Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Food Engineering 72 (2006) 234–241
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Electrolyzed oxidizing water treatment for decontamination of raw salmon inoculated with *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A and response surface modeling

Nil P. Ozer [a], Ali Demirci [b,*]

[a] *Faculty of Engineering, Kocaeli University, Izmit, Kocaeli, Turkey*
[b] *The Huck Institutes of Life Sciences, Department of Agricultural and Biological Engineering, Pennsylvania State University, University Park, PA 16802, United States*

Received 10 November 2003; revised 24 June 2004; accepted 15 November 2004
Available online 16 February 2005

Abstract

Raw fish is prone to the risk of microbial outbreaks due to contamination by pathogenic microorganisms, such as *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Therefore, it is essential to treat raw fish to inactivate pathogenic microorganisms. Electrolyzed Oxidizing Water (EO) is a novel antimicrobial agent containing acidic solution with a pH of 2.6, Oxidation Reduction Potential (ORP) of 1150 mV, and 70–90 ppm free chlorine, and alkaline solution with a pH of 11.4 and ORP of −795 mV. This study was undertaken to evaluate the efficacy of acidic EO water treatment and alkaline EO water treatment followed by acidic EO water treatment at various temperatures for the inactivation of *E. coli* O157:H7 and *L. monocytogenes* Scott A on the muscle and skin surfaces of inoculated salmon fillets. Inoculated salmon fillets were treated with acidic EO water at 22 and 35 °C and 90 ppm free-chlorine solution as control at 22 °C for 2, 4, 8, 16, 32, and 64 min. The acidic EO water treatments resulted in a reduction of *L. monocytogenes* Scott A population in the range of 0.40 $\log_{10}$ CFU/g (60%) at 22 °C to 1.12 $\log_{10}$ CFU/g (92.3%) at 35 °C. Treatment of inoculated salmon fillets with acidic EO water reduced *E. coli* O157:H7 populations by 0.49 $\log_{10}$ CFU/g (67%) at 22 °C and 1.07 $\log_{10}$ CFU/g (91.1%) at 35 °C. The maximum reduction with chlorine solution (control) was 1.46 $\log_{10}$ CFU/g (96.3%) for *E. coli* O157:H7 and 1.3 $\log_{10}$ CFU/g (95.3%) for *L. monocytogenes* Scott A at 64 min. A response surface model was developed for alkaline treatment followed by acidic EO water treatment to predict treatment times in the range of 5–30 min and temperatures in the range of 22–35 °C for effective treatment with alkaline EO water followed by acidic water, alkaline and acidic water treatments. Response surface analysis demonstrated maximum log reductions of 1.33 $\log_{10}$ CFU/g (95.3%) for *E. coli* O157:H7 and 1.09 $\log_{10}$ CFU/g (91.9%) for *L. monocytogenes* Scott A. Data collected from the treatments was used to develop empirical models as a function of treatment times and temperature for prediction of population of *E. coli* O157:H7 and *L. monocytogenes* Scott A. Correlations (R^2) of 0.52 and 0.77 were obtained between model predicted and experimental $\log_{10}$ reduction for *E. coli* O157:H7 and *L. monocytogenes* Scott A reductions, respectively. These results clearly indicated that EO water has a potential to be used for decontamination of raw fish.

Keywords: Salmon fillet; Electrolyzed oxidizing water; Decontamination; *E. coli* O157:H7 and *L. monocytogenes* Scott A

* Corresponding author. Tel.: +1 814 863 1098; fax: +1 814 863 1031.
E-mail address: demirci@psu.edu (A. Demirci).

0260-8774/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jfoodeng.2004.11.038

1. Introduction

Various fish and fish products have been reported to be associated with outbreak of food borne diseases. During 1980–1994, 339 seafood associated outbreaks were reported, resulting in 3959 illnesses, 76 hospitalizations, and four deaths in New York (Wallace, Guzewich, Cambridge, Altekruse, & Morse, 1999). Center for Science in the Public Interest (CSPI) documented 2472 outbreaks between 1990 and 2002, seafood caused the most outbreaks, 539 and 6781 cases of illness (CSPI, 2003). Public health and regulatory agencies in the US have established a zero tolerance policy for *Listeria monocytogenes* in cooked and ready-to-eat seafood (Shank, Elliot, Wachsmuth, & Losikoff, 1996). Between 1987 and 1998 there were 112 Class I recalls, which indicate serious health problems or death, for domestic or imported ready-to-eat seafood products contaminated with *L. monocytogenes* and more than 280,000£ products were affected. Even though in many instances there had been no known death case or outbreak of *L. monocytogenes* contaminated processed seafood, FDA required the plants to develop and operate under Hazard Analysis and Critical Control Point (HACCP) plans and the underlying Good Manufacturing Practices (GMPs) can eliminate or reduce *L. monocytogenes* to non-detectable levels in the seafood products (Elliot & Kvenberg, 2000). During the last 15–20 years smoked salmon and smoked rainbow trout have been considered to be risk products for human listeriosis, and *L. monocytogenes* contamination is of great concern to the smoked fish industry. The overall risk of listeriosis to the human population appears to be around 1–10 per million per year based on internationally published incidence data (Feldhusen, 2000). Even though there are no reports on epidemic outbreaks due to consumption of *L. monocytogenes* contaminated seafood, but *L. monocytogenes* should be taken seriously to prevent any catastrophe. On the other hand, between 1990 and 2002, four seafood (fish and shrimp) associated outbreaks with *E. coli* O157:H7 with 39 cases were reported (CSPI, 2003). An estimated 73,000 cases, with *Escherichia coli* O157:H7, occur annually in the United States and most of vehicle was unknown (CDC, 2003). Where animal manures, particularly bovine, are used as pond fertilizers, there is a risk of pathogenic strains of *E. coli* to be present in the pond water.

So far, several chemical solution (aqueous) treatments have been investigated such as hypochlorite (OCl^-), chlorine, chlorine dioxide (ClO_2), trisodium phosphate solution (TSP) and, acidified sodium chlorite (ASC) to eliminate pathogens from seafood (Bremer & Osborne, 1998; Kim, Huang, Marshall, & Wei, 1999; Lin, Huang, Cornell, Lin, & Wei, 1996; Mu, Huang, Gates, & Wu, 1997; Park, Rua, & Acker, 1991; Su & Morrissey, 2003). Lin et al. (1996) determined bactericidal effect of aqueous chlorine and chlorine dioxide on the *E. coli* O157:H7, *L. monocytogenes*, and streptomycin-resistant (Str^R)-*L. monocytogenes*, inoculated fish cubes (mangrove snapper). They compared effectiveness of chlorinating solutions (aqueous chlorine, chlorine dioxide and CDC (chlorine dioxide from Oxine® Concentrate, OC)) at various concentrations (40, 100, 200, and 400) for two levels of *Listeria* inoculum (low and high). They obtained maximum percent reduction of 60% (for high inoculation) with CDC (chlorine dioxide from Oxine® Concentrate, OC) solution at 400 ppm. Mu et al. (1997) explored the potential of using trisodium phosphate (TSP) to reduce bacterial populations in fresh fishery products. They inoculated shrimp and trout fillets with *L. monocytogenes* before dipping in tap water, 10% TSP, or 20% TSP solutions for 10 min. They showed that, the inhibitory effect of 20% TSP treatment on *Listeria* count ($\sim$1.7 logs CFU/cm^2 log reduction) of trout fillets was more significant ($p < 0.05$) compared to 10% TSP or tap water treatments. Kim et al. (1999) treated various seafood with fresh chlorine dioxide (ClO_2) solutions (20, 40, 100, and 200 ppm total available ClO_2) for 5 min. They showed that after 200 ppm ClO_2 treatment of salmon fillets, bacterial loads reduced by $\sim 1.07 \log_{10}$ CFU/g. However, treated salmon and red grouper fillets treated with 100 and 200 ppm ClO_2 developed skin discoloration (lighter color). The antimicrobial activity of acidified sodium chlorite (ASC) against *L. monocytogenes* in salmon fillets was studied very recently (Su & Morrissey, 2003). *L. monocytogenes* (10^4 CFU/g) inoculated raw salmon fillets were washed with ASC solution (50 ppm) for 1 min and *L. monocytogenes* reduction was found to be $0.5 \log_{10}$ CFU/g.

On the other hand, Electrolyzed Oxidizing (EO) water is a novel antimicrobial agent and has been used in Japan for several years to disinfect medical instruments. In recent years, it has gained interest as a disinfectant in the food industry. EO water is generated through the electrolysis of a dilute solution of NaCl and softened tap water passed through on electrolysis chamber. As the current passes between the electrodes, two solutions with different properties are generated (Kim, Hung, & Brackett, 2000). The sodium ions are drawn to the cathode (NaOH) and the chlorine ions are drawn to the anode (HOCl). The alkaline EO water so collected has a pH of approximately 11.4 and ORP of -795 mV, while acidic EO water has a pH of approximately 2.6, ORP of 1150 mV and a chlorine concentration 40 and 90 ppm. EO water has been utilized to disinfect kitchen cutting boards, and other surfaces, fresh cut vegetables, alfalfa seeds and sprouts, broccoli, strawberry, lettuce, tomatoes, apple and poultry (Bari, Sabina, Isobe, Uemura, & Isshiki, 2003; Fabrizio, Sharma, Demirci, & Cutter, 2002; Izumi, 1999; Kim, Hung, Brackett, & Lin, 2003; Koseki, Yoshida,

Seiichiro, & Itoh, 2001; Park, Hung, & Kim, 2002; Sharma & Demirci, 2003; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999). Izumi (1999) studied using acidic EO water to treat fresh-cut vegetables, and achieved up to a 2.6 $\log_{10}$ CFU/g reduction in bacterial population. In another study, EO water was used to disinfect plastic kitchen cutting board, which reduced *E. coli* O157:H7 populations more than 5.0 log CFU/100 cm^2 (Venkitanarayanan et al., 1999). Using acidic EO water against pure cultures of *Enterobacter aerogenes* and *Staphylococcus aureus* yielded 9 $\log_{10}$ CFU/g reduction after 30 s treatment (Park et al., 2002).

EO water also has the potential to be more cost effective than traditional disinfectants. It is less dangerous and less expensive than most traditional preservation methods. Therefore the purpose of this research is to evaluate effectiveness of acidic EO water treatment, and both alkaline and acidic EO water treatment to inactive pathogenic microorganisms on raw salmon fillets.

2. Material and methods

Salmon fillets were purchased from a local grocery store in State College, PA. Fillets were stored in a freezer at −20 °C until used. Before the experiments, the frozen fillets were thawed at 4 °C for 24 h and cut into 3×8 cm^2 pieces prior to inoculation and EO water treatments. Average weight of treated fillets were 48 g.

2.1. Preparation of inoculum

Three strains of enterohemorrhagic *E. coli* O157:H7 strains resistant to nalidixic acid were obtained from the Center for Food Safety, University of Georgia. The strains were 932 (human isolate), 944 (salami isolate), and E0018 (calf fecal isolate). Cells were grown in tryptic soy broth (Difco, Detroit, MI) supplemented with 50 μg/ml nalidixic acid (Fisher, Fair Lawn, N.J.) and 0.1% dextrose (TSBN) at 37 °C for 18 h. The use of nalidixic acid minimized growth of microorganisms other than *E. coli* O157:H7 in the selective agar. A cocktail of the three *E. coli* O157:H7 strains was prepared by mixing 100 ml of each. After centrifuging (Sorvall STH750, Kendro Lab Product, Newton, CO) the culture broth at 4 °C and 4000*g* for 60 min. The supernatant was decanted and pellet was resuspended in 200 ml of sterile 0.1% buffered peptone water.

L. monocytogenes Scott A (ATCC 49594) was obtained from the culture collection in the Department of Food Science, Penn State University. Cells were grown in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) at 37 °C for 24 h. After centrifuging at 4 °C and 4000*g* for 60 min, the supernatant was decanted and the pellet was resuspended in 100 ml of sterile 0.1% buffered peptone water. Inoculum's strength was ~8.7 $\log_{10}$ CFU/ml for *E. coli* O157:H7 and *L. monocytogenes* Scott A suspensions.

2.2. Inoculation of fish fillets

Each side of fish fillets (skin and muscle) was inoculated by spreading 0.1 ml of the prepared *E. coli* O157:H7 or *L. monocytogenes* inoculum with the pitetor tip. Inoculated fish fillet were kept in laminar flow hood at room temperature for 1 h. The resulting *E. coli* O157:H7 and *L. monocytogenes* populations were about 7 $\log_{10}$ CFU/g of fish fillet.

2.3. Preparation of electrolyzed oxidizing water

Electrolyzed oxidizing (EO) water was prepared using a continuous EO water generator (Model ROX 20TA, Hoshizaki Electric Co. Ltd., Japan) at 10 V and 19 A. A continuous supply of softened tap water and 12% sodium chloride solution at room temperature was pumped into the equipment. The generator was allowed to run for about 20 min before collecting water for the treatment. The pH and ORP were determined using a pH/ORP meter (model pH 430, Corning Inc., NY) equipped with pH and ORP probes. Free chlorine concentrations were determined by DPD-FEAS (*N,N*-diethyl-*p*-phenylenediamine-ferrous ethylenediammonium sulfate) test kit according to manufacturer's specification (Hach Co., Ames, IA). The acidic EO water had a pH of 2.6, ORP of 1150 mV, and 76–90 ppm free chlorine, while the alkaline EO water had a pH of 11.4 and ORP of −795 mV. The solutions were heated using a hot plate and then placed in a pre-heated water bath to ensure consistent desired treatment temperatures (22, 28.5, and 35 °C). After heating, paper test strips (Advantec MHS, Inc., Dublin, CA) were used to verify that the chlorine content of the acidic EO water was above 50 ppm. For control, sodium hypochlorite (Aldrich, Milwaukee, WI) solution was used (90 ppm free chlorine) at 22 °C.

2.4. Treatment of inoculated fish fillets with acidic EO water

As depicted in Fig. 1, artificially inoculated fish fillets were hung with a hook in one liter of treatment solution (alkaline, acidic EO water or chlorine solution, which was placed in a water bath equipped with immersion heater (Model 1112, VWR., Buffalo Grove, IL)). The treatment solution was agitated by using a magnetic stirrer (Corning Inc., NY). Two different temperatures (22 and 35 °C) were evaluated for acidic EO water. At each temperature, treatments were conducted for 2, 4, 8, 16, 32, and 64 min to determine the effect of treatment time. Samples from acid EO water were also taken for micro-

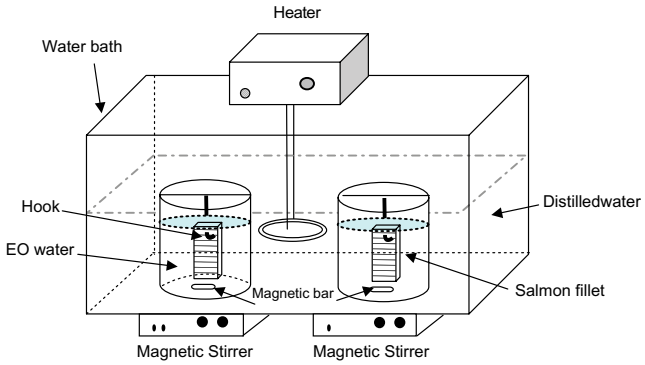

Fig. 1. Schematic diagram of experimental setup.

biological analysis. Also, inoculated salmon fillets were treated with sodium hypochlorite solution (90 ppm free chlorine) as control at 22 °C for 2, 4, 8, 16, 32, and 64 min.

In order to determine minimum and maximum limits for each parameters, a series of preliminary experiments were preformed, which suggested treatment time limits as 5–30 min and temperature limits as 22–35 °C. For alkaline EO water treatment followed by acidic EO water treatment, a Box Benken response surface design (Table 1) was used to select combinations of treatment time in alkaline EO water, treatment in acidic EO water, and treatment temperature in order to keep experimental trial numbers to a minimum. A series of preliminary experiments were performed to select the treatment time limits of 5–30 min, and a temperature range of 22–35 °C.

2.5. Microbiological analysis

The populations of *E. coli* O157:H7 and *L. monocytogenes* on treated and untreated fish fillets were determined by placing fillets in 100 ml of sterile 0.1% buffered peptone water in a sterile stomacher® 400 bag fitted with integral filter (Model P, Interscience, Saint-Nom, France) and pummeled for 30 s at 230 rpm with a stomacher® (Model 400, Seward, England). Then the solution was serially diluted in sterile 0.1% buffered peptone water and spiral plated on TSAN for *E. coli* O157:H7 and Palcam agar (*Listeria* Agar Base, EM Science, Gibbstown, NJ) for *L. monocytogenes* using a spiral plater (Autoplate 4000, Spiral Biotech, Norwood, MA). After incubating the agar plates at 37 °C for 36 h, the colonies were enumerated by using Q-count™ system (Spiral Biotech, Norwood, MA) and confirmed with an *E. coli* O157:H7 latex agglutination test (Remel Microbiological Products, Lenexa, KS), *L. monocytogenes* colonies were evaluated by Gram staining. Uninoculated fish yielded no colonies for either of microorganisms on the selective agar.

Table 1
Box-Benken response surface design

Trial	Time in alkaline EO water (min)	Time in acidic EO water (min)	Temperature (°C)
1	17.5	30.0	35.0
2	5.0	17.5	22.0
3	30.0	17.5	22.0
4	5.0	5.0	28.5
5	5.0	30.0	28.5
6	30.0	30.0	28.5
7	30.0	5.0	28.5
8	17.5	17.5	28.5
9	17.5	17.5	28.5
10	17.5	5.0	22.0
11	17.5	5.0	35.0
12	5.0	17.5	35.0
13	30.0	17.5	35.0
14	17.5	17.5	28.5
15	17.5	30.0	22.0

2.6. Statistical analysis

Bacterial numbers in CFU/g raw salmon were transformed into $\log_{10}$ for statistical analysis. All treatments were replicated three times and results were reported as means. An analysis of variance (ANOVA) was performed using the general linear models procedure of MINITAB 13.30 (Minitab Inc., State College, PA., USA). Models to predict inactivation of *E. coli* O157:H7 and *L. monocytogenes* Scott A on salmon fillets were developed in terms of treatment times and temperature using the response surface approach in MINITAB. Tukey test was used to compare the average log reduction between pairs of treatment times and treatment temperatures. Pairwise comparison among means, with a 95% confidence level was used.

3. Results and discussion

Effect of acidic EO water treatment on inactivation of *E. coli* O157:H7 and *L. monocytogenes* Scott A on salmon fillets.

Inoculated salmon fillets were treated with acidic EO water for 2, 4, 8, 16, 32, and 64 min at room temperature (22 °C) and 35 °C. The acidic EO water treatment reduced *E. coli* O157:H7 populations by 0.49 $\log_{10}$ CFU/g (67%) at 22 °C for 2 min and 1.07 $\log_{10}$ CFU/g (91.1%) at 35 °C for 64 min, respectively. The reduction with chlorine solution (control) was 1.46 $\log_{10}$ CFU/g (96.3%) at 64 min for *E. coli* O157:H7. The difference between the log reductions of acidic EO water at 35 °C and chlorine solution was not significant ($p > 0.05$) at 64 min. However, the difference between acidic EO water at 22 °C and chlorine solution was significant (Table 2).

On the other hand, acidic EO water treatments resulted in reductions of population of *L. monocytogenes* ranging from 0.40 $\log_{10}$ CFU/g (60%) at 22 °C for 2 min to 1.12 $\log_{10}$ CFU/g (92.3%) at 35 °C for 64 min (Table 3). The reduction in chlorine solution (control)

Table 2
Log reduction in population *E. coli* O157:H7 on salmon fillets with acidic EO water at 22–35 °C

Treatment time (min)	Log reduction (CFU/g)[a,b]		
	Control[c]	22 °C	35 °C
2	c 0.53 A	a 0.49 A	c 0.59 A
4	c 0.53 A	a 0.53 A	bc 0.61 A
8	bc 0.56 AB	a 0.53 B	abc 0.95 A
16	bc 0.68 A	a 0.63 A	ab 1.00 A
32	b 0.93 A	a 0.64 A	bc 0.64 A
64	a 1.46 A	a 0.84 B	a 1.07 AB

[a] Within the same row, values not followed by the same capital letter are significantly different ($p < 0.05$).
[b] Within the same column, values not preceded by the same letter are significantly different ($p < 0.05$).
[c] Control is chlorine solution (90 ppm) at 22 °C.

Table 3
Log reduction in population *L. monocytogenes* Scott A on salmon fillets with acidic EO water at 22–35 °C

Treatment time (min)	Log reduction (CFU/g)[a,b]		
	Control[c]	22 °C	35 °C
2	b 0.33 A	a 0.40 A	a 0.79 A
4	b 0.37 A	a 0.48 A	a 0.88 A
8	b 0.37 A	a 0.52 A	a 0.90 A
16	ab 0.65 A	a 0.58 A	a 0.93 A
32	ab 0.77 A	a 0.74 A	a 0.99 A
64	a 1.30 A	a 0.86 A	a 1.12 A

[a] Within the same row, values not followed by the same capital letter are significantly different ($p < 0.05$).
[b] Within the same column, values not preceded by the same letter are significantly different ($p < 0.05$).
[c] Control is chlorine solution (90 ppm) at 22 °C.

was 1.3 $\log_{10}$ CFU/g (95.3%) at 64 min, which was no significant difference for 35 °C acidic EO water treatment at 64 min. However, the difference between the bactericidal activity of EO water (35 °C) and chlorine solution was not significantly different ($p > 0.05$) for *L. monocytogenes* at all treatment times.

After acidic EO water treatment, one milliliter sample of acidic EO water was taken from each beaker of 2, 4, 8, 16, 32, and 64 min at room temperature. *E. coli* O157:H7 and *L. monocytogenes* were not detected in the acidic EO water.

3.1. Response surface model for EO water treatment

For the treatment with alkaline EO water followed by acidic EO water, a response surface model was developed to determine effective times in the range of 5–30 min and temperatures in the range of 22–35 °C for both alkaline and acidic water treatments. A full quadratic model to mean *E. coli* O157:H7 and *L. monocytogenes* reductions obtained from 15 alkaline-acidic EO water (12 different and 3 replicate) treatments (Table 1). Response surface analysis demonstrated reduction ranging from 0.39 to 1.09 $\log_{10}$ CFU/g for *L. monocytogenes* and from 0.65 to 1.33 $\log_{10}$ CFU/g for *E. coli* O157:H7.

The response surface diagram given in Fig. 2A shows that as treatment time of alkaline and acidic EO water increases, the reduction in *E. coli* O157:H7 population increases. The inactivation of *E. coli* O157:H7 is inversely related to increase in temperature but slightly increases with increase in alkaline and acidic soaking time (Fig. 2B and C). The log reduction of population of *L. mono-*

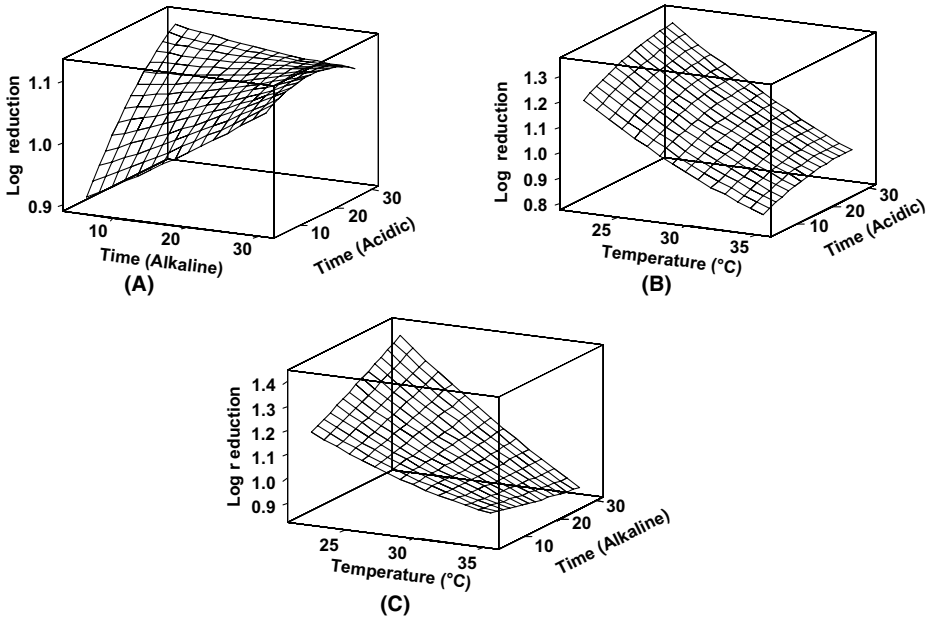

Fig. 2. Response surface diagrams generated (A) using 5, 17.5, 30 min data for the effect of time (alkaline) and time (acidic), (B) effect of temperature (°C) and time (acidic), (C) effect of temperature (°C) and time (alkaline) for *E. coli* O157:H7.

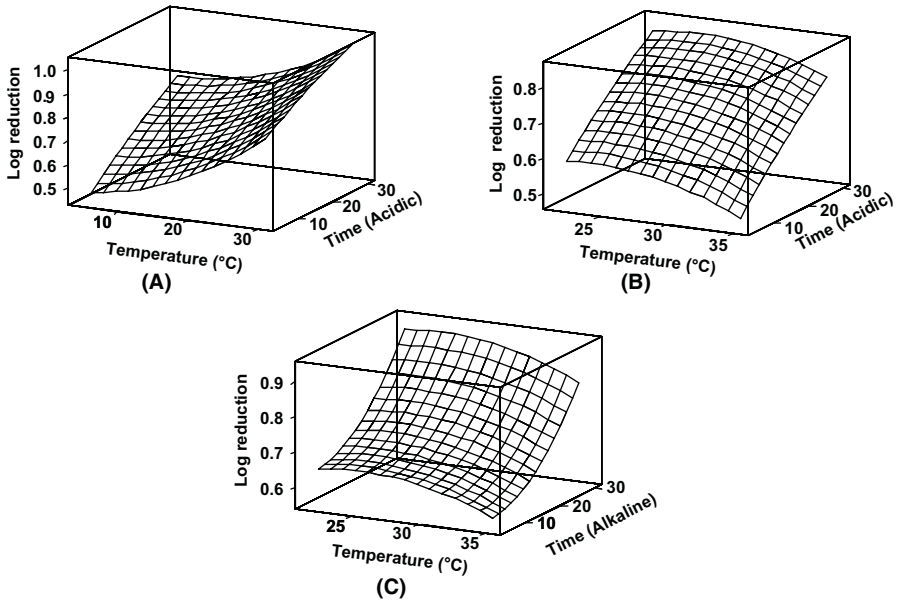

Fig. 3. Response surface diagrams generated (A) using 5, 17.5, 30 min data for the effect of time (alkaline) and time (acidic), (B) effect of temperature (°C) and time (acidic), (C) effect of temperature (°C) and time (alkaline) for *L. monocytogenes*.

cytogenes, increases directly with increase in alkaline and acidic EO water treatment time (Fig. 3A). Fig. 3B and C shows that the log reduction of *L. monocytogenes* is inversely related to increase in temperature but increases with increase in alkaline and acidic soaking time.

3.2. Development and validation of models

Empirical models were developed to predict the $\log_{10}$ reduction of *E. coli* O157:H7 and *L. monocytogenes* Scott A on fish fillets treated with EO water. Variables used to develop the models included treatment time in alkaline water, treatment time in acidic water, and treatment temperature. Response surface regression analysis was done using uncoded units to estimate the regression coefficients (Pond, Wood, Mumin, Barbut, & Griffiths, 2001). The general model equation was

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \alpha_4 x_1 x_2 + \alpha_5 x_1 x_3 + \alpha_6 x_2 x_3 \quad (1)$$

where $y = \log_{10}$ reduction of *E. coli* O157:H7 or *L. monocytogenes* Scott A on fish fillets, α_0 is the constant term, $\alpha_{1,\ldots,6}$ are coefficients, x_1 is the time in alkaline EO water, x_2 is the time in acidic EO water, and x_3 is the temperature of treatment.

3.3. Model for E. coli O157:H7 reduction

The RSM described the alkaline water treatment time (t_{al}) and acidic EO water treatment time (t_{ac}) at various temperatures (T) and the interactions between the variables for *E. coli* O157:H7 reduction as

$$\log_{10}\text{reduction} = 1.17783 + 0.03411t_{al} + 0.01446t_{ac} - 0.01169T - 0.00034t_{al}t_{ac} - 0.00089t_{al}T - 0.00015t_{ac}T \quad (2)$$

Analysis of variance for $\log_{10}$ reductions showed that none of the terms in the model equation have significant effect ($p > 0.05$) on the model prediction. The model was validated by back predicting $\log_{10}$ reduction values resulting from treatments and correlating them to the experimental data. A linear regression resulted in R^2 of 0.52 (Fig. 4). This indicated that the model could not sufficiently explain the variability in $\log_{10}$ reduction values.

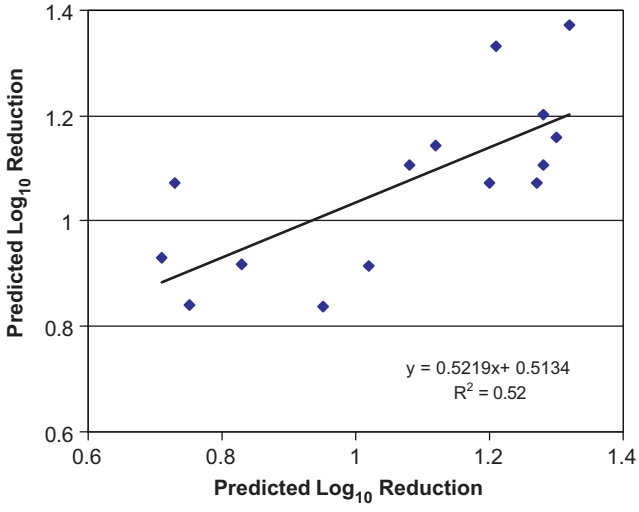

Fig. 4. Linear regression analysis between experimental $\log_{10}$ reduction and predicted $\log_{10}$ reduction for *E. coli* O157:H7.

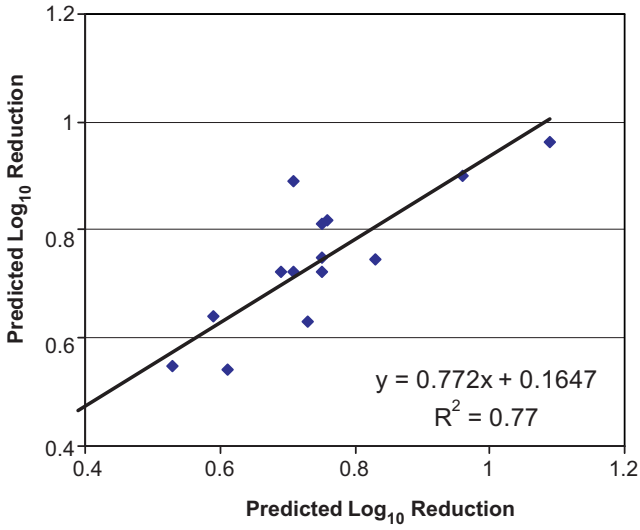

Fig. 5. Linear regression analysis between experimental $\log_{10}$ reduction and predicted $\log_{10}$ reduction for *L. monocytogenes* Scott A.

3.4. Model for L. monocytogenes Scott A

RSM described the variables as treatment times (t_{al} and t_{ac}) and the interactions between the variables for *L. monocytogenes* Scott A reduction as

$$\log_{10}\text{reduction} = 0.52037 + 0.01438t_{al} + 0.01067t_{ac} - 0.00762T - 0.00016t_{al}t_{ac} - 0.00003t_{al}T + 0.00009t_{ac}T \quad (3)$$

Analysis of variance for $\log_{10}$ reductions also showed that none of the terms in the model equation have sugnificant effect ($p > 0.05$) on the model prediction. Validation of the model by back prediction of $\log_{10}$ reduction values for demonstrated better R^2(0.77) for the prediction *L. monocytogenes* Scott A (Fig. 5).

4. Conclusion

This study shows the potential of EO water for washing fresh salmon fillets. Acidic EO water demonstrated 1.07 $\log_{10}$ CFU/g *E. coli* O157:H7 reduction and 1.12 $\log_{10}$ CFU/g *L. monocytogenes* reduction on salmon fillets inoculated with the pathogens. In fact most of the published literature concentrates on the studying of use of acidic EO water as a disinfectant conventional treatment. A maximum log reduction of 1.33 $\log_{10}$ CFU/g (95.3%) for *E. coli* O157:H7 was obtained after treatment with alkaline EO water for 17.5 min and then acidic EO water for 30 min at 22 °C. The reduction was 1.09 $\log_{10}$ CFU/g (91.9%) for *L. monocytogenes*, after washing with alkaline EO water for 30 min followed by acidic EO water for 30 min at 28 °C with response surface model, which indicated that utilization of alkaline EO water did not improve the decontamination rate significantly. However comparisons of EO water treatments with other treatments reported in literature indicated that EO water has a potential to be used for decontamination for raw salmon. Data collected from the treatments was used to develop empirical models as a function of treatment times and temperature for prediction of population of *E. coli* O157:H7 and *L. monocytogenes* Scott A. Correlations (R^2) of 0.52 and 0.77 were obtained between model predicted and experimental $\log_{10}$ reduction for *E. coli* O157:H7 and *L. monocytogenes* Scott A reductions, respectively.

References

Bari, M. L., Sabina, Y., Isobe, S., Uemura, T., & Isshiki, K. (2003). Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surfaces of tomatoes. *Journal of Food Protection, 66*, 542–548.

Bremer, P. J., & Osborne, C. M. (1998). Reducing total aerobic count and *Listeria monocytogenes* on the surface of king salmon (*Oncorhynchus tshawytscha*). *Journal of Food Protection, 61*, 849–854.

Centers for Disease Control and Prevention (CDC). 2003. Division of bacterial and mycotic diseases. Disease information. Available from http://www.cdc.gov/ncidod/dmbd/diseaseinfo/escherichia-coli_t.htm. Accessed on: 7 July 2003.

Center for Science in the Public Interest (CSPI). 2003. Seafood and produce top food poisoning culprits. Available from http://www.cspinet.org/reports/outbreak_report.pdf. Accessed on: 7 July 2003.

Elliot, E. L., & Kvenberg, J. E. (2000). Risk assessment used to evaluate the US position on *Listeria monocytogenes* in seafood. *International Journal of Food Microbiology, 62*, 253–260.

Fabrizio, K. A., Sharma, R. R., Demirci, A., & Cutter, C. N. (2002). Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce Salmonella species on poultry. *Poultry Science, 81*, 1598–1605.

Feldhusen, F. (2000). The role of seafood in bacterial foodborne diseases. *Microbes and Infection, 2*, 1651–1660.

Izumi, H. (1999). Electrolyzed water as a disinfectant for fresh-cut vegetables. *Journal of Food Science, 64*, 536–539.

Kim, C., Hung, Y.-C., & Brackett, R. E. (2000). Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for inactivation of food-related pathogens. *Journal of Food Protection, 63*, 19–24.

Kim, C., Hung, Y.-C., Brackett, R. E., & Lin, C.-S. (2003). Efficacy of electrolyzed oxidizing water inactivating Salmonella on alfalfa seeds and sprouts. *Journal of Food Protection, 66*, 208–214.

Kim, J. M., Huang, T.-S., Marshall, M. R., & Wei, C. I. (1999). Chlorine dioxide treatment of seafoods to reduce bacterial loads. *Journal of Food Science, 64*, 1089–1093.

Koseki, S., Yoshida, K., Seiichiro, S., & Itoh, K. (2001). Decontimation of lettuce using acidic electrolyzed water. *Journal of Food Protection, 64*, 652–658.

Lin, W.-F., Huang, T.-S., Cornell, J. A., Lin, C.-M., & Wei, C.-I. (1996). Bactericidal activity of aqueous chlorine and chlorine dioxide solutions in a fish model system. *Journal of Food Science, 61*, 1030–1034.

Mu, D., Huang, Y. W., Gates, K. W., & Wu, W.-H. (1997). Effect of Trisodium Phosphate on *Listeria monocytogenes* attached to rainbow trout (*Oncorhynchus mykiss* and shrimp

Penaeus spp.) during refrigerated storage. *Journal of Food Safety, 17*, 37–46.

Park, H., Hung, Y. C., & Kim, C. (2002). Effectiveness of electrolyzed water as a sanitizer for treating different surfaces. *Journal of Food Protection, 65*, 1276–1280.

Park, D. L., Rua, S. M., & Acker, R. F. (1991). Direct application of a new hypochlorite sanitizer for reducing bacterial contamination on foods. *Journal of Food Protection, 54*, 960–965.

Pond, T. J., Wood, D. S., Mumin, I. M., Barbut, S., & Griffiths, M. W. (2001). Modeling the survival of *Escherichia coli* O157:H7 in uncooked semidry, fermented sausage. *Journal of Food Protection, 64*, 759–766.

Shank, F. R., Elliot, E. L., Wachsmuth, I. K., & Losikoff, M. E. (1996). US position on *Listeria monocytogenes* in foods. *Food Control, 7*, 229–234.

Sharma, R. R., & Demirci, A. (2003). Treatment of *Escherichia coli* O157:H7 inoculated alfalfa seeds and sprouts with electrolyzed oxidizing water. *International Journal of Food Microbiology, 86*, 231–237.

Su, Y. C., & Morrissey, M. T. (2003). Reducing levels of *Listeria monocytogenes* contamination on raw salmon with acidified sodium chlorite. *Journal of Food Protection, 66*, 812–818.

Venkitanarayanan, K. S., Ezeike, G. O., Hung, Y. C., & Doyle, M. P. (1999). Inactivation of *Escherichia coli* O157:H7, and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolyzed oxidizing water. *Journal of Food Protection, 62*, 857–860.

Wallace, B. J., Guzewich, J. J., Cambridge, M., Altekruse, S., & Morse, D. L. (1999). Seafood-associated disease outbreaks in New York. 1980–1994. *American Journal of Preventive Medicine, 17*, 48–54.