Journal of Food Protection, Vol. 77, No. 12, 2014, Pages 2176–2180
doi:10.4315/0362-028X.JFP-14-161

Research Note

Efficacy of Neutral Electrolyzed Water for Reducing Pathogenic Bacteria Contaminating Shrimp

PATTAMA RATANA-ARPORN* AND NARUEMON JOMMARK

Department of Fishery Products, Faculty of Fisheries, Kasetsart University, 50 Ngamwongwan Road, Ladyao, Chatuchak Bangkok, 10900 Thailand; and Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok, 10900 Thailand

MS 14-161: Received 7 April 2014/Accepted 14 July 2014

ABSTRACT

Pathogenic contamination is a food safety concern. This study was conducted to investigate the efficacy of neutral electrolyzed water (NEW) in killing pathogens, namely, *Vibrio parahaemolyticus, Vibrio vulnificus, Salmonella* Enteritidis, and *Escherichia coli* in shrimp. Pure cultures of each pathogen were submerged separately in NEW containing five different chlorine concentrations: 10, 30, 50, 70, and 100 ppm. For each concentration, three submersion times were tested: 1, 3, and 5 min. The population of *V. parahaemolyticus* was rapidly reduced even at low concentrations, but prolonged contact times caused only a slight reduction. *V. vulnificus* was gradually inhibited with increasing NEW concentrations and contact times. For the *V. parahaemolyticus* applications of 70 ppm for 5 min and of 100 ppm for 3 min, each eliminated 7 log CFU/ml. For *V. vulnificus,* applications of 50 ppm for 3 min and 100 ppm for 1 min, each eliminated 7 log CFU/ml. *Salmonella* Enteritidis and *E. coli* were slightly reduced by NEW. Applications of 50 ppm for 15 min and 10 ppm for 30 min completely eliminated 4.16 log CFU/g of *V. parahaemolyticus* in inoculated shrimp, while only a 1-log CFU/g reduction of *V. vulnificus* was detected. Soaking shrimp in 10 ppm NEW for 30 min did not affect its sensory quality. Our results suggest NEW could be an alternative sanitizer to improve the microbiological quality of seafood.

Sodium hypochlorite (NaOCl) is an effective chlorine disinfectant that has long been used in the food industry *(6),* but it can incompletely oxidize food constituents, producing byproducts unsafe for humans, such as chloroform ($CHCl_3$), haloacetic acid, and other trihalomethanes, which are known or suspected carcinogens and mutagens *(11).* An alternative disinfectant is electrolyzed water (EW), which is formed by electrolyzing a dilute salt (NaCl) solution in an electrolysis chamber, where anode and cathode electrodes are separated by a membrane, and has a strong effect against pathogens in food *(4, 10).*

EW disinfectants include acidic EW (AEW) and neutral EW (NEW). AEW can effectively reduce bacterial populations in some seafood *(13),* but its use is limited because of its low pH value (≤2.7), its decreasing biocidal effectiveness over time due to the rapid volatilization of dissolved Cl_2 gas, and its effect on the sensory characteristics of food products *(7, 9).*

NEW also has strong antimicrobial activity, but it is a neutral solution (pH 6.5 to 8.5), so its application on food does not affect the food's pH, surface color, or general appearance, and it does not corrode processing equipment or irritate hands as severely as AEW does *(1).* Despite these advantages, there have been few studies on the effects of NEW in seafood.

* Author for correspondence. Tel: 6629428644-5; Fax: 6629428644-5, Ext 12; E-mail: ffispmn@ku.ac.th.

The objectives of this study were to determine the effectiveness of NEW on pure cultures of pathogens frequently found in shrimp, namely, *Vibrio parahaemolyticus, Vibrio vulnificus, Salmonella* Enteritidis, and *Escherichia coli (2, 5, 8)* and to evaluate its efficacy against selected pathogens artificially inoculated in shrimp and its effects on the sensory characteristics of shrimp.

MATERIALS AND METHODS

Cell culture preparations. *V. parahaemolyticus* ATCC 17802, *V. vulnificus* DMST 5852, *Salmonella* Enteritidis DMST 15676, and *E. coli* ATCC 25922 stock cultures were obtained from the Department of Medical Sciences, Ministry of Public Health of Thailand, and all microbiological media were purchased from Difco (BD). *V. parahaemolyticus* and *V. vulnificus* were cultured individually on thiosulfate citrate bile salt sucrose agar. *Salmonella* Enteritidis and *E. coli* were cultured on xylose lysine desoxycholate agar and eosin methylene blue agar, respectively. *V. parahaemolyticus* was precultured by transferring the cell from agar plate to 10 ml of tryptic soy broth (TSB), supplemented with 1.5% NaCl using a 10-μl inoculation loop at 37°C for 24 h, and a loopful of *V. parahaemolyticus* was transferred to 10 ml of TSB salt before being inoculated at 37°C for 8 h. Then, 1 ml of culture was resuspended into 100 ml of TSB salt and incubated at 37°C for 3.5 h, for cells in the log phase of growth. *Salmonella* Enteritidis and *E. coli* were also precultured following the same procedure used for *V. parahaemolyticus,* except they were cultured in TSB without NaCl for 4 h. *V. vulnificus* was precultured in alkaline peptone water containing 3% NaCl by selecting a single colony on

TABLE 1. *Physicochemical properties of treated neutral electrolyzed water (NEW) as used in treatment of pure culture and inoculated shrimp*[a]

Experiments	Treatment solution	ACC (ppm)	pH	ORP (mV)
Pure cultures	NEW10	10.92 ± 0.00	7.16 ± 0.01	863 ± 4
	NEW30	30.99 ± 0.16	7.14 ± 0.01	875 ± 4
	NEW50	50.08 ± 0.09	7.13 ± 0.02	882 ± 5
	NEW70	67.98 ± 0.23	7.11 ± 0.01	888 ± 2
	NEW100	101.07 ± 0.34	7.11 ± 0.01	899 ± 2
Inoculated shrimp	TW	1.34 ± 0.00	7.14 ± 0.01	198 ± 3
	NEW10	10.27 ± 0.12	7.11 ± 0.01	841 ± 2
	NEW50	50.67 ± 0.29	7.05 ± 0.03	863 ± 5

[a] Values are means ± standard deviations of determinations.

thiosulfate citrate bile salt sucrose agar to 10 ml of alkaline peptone water before being incubated at 37°C for 12 h. Then, the cells were grown to log phase by transferring 1 ml of cells to 99 ml of alkaline peptone water and shaking at 100 rpm at 28 to 30°C for 9 h. Finally, all the activated cells were used as the stock inoculums, with an initial number of 8 to 9 log CFU/ml.

Preparation of inoculated shrimp. White shrimp (*Penaeus vannamei*) of the same crop were obtained from a farm in Thailand and stored at 4°C until use. Head-on shrimp (66 to 70 shrimp per kg) were washed twice with tap water (TW) and inoculated with the pathogen cells (selected from the effective results on pure cultures) in a saline solution (0.85% NaCl) containing 6.0 to 6.5 log CFU/ml by immersing (1 g of shrimp per 5 ml of bacteria cell) and stirring with a glass rod 45 times per minute in a sterile beaker for 2 min. The inoculated shrimp were then air dried on a sterile wire screen under a laminar flow hood for 10 min at room temperature. Using this procedure, approximately 4.0 to 4.5 log CFU/g of pathogens were expected to contaminate the shrimp. The artificial inoculation counts refer to bacterial populations in nontreated samples. All treatments were conducted twice.

NEW preparation. An electrolysis device with a semipermeable membrane (model 80, Tip Stel; Aquaeca, Saint Petersburg, Russia) was used to produce NEW from the anode side. The 12% NaCl solution was pumped into the electrolysis chamber at a flow rate of 80 liters/h using 10 A of electric current. NEW with different available chlorine concentrations (ACC) can be prepared by diluting the freshly produced NEW with distilled water. The pH and the oxidation reduction potential (ORP) were measured using a pH meter (model 744, Metrohm, Herisau, Switzerland) equipped with pH and ORP probes. The ACC was measured by iodometric titration *(3)*.

Treatment of pure cultures. Activated cell cultures of approximately 8 log CFU/ml were prepared in each broth media. Fifteen pure cultures of each pathogen were prepared, and three cultures from each were submerged in NEW containing one of five different chlorine concentrations: 10, 30, 50, 70, and 100 ppm. For each concentration, 1 ml of bacteria cells was added to 9 ml of NEW; the cultures were submerged for 1, 3, or 5 min. After each submersion, 1 ml of each sample was immediately transferred to 9 ml of the neutralizing solution (0.5% sodium thiosulfate) and left for 5 min before making a series of dilutions in 0.85% NaCl solution. Survival of *Vibrio* spp. were determined on tryptic soy agar (TSA), supplemented with 1.5% NaCl (wt/vol), while the others were determined on TSA without supplement, using the pour plate technique. All duplicate plates were then incubated at 37°C for 24 h, and colonies formed were counted. The 1-ml dilution of bacteria added to 9 ml of sterile distilled water represents the initial cell culture for this experiment. The experiment was repeated three times.

Treatment of inoculated shrimp. Inoculated shrimp (about 70 g) were put into a sterile Erlenmayer flask and separately immersed in twice the volume of NEW 10 ppm, NEW 50 ppm, or TW, and soaked at room temperature (25 ± 2°C) for 15, 30, or 45 min. The 25-g samples of treated shrimp were immediately taken and homogenized in 225 ml of 0.85% NaCl solution with a stomacher (model BA 7021, Seward, London, UK) for 2 min and then serially diluted, using the spread-plated technique, and incubated as described previously. Surviving populations of *V. parahaemolyticus* and *V. vulnificus* were counted on thiosulfate citrate bile salt sucrose agar, and those of *Salmonella* Enteritidis and *E. coli* were counted on xylose lysine desoxycholate agar and eosin methylene blue agar, respectively.

Sensory evaluation of the treated shrimp. Shrimp were immersed in 10 ppm of NEW for 30 min, which was found to be an effective treatment (described in the following in ''Results''), and TW was used as a control condition. Sensory evaluation were done by 18 panelists using the hedonic 9-point scale, with 9 representing ''extremely liked'' and 1 representing ''extremely disliked.'' Noninoculated samples were used to ensure the panelists' safety. Both raw, peeled and cooked shrimp were evaluated for their appearance, color, odor, texture, overall acceptability, and flavor (for only cooked shrimp).

Statistical analysis. Data from the bacteria populations in pure cultures and in inoculated shrimp were compared using Duncan's multiple range tests, and sensory evaluation data were analyzed using a *t* test. Both tests were performed using SPSS software (SPSS Inc., Chicago, IL), with the 0.05 level of significance.

RESULTS AND DISCUSSION

Treatment of pure cultures. The effectiveness of NEW against microorganisms in pure cultures was assessed using NEW, with different ACC levels and contact times. All NEW solutions had pH values and ORPs ranging from 7.11 to 7.16 and 863 to 899 mV, respectively (Table 1). Surviving *V. parahaemolyticus* and *V. vulnificus* populations from the initial amount of about 7 log CFU/ml, as a result of NEW treatments, are shown in Figure 1A and 1B. For *V. parahaemolyticus,* a rapid

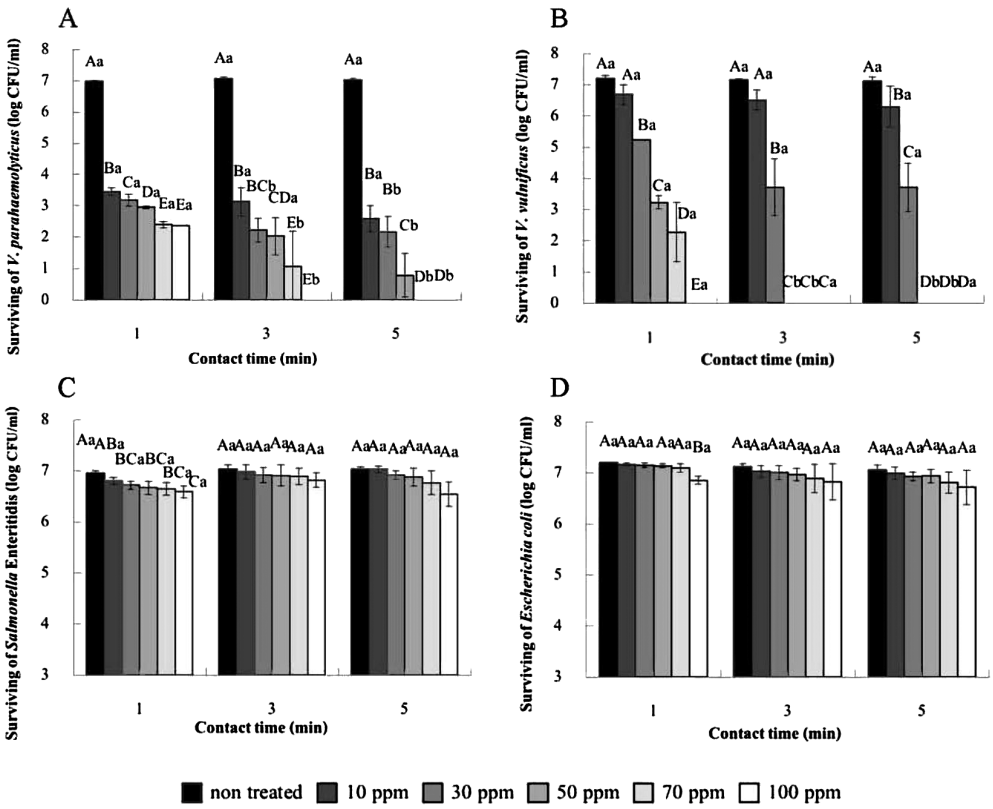

FIGURE 1. *Inhibition effect of NEW on pure culture of (A)* Vibrio parahaemolyticus, *(B)* Vibrio vulnificus, *(C)* Salmonella *Enteritidis, and (D)* Escherichia coli. *Surviving population numbers are the means of triplicate measurements ± standard deviations. Uppercase letters indicate significant differences (*$P < 0.05$*) in ACC. Lowercase letters indicate significant differences (*$P < 0.05$*) in contact time. A value of 0.00 log CFU/ml indicates no detectable survivors by the pour plate method, with a dilution of 1:10. A mean less than 1 log CFU/ml indicates that at least one of the triplicates was below the detection limit (10 CFU/ml).*

reduction of approximately 4 log CFU/ml was detected after contact with 10 ppm. The results indicate that a certain amount of *V. parahaemolyticus* was reduced immediately after contact with NEW ($P < 0.05$), even at low concentration; thereafter, increasing the concentration or contact time had only a slight effect. NEW treatments of 70 ppm for 5 min and 100 ppm for 3 min each eliminated 7 log CFU/ml of the initial culture of *V. parahaemolyticus*.

For *V. vulnificus,* the 10-ppm NEW treatment caused less than 1-log CFU/ml reduction for all contact time durations (Fig. 1B). Increasing the ACC from 10 to 30, 50, 70, and 100 ppm gradually reduced the survival by about 1 log at each interval concentration for the 1-min contact time, while a reduction of about 3 log CFU/ml was detected at the 3- and 5-min contact times. Applying 50 ppm of NEW for 3 min or 100 ppm for 1 min eliminated 7 log CFU/ml of the initial culture. This study demonstrated that the bactericidal effectiveness of NEW depended more on ACC than on treatment time.

The inhibitory results of *V. parahaemolyticus* and *V. vulnificus* in this study were similar to those reported by Ren and Su *(13),* who used AEW, and Quan et al. *(12),* who used a weakly AEW, and the gradual reduction pattern of *V. vulnificus* in this study agreed with the results of a previous study of AEW *(13)*.

In this study, we found only slight reductions of *Salmonella* Enteritidis and *E. coli* (Fig. 1C and 1D). Limited bacterial reductions of approximately 0.5 log CFU/ml were observed for both species. Increasing the NEW concentration slightly increased its effectiveness, while increasing the contact time seemed to have no effect on reduction. This study suggests that NEW is less effective against *Salmonella* Enteritidis and *E. coli* than it is against *V. parahaemolyticus* and *V. vulnificus*. The difference in efficacy against microbial species may have been due to the pH-dependent sensitivity to the lethal effect of each species.

Treatment of inoculated shrimp. *V. parahaemolyticus* and *V. vulnificus* were selected as target pathogens to study their effects on shrimp. Shrimp inoculated with either *V. parahaemolyticus* or *V. vulnificus* to about 4.16 to 4.31 log CFU/g was individually immersed in 10 or 50 ppm of NEW, for different contact times of 15, 30, or 45 min at each concentration. TW was used for comparison because it is typically used for cleaning shrimp in processing plants. Compared with TW (1.34 ppm of ACC), NEW had a similar pH range (7.05 to 7.11), but its ORP was four times higher, and its ACC was 8 and 38 times higher for 10 ppm and 50 ppm, respectively (Table 1). The bactericidal effect of NEW was higher than those of TW on both species. Samples treated with 50 ppm of NEW for 15 min showed remarkably reduced *V. parahaemolyticus* cells by more than 4.16 log CFU/g, while 2.07 log CFU/g of *V. parahaemolyticus* cells were reduced with NEW 10 ppm for the same

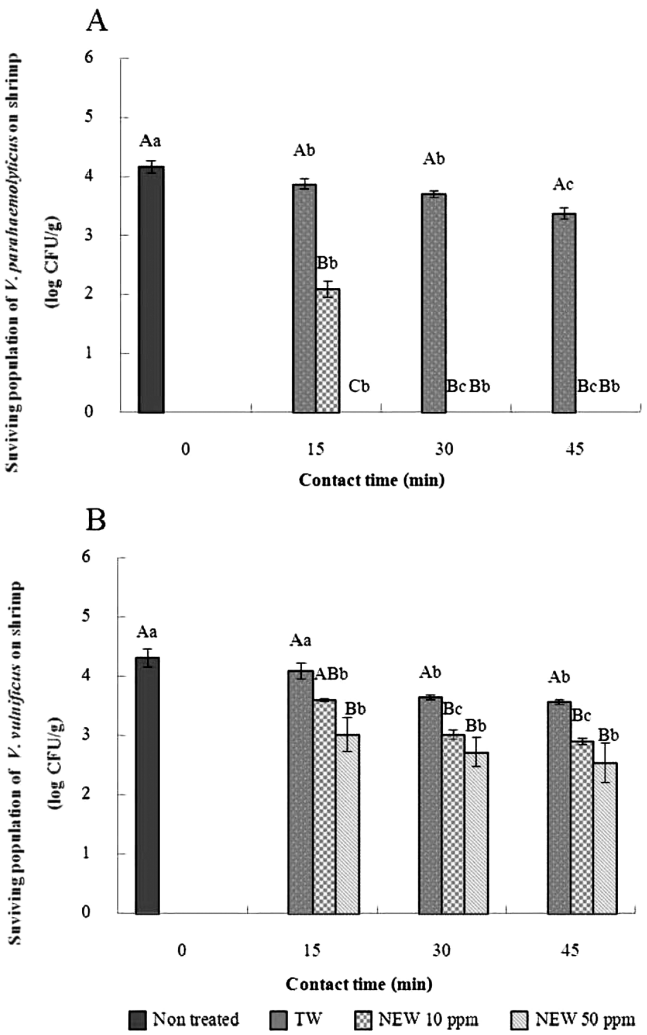

FIGURE 2. *Surviving population of (A)* Vibrio parahaemolyticus *and (B)* Vibrio vulnificus *on inoculated shrimp treated with neutral electrolyzed water (NEW) and tap water (TW). Surviving population numbers are the means of duplicate measurements ± standard deviations. Uppercase letters indicate significant differences (*$P < 0.05$*) in treatment solutions. Lowercase letters indicate significant differences (*$P < 0.05$*) in contact time. A value of 0.00 log CFU/g indicates no detectable survivors by the spread-plated method, with a dilution of 1:10. A mean less than 2 log CFU/g indicates that at least one of the duplicates was below the detection limit (100 CFU/g).*

period of time, and complete inactivation was detected after 30 min (Fig. 2A).

V. vulnificus survival after immersion for 15 min did not significantly ($P > 0.05$) decrease for either the TW or 10 ppm of NEW (Fig. 2B) treatments, but the 50-ppm NEW treatment caused about a 1.3-log CFU/g reduction. Increasing the contact time to 30 min resulted in a significant reduction of a 1.3-log CFU/g population for the 10-ppm NEW treatment, but no reduction in the 50-ppm NEW treatment. Thereafter, there were no significant changes in the populations after prolonging contact to 45 min with both 10- and 50-ppm levels of NEW. These results indicate that NEW was more effective in the pure cultures than the shrimp samples, as theoretically expected.

TABLE 2. *Sensory scores for raw and cooked shrimp treated with tap water (TW) and neutral electrolyzed water (NEW)*[a]

Sensory attributes	Scores	
	TW	NEW[b]
Raw shrimp		
Appearance	6.94 ± 1.35	7.00 ± 1.24
Color	6.67 ± 1.19	6.94 ± 1.16
Odor	6.78 ± 0.94	6.94 ± 1.16
Texture	7.22 ± 1.00	7.11 ± 1.02
Overall acceptability	6.89 ± 1.23	7.00 ± 1.08
Cooked shrimp		
Appearance	6.78 ± 1.22	6.94 ± 1.11
Color	7.11 ± 0.68	7.00 ± 0.97
Odor	6.56 ± 1.04	7.11 ± 1.02
Flavor	6.89 ± 0.90	6.61 ± 1.42
Texture	6.61 ± 1.04	6.72 ± 1.07
Overall acceptability	6.83 ± 0.98	6.94 ± 1.11

[a] Values are means ± standard deviations from 18 panelists.
[b] No significant difference was observed between means of treatments in all characteristics ($P > 0.05$).

Our results suggest that the effective treatment for reducing *V. parahaemolyticus* and *V. vulnificus* contamination in shrimp is a NEW concentration of 10 ppm for 30 min. TW did not significantly ($P > 0.05$) reduce either pathogen loads.

Sensory evaluation of the treated shrimp. The sensory characteristics of shrimp soaked in 10 ppm of NEW for 30 min were compared with those of shrimp soaked in TW (Table 2). There were no significant differences ($P > 0.05$) of sensory scores for all attributes between NEW-treated and TW-treated samples. The scores of all attributes were in the range of 6.5 to 7.5, which equates to ''slightly liking'' to ''moderate liking.'' The present results agree with those of Loi-Braden et al. *(10)* who used EO water for washing shrimp prior to freezing. This indicates that the NEW treatment did not cause any odors, aftertaste, or other adverse effects on the sensory quality of shrimp samples.

In conclusion, NEW was highly efficient in killing pure cultures of *V. parahaemolyticus* and *V. vulnificus* and less efficient for *Salmonella* Enteritidis and *E. coli*. At the 10-ppm chlorine level, which is normally recommended for water used in contact with food *(6),* NEW did not alter the sensory quality of shrimp. It may be considered as an alternative sanitizer to improve microbiological quality in shrimp, which is commonly contaminated with *Vibrio* spp. and would also provide an option for reducing the excessive use of chlorine.

ACKNOWLEDGMENTS

This work was partially supported by the Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand. The authors also thank

JCW International Co. Ltd., Thailand, for supporting NEW and Vivian Raksakulthai and John Bower for their English language editing.

REFERENCES

1. Abadias, M., J. Usall, M. Oliveira, I. Alegre, and I. Vinas. 2008. Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally processed vegetables. *Int. J. Food Microbiol.* 123:151–158.
2. Amagliani, G., G. Brandi, and G. F. Schiavano. 2012. Incidence and role of *Salmonella* in seafood safety. *Food Res. Int.* 45:780–788.
3. American Public Health Association. 2005. Standard methods for the examination water and wastewater, 21st ed. American Public Health Association, Washington, DC.
4. Bari, M. L., Y. Sabina, S. Isobe, T. Uemura, and K. Isshiki. 2003. Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* on the surfaces of tomatoes. *J. Food Prot.* 66:542–548.
5. Chitov, T., S. Wongdao, W. Thatum, T. Puprae, and P. Sisuwan. 2009. Occurrence of potentially pathogenic *Vibrio* species in raw, processed, and ready-to-eat seafood and seafood products. *Maejo Int. J. Sci. Technol.* 3:88–98.
6. Codex. 2000. Discussion paper on the use of chlorinated water. CX/FFP 00/13. Presented at the Codex Committee on Fish and Fishery Products, Twenty-Fourth Session, Alesund, Norway, 5 to 9 June 2000.
7. Guentzel, J. L., K. L. Lam, M. A. Callan, S. A. Emmons, and V. L. Dunham. 2008. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiol.* 25:36–41.
8. Hatha, A. A. M., T. K. Maqbool, and S. S. Kumar. 2003. Microbial quality of shrimp products of export trade produced from aqua-cultured shrimp. *Int. J. Food Microbiol.* 82:213–221.
9. Len, S. V., Y. C. Hung, D. Chung, J. L. Anderson, M. C. Erickson, and K. Morita. 2002. Effects of storage conditions and pH on chlorine loss in electrolyzed oxidizing (EO) water. *J. Agric. Food Chem.* 50: 209–212.
10. Loi-Braden, M. H., T. S. Huang, J. H. Kim, C. I. Wei, and J. Weese. 2005. Use of electrolyzed oxidizing water for quality improvement of frozen shrimp. *J. Food Sci.* 70:M310–M315.
11. Nieuwenhuijsen, M. J., M. B. Toledano, and P. Elliot. 2000. Uptake of chlorination disinfection by-products; a review and a discussion of its implications for exposure assessment in epidemiological studies. *J. Expo. Anal. Environ. Epidemiol.* 10:586–599.
12. Quan, Y. R., K. D. Choi, D. Chung, and I. S. Shin. 2010. Evaluation of bactericidal activity of weakly acidic electrolyzed water (WAEW) against *Vibrio vulnificus* and *Vibrio parahaemolyticus*. *Int. J. Food Microbiol.* 136:255–260.
13. Ren, T., and Y. Su. 2006. Effect of electrolyzed oxidizing water treatment on reducing *Vibrio parahaemolyticus* and *Vibrio vulnificus* in raw oysters. *J. Food Prot.* 69:1829–1834.