherichia coli* K12NSR[4]
Alkaline EO water[5]	1.7 ± 0.59[2,a]	3.6 ± 0.31[2,a]
Acidic EO water[5]	≥2.1 ± 0.31[3,b]	≥2.3 ± 0.13[3,b]
EO water (alkaline followed by acidic EO water)[6]	≥2.1 ± 0.31[3,c]	≥2.3 ± 0.13[3,c]
Detergent-sanitizer[6]	1.7 ± 0.57[2,d]	2.0 ± 0.48[2,d]

[a-d]Values within a column without a common superscript are significantly different ($P < 0.05$).

[1]Average initial counts were 5.2 log_{10}.

[2]Average log_{10} reductions.

[3]Treatments yielded no colonies on the plates. Log_{10} reductions were obtained by subtracting the minimum detection limit from the initial log_{10} population.

[4]n =3 per repetition.

[5]EO = electrolyzed oxidizing. Treatment at 45°C for 3 min.

[6]Treatment at 45°C for 3 min followed by 45°C for 3 min.

Effect of Alkaline and Acidic EO Water Alone

The effects of alkaline EO water alone were examined using *S.* Enteritidis PT8NSR and *E. coli* K12NSR. Alkaline EO water treatment of 3 min at 45°C yielded log_{10} reductions of 1.7 and 3.6 for *S.* Enteritidis PT8NSR and *E. coli* K12NSR, respectively. Acidic EO water treatment of 3 min at 45°C yielded log_{10} reductions of ≥ 2.1 and ≥ 2.3 for *S.* Enteritidis PT8NSR and *E. coli* K12NSR, respectively (Table 3). Although it appeared that alkaline EO water might be more effective, the contrary was true. TSAYE plates for the acidic EO water treatment had undetectable levels of bacteria, whereas plates for alkaline EO water treatment all had detectable levels of bacteria. Therefore the acidic EO water treatment was more effective at killing the bacteria than the alkaline EO water treatment. However, the alkaline treatment is still an integral part of the washing process, because it can be used to remove the soil that may be present on the eggs.

Comparison of EO Water and Commercial Detergent-Sanitizer Treatments

A comparison of the detergent-sanitizer treatment and the EO water treatment was done using both bacteria to show whether there was a statistically significant difference between the 2 treatments. A log_{10} reduction of greater than 2 was observed by EO water treatment of *S.* Enteritidis PT8NSR and *E. coli* K12NSR (Table 3). Statistical analysis of the EO water treatment suggested significantly greater log reductions than the detergent-sanitizer treatment. This would represent the worst-case scenario, because no agitation, brushing, or other factors involved in commercial egg washing were used in the in vitro trials.

Pilot-Scale Study

Microbial Analysis. A microbial analysis was performed on eggs treated with EO water and commercial detergent-sanitizer methods after being washed in a pilot-scale washer. Average log_{10} reductions of ≥2.98 and ≥2.91 were obtained for the EO water treatment and commercial detergent-sanitizer treatment, respectively (Table 4). TSAYE plates for EO water treatment did not produce any colonies, whereas 5% of plates (3 plates) analyzed for commercial detergent-sanitizer treatment did have colonies. In addition to plating, each sample was enriched in nonselective and selective broths. The nonselective enrichment for both EO water and commercial detergent-sanitizer treatments were negative 69% of the time. All selective enrichments were negative, indicating that cells were killed or injured during treatment. Statistical analysis indicated that there was no significant difference between log_{10} reduction for EO water treatment and commercial detergent-sanitizer treatment. When these results are compared with those from the in vitro experiments, it seems that agitation, brushing, and spraying may be important factors during washing, as well as the increased temperature of the acidic EO-sanitizer rinse.

Effect of Washing Treatment on Egg Quality

Albumen Height. The average albumen heights were 7.05 and 6.41 mm for EO water and detergent-sanitizer treated eggs, respectively, whereas the average albumen height for untreated eggs was 6.42 mm (Table 4). Although it appears that EO water treatment increased the albumen height when compared with untreated and detergent-sanitizer treated eggs, there was no significant difference between treated and untreated eggs.

Eggshell Strength. For untreated eggs the average failure forces were 33.4 and 33.9 N for polar and equatorial orientations, respectively (Table 4). The failure forces for EO water treated eggs were 36.4 and 26.9 N, respectively, and for detergent-sanitizer washed eggs were 33.3 and 33.7 N, respectively. Although it does appear that EO water treatment decreased the force needed to cause failure, statistical analysis showed that there was no significant difference between the treatments.

The deformation of the egg at failure was also determined. Deformations of 0.69, 0.83, and 0.82 cm for untreated, EO water treated, and commercial detergent-

TABLE 4. Mean results for egg quality attributes for untreated and pilot-scale treated eggs[1]

Parameter	Untreated	EO water	Detergent-sanitizer
Log_{10} reduction[3] (*E. coli* K12NSR)	N/A[2]	≥ 2.98 ± 0.02[a]	≥ 2.91± 0.09[a]
Albumen height (mm)	6.42 ± 1.10[a]	7.05 ± 1.60[a]	6.41 ± 1.35[a]
Failure force at equator (N)	33.9 ± 3.10[a]	26.9 ± 1.70[a]	33.7 ± 1.20[a]
Failure force at pole (N)	33.4 ± 10.3[a]	36.4 ± 7.60[a]	33.3 ± 6.50[a]
Deformation at equator (cm)	3.31 ± 0.09[a]	3.30 ± 0.08[a]	3.10 ± 0.24[a]
Deformation at pole (cm)	0.69 ± 0.02[a]	0.83 ± 0.16[a]	0.82 ± 0.21[a]
CIELAB a* value	–34.4 ± 4.46[a]	–25.4 ± 4.80[b]	–32.6 ± 6.86[b]
CIELAB b* value	23.6 ± 5.18[a]	26.7 ± 2.62[b]	31.3 ± 2.52[b]

[a,b]Means within a row without a common superscript are significantly different ($P < 0.05$).

[1]n = 30 per repetition.

[2]Not applicable.

[3]Average initial counts were 5.9 log_{10}.

sanitizer treated eggs, respectively, were found at the polar orientation (Table 4). For the equatorial orientation deformations of 3.3, 3.3, and 3.1 cm were determined for untreated, EO water treated, and commercial detergent-sanitizer treated eggs, respectively. In polar orientation it appears that treating eggs with either EO water or detergent-sanitizer increases the deformation at failure, but when a statistical analysis was performed it was determined that there was no significant difference between the treatments for either the polar or equatorial orientations.

Cuticle Presence. The presence of the cuticle was examined qualitatively and quantitatively. After being dried, the eggs were visually inspected, and it was observed that untreated eggs had a consistent green color, whereas EO water and detergent-sanitizer treated eggs were spotty green and white. A full green color indicated the presence of an intact cuticle. A CIELAB color space was used to quantify the color (Table 4). Green color is indicated by –a* and a yellow color by +b*. Untreated eggs had a* and b* values of –34.4 and 23.6, respectively, and EO water treated eggs had a* and b* values of –25.4 and 26.7, respectively. Statistical analysis performed on the data indicated that there was a significant difference between untreated and treated eggs. However, there was no significant difference between EO water treated and detergent-sanitizer treated eggs.

Electrolyzed oxidizing water shows potential as a washing and sanitizing agent for washing shell eggs. The regression model indicated that there is little or no effect of treatment temperature, alkaline EO water wash time, or acidic EO water wash time individually or in combination. Models of bacterial reductions had poor fits and could not be used with confidence. A treatment combination of 45°C, 3-min alkaline EO water treatment, and 3-min acidic EO water treatment was used for in vitro and pilot-scale egg washing experiments. Additional experiments showed that acidic EO water was more effective at reducing *S.* Enteritidis PT8NSR than alkaline EO water, yielding log_{10} reductions of ≥2.15 and 1.68 for acidic and alkaline EO water, respectively, in the in vitro experiments. When the EO water treatment was compared with the commercial detergent-sanitizer treatment in vitro log_{10} reductions of ≥2.15 and ≥2.31 were observed for EO water, whereas the commercial detergent-sanitizer treatment yielded reductions of 1.67 and 2.00 for *S.* Enteritidis PT8NSR and *E. coli* K12NSR, respectively.

A pilot-scale washing study was conducted using EO water and commercial detergent-sanitizer treatments. A ≥2.95 log_{10} reduction was achieved using EO water and a ≥2.86 log_{10} reduction using commercial detergent-sanitizer treatment. Statistical analysis showed that there was no significant difference in the microbial reduction of these treatments. There were no negative effects on egg quality except for cuticle destruction; however, EO water treatment and commercial detergent-sanitizer treatment affected cuticle quality similarly.

Based on these results alkaline and acidic EO water have the potential to fit into the Pennsylvania Egg Quality Assurance Program (PEQAP) program, which is a pre-Hazard Analysis Critical Control Point (pre-HACCP) program that incorporates egg processing into a larger egg quality control program. This program requires a wash water temperature of at least 32°C, a pH of 11, and a sanitizer containing between 50 and 200 ppm of free chlorine. EO water has a pH of 11.4 and between 50 and 80 ppm of free chlorine. Through the experience of working with EO water it was concluded that low levels of egg solids have very little effect on the pH of EO water, only at concentrations of 2% were effects noticed. The results of this study indicate that EO water has a potential to be used as a sanitizing agent for egg washing.

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