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Efficacy of electrolyzed oxidizing water against *Listeria monocytogenes* and *Morganella morganii* on conveyor belt and raw fish surfaces

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ABSTRACT

Listeria monocytogenes and *Morganella morganii* have been implicated in listeriosis outbreaks and histamine fish poisoning, respectively. Possible sources of contamination of food products include processing equipment, food handlers, and fish smokehouses. Treatment of food preparation surfaces and of whole fish during handling with agents such as, electrolyzed oxidizing (EO) water, could reduce biofilm formation on seafood products and in seafood processing plants. We examined the efficacy of EO water against *L. monocytogenes* and *M. morganii* biofilms using the MBEC™ Assay System (Innovotech Inc.), conveyor belt coupons, and raw fish surfaces. The MBEC™ Assay System was used to assess the activity of EO water against 24-h biofilms of 90 *L. monocytogenes* strains and five *M. morganii* strains. Biofilms were exposed to PBS or EO water for 0 (control), 5, 15, and 30 min. All bacterial isolates were susceptible (reduction of 7 $\log_{10}$CFU) to treatment with EO water for 5 min based on results obtained using this assay system. EO water was used to treat four *L. monocytogenes* strains and one *M. morganii* strain attached to conveyor belt coupons and fish surfaces. Three *L. monocytogenes* strains and one *M. morganii* strain on belt coupons were reduced by 1–2.5 $\log_{10}$CFU/cm^2 by exposure (5 min) to EO water compared to exposure to sterile distilled water. Strain to strain variability in susceptibility to EO water was evidenced by the fact that numbers of one *L. monocytogenes* strain were not reduced by EO water treatment of belt surfaces. EO water was not effective against *L. monocytogenes* and *M. morganii* on fish surfaces as growth occurred during cold storage. These results suggest that exposure of conveyor belts to EO water for a minimum of 5 min could assist in the removal of some biofilms. Removal of food residue with continuous or intermittent spraying of food processing equipment (e.g., conveyor belts, slicers) could reduce or prevent further biofilm formation. Additional sanitizers must be investigated for activity against bacteria associated with raw fish.

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1. Introduction

Listeria monocytogenes is a food-borne pathogen that can cause listeriosis in the elderly, newborns, and immunocompromised individuals (FAO–WHO, 2004). Listeriosis has a mortality rate of about 24% (Farber & Peterkin, 1991) and accounts for 28% of deaths from food-borne pathogens (Scallan, E., R.M. Hoekstra, F.J. Angulo, R.V. Tauxe, M.-A. Widdowson, S.L. Ray, et al., 2011). The U.S. Food and Drug Administration (FDA) maintains zero tolerance for *L. monocytogenes* in ready-to-eat (RTE) seafood. However, RTE products, such as smoked salmon, smoked trout, cooked crawfish, and seafood salad, have been found to be contaminated with *L. monocytogenes* (Gombas, Chen, Clavero, & Scott, 2003; Inoue et al., 2000; Miettinen et al., 1999; Thimothe et al., 2002). *L. monocytogenes* has also been found in raw catfish (Chou, Silva, & Wang, 2006) and raw shrimp (Gudmundsdóttir, Gudbjörnsdóttir, Einarsson, Kristinsson, & Kristjánsson, 2006). Outbreaks of listeriosis have been linked to the consumption of cold-smoked rainbow trout in Sweden (Ericsson et al., 1997), smoked mussels in New Zealand (Brett, Short, & McLauchlin, 1998), imitation crabmeat in Canada (Farber, Daley, Mackie, & Limerick, 2000), and shrimp in the U. S. (Riedo et al., 1994). *L. monocytogenes* is commonly isolated from seafood processing plants and smokehouses where specific ribotypes can persist for months to years (Gudmundsdóttir et al., 2006; Hansen, Vogel, & Gram, 2006; Wulff, Gram, Ahrens, & Vogel, 2006). Contamination of seafood processing plant equipment occurs where raw materials are handled (Gudmundsdóttir et al., 2006; Kim et al., 2003), making the food processing environment the primary source of *L. monocytogenes* contamination of

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seafood products (Thimothe et al., 2002; Vogel, Huss, Ojeniyi, Ahrens, & Gram, 2001).

Histamine fish poisoning (HFP), also known as scombroid poisoning, is a common form of toxicity due to ingestion of fish and accounts for approximately 35% of seafood-associated disease outbreaks (CSPI, 2009). More than 16,000 cases of HFP have been reported worldwide (Emborg & Dalgaard, 2008). HFP is a mild illness with symptoms of rash, nausea, abdominal cramps, headache, and oral burning. Histamine is regulated by the U. S. FDA Hazard Analysis and Critical Control Point (HACCP) principles (FDA, 2011) that recommend storage of all fish at temperatures $\leq$4.4 °C to prevent growth of histamine-producing bacteria (HPB). Scombroid fish (e.g., tuna and mackerel) and non-scombroid species (e.g., mahi-mahi, sardines, and bluefish) are associated with HFP due to high levels of free histidine in their muscle. Post-harvest contamination, including that from fish intestines, is considered the primary source of HPB (Lehane & Olley, 2000). Exposure of fish to elevated temperatures between harvest and consumption allows the growth of HPB with production of histamine (Lehane & Olley, 2000). Once formed, histamine cannot be eliminated by post-harvest treatments. *Morganella morganii* is one of the most prevalent and potent histamine producers associated with fish (Kim, Field, Chang, Wei, & An, 2001). It is most frequently associated with gills and skin and has been detected on the surfaces of conveyer belts and plastic totes during processing (Kim et al., 2003).

Control of pathogenic bacteria such as, *L. monocytogenes*, and endogenous spoilage bacteria, such as *M. morganii*, in processing plants depends on proper cleaning and disinfection of equipment, prevention of contamination of RTE products, and control of microbial growth in refrigerated products. Electrolyzed oxidizing (EO) water was reported to exhibit antimicrobial activity against cell suspensions of several bacterial species and against food-borne pathogens attached to fruits and vegetables (Bari, Sabina, Isobe, Uemura, & Isshiki, 2003; Guentzel, Lamb, Callan, Emmons, & Dunhamb, 2008; Udompijitkul, Daeschel, & Zhao, 2007), eggs (Russell, 2003), tableware (Handojo, Lee, Hipp, & Pascall, 2009), cutting boards (Chiu, Duan, Liu, & Su, 2006), food processing equipment (Park, Hung, & Kim, 2002), food processing gloves (Liu & Su, 2006) and ceramic tile, floor tile, and stainless steel surfaces (Liu, Duan, & Su, 2006; Phuvasate & Su, 2010). EO water was also found to be effective against pathogens associated with poultry processing (Hinton, Northcutt, Smith, Musgrove, & Ingram, 2007) but was ineffective in reducing pathogens on fresh pork (Fabrizio & Cutter, 2004) and RTE meats (Fabrizio & Cutter, 2005).

The potential exists for raw fish, as well as RTE seafood products, to become contaminated with pathogenic or spoilage bacteria present in a seafood processing environment. Cross-contamination of seafood usually results from contact with sources within the plant (e.g., knives, slicers, food preparation surfaces, conveyor belts, brining solutions, personnel). Reduction of bacterial populations on fish surfaces has been addressed by several researchers. Mahmoud et al. (2004) found that numbers of aerobic bacteria were reduced by soaking whole carp in EO water, while Kim et al. (2006) reported that storage of Pacific saury in ice prepared with EO water inhibited the growth of aerobic and psychrotrophic bacteria during refrigerated storage. *Escherichia coli* O157:H7 and *L. monocytogenes* numbers were reduced by up to 1.1 $\log_{10}$CFU/g following EO water treatment of raw salmon muscle and skin surfaces at 35 °C (Ozer & Demirci, 2006), while treatment of salmon skin with EO water for 2 h resulted in 1.3 and 2.2 $\log_{10}$CFU/cm^2 reductions of *Enterobacter aerogenes* and *M. morganii*, respectively; and treatment of tuna skin with EO ice for 24 h reduced *E. aerogenes* and *M. morganii* by 2.4 and 3.5 $\log_{10}$CFU/cm^2, respectively (Phuvasate & Su, 2010).

Reducing contamination in seafood processing environments could prevent cross-contamination of raw and RTE products. The efficacy of EO water against food-borne bacteria on conveyor belts has not been previously reported. The objectives of this study were to evaluate the effectiveness of EO water against *L. monocytogenes* and *M. morganii* cells attached to conveyor belts commonly used in seafood processing, and to determine the bactericidal/bacteriostatic activity of EO water against bacterial cells on raw salmon (*L. monocytogenes*) and raw mahi-mahi (*M. morganii*) fillets during storage at 4 °C.

2. Materials and methods

2.1. Bacterial strains

Ninety *L. monocytogenes* and five *M. morganii* isolates were used in this study. The bacterial isolates, number of strains, and sources are listed in Table 1. All strains are from FDA culture collections and are maintained at the FDA, Gulf Coast Seafood Laboratory. *L. monocytogenes* cultures in tryptic soy broth (Difco, Sparks, MD) + 0.6% yeast extract (Difco; TSBYE) and *M. morganii* cultures in TSBYE + 1% NaCl (Fisher Scientific, Fair Lawn, NJ) were maintained in 96-well plates containing 10% glycerol (Fisher; final concentration) at −80 °C and on tryptic soy agar (Difco) + 0.6% yeast extract (TSAYE) or TSAYE + 1% NaCl slants overlaid with sterile mineral oil at 4 °C. Cultures were transferred to new 96-well plates containing 100 μl of appropriate broth and incubated overnight at 35 °C or inoculated into 150 ml of broth in a 250 ml flask and incubated overnight at room temperature on a shaking platform (Model 2314FS, Fisher Scientific, Dubuque, IA). Cultures were transferred three times in broth before use.

2.2. EO water preparation

Electrolyzed oxidizing (EO) water was prepared using a continuous EO water generator (ElectroCide System (Electrolyzer Corp., Woburn, MA). The electrolyzer was allowed to run for 20 min before collecting the acidic EO water which was used within 1 h of collection. The pH of the solution was measured using a pH meter (Orion, Fisher Scientific, Pittsburgh, PA); oxidation-reduction potential (ORP) was measured with an ORP meter (ORPTestr 10, Oakton Instruments, Vernon Hills, IL); and free chlorine concentration was determined with a chlorine detection kit (Hach Pocket

Table 1
Bacterial strains used in this study.

Bacterium/Serotype	No. of strains	Source
Listeria monocytogenes/1	5	ATCC 15313, smoked mussels, raw milk, Jalisco cheese, human case
Listeria monocytogenes/1a1	8	Popsicle, shrimp, crab, surimi scallops, pollack, patient, unknown
Listeria monocytogenes/1a2	3	Lobster, cooked snow crab
Listeria monocytogenes/1/2a	3	Hot dog, human cases
Listeria monocytogenes/1/2b	2	Chocolate milk, human case
Listeria monocytogenes/3b	1	Turkey ham
Listeria monocytogenes/4	7	Cheese, amniotic fluid, blood culture, patient, human cases
Listeria monocytogenes/4a	1	ATCC 19114 (Animal tissue)
Listeria monocytogenes/4a7,9	1	FDA culture collection
Listeria monocytogenes/4b	19	Cheese, coleslaw, pate, deli meat, RTE meat product, human case, environment
Listeria monocytogenes/4b6	7	Scott A, raw shrimp, cooked snow crab, smoked salmon, scallops, Jalisco cheese, red bean ice bar
Listeria monocytogenes/4c	1	ATCC 19116 (Chicken)
Listeria monocytogenes	35	FDA culture collection
Morganella morganii	2	Mahi-mahi muscle
Morganella morganii	3	Mahi-mahi gills

Colorimeter™II, HACH Company, Loveland, CO) according to the manufacturers' instructions. The acidic EO water had a pH of 2.8, ORP of 1080 mV, and contained 50 ppm free chlorine.

2.3. MBEC™ Assay

The Minimal Biofilm Eradication Concentration (MBEC™) Physiology and Genetics (P & G) Assay (MBEC Bioproducts Inc., Edmonton, Alberta, Canada) was used for the formation of bacterial biofilms on 96 identical polystyrene pegs to determine susceptibility of biofilms to EO water. This is a rapid and reproducible assay for determining biofilm susceptibilities to antimicrobials (Ali, Khambaty, & Diachenko, 2006). Ninety *L. monocytogenes* and five *M. morganii* isolates were used for the MBEC™ P & G Assay. Wells containing 200 μl of 1/3 TSBYE medium (0.3% TSB + 0.18% YE; Ali et al., 2006) were inoculated with *L. monocytogenes*, while wells containing 200 μl of 1/3 TSBYE + 0.6% NaCl medium were inoculated with *M. morganii* using a 96-prong replicator. Biofilms were allowed to form on pegs for 24 h with incubation at room temperature on a shaking platform. Biofilms on pegs were rinsed four times for 2 min in fresh 96-well plates containing 200 μl phosphate buffered saline (PBS: NaCl, 7.65 g; Na_2HPO_4, 0.724 g; KH_2PO_4, 0.21 g; deionized water, 1 L; pH 7.2) per well to release loosely attached cells. Rinsed pegs were transferred to 96-well plates containing 200 μl of EO water or PBS (control) for five, 15, or 30 min with shaking. Treated pegs were transferred to 200 μl of neutralizing buffer (PBS + 0.1% sodium thiosulfate [Fisher]) for 2 min; neutralizer was removed by washing for 1 min in PBS. Pegs were then sonicated (VWR Model 250 HT, VWR, West Chester, PA) in 200 μl of wash buffer (PBS + 0.5% Tween 20 [Sigma Chemical Co., St. Louis, MO] + 0.1% glycine [Sigma]) for 10 min to remove attached cells. All treatments were performed at room temperature. Surviving cells in 100 μl of wash buffer were transferred to wells containing 100 μl of 1/3 TSBYE or 1/3 TSBYE + 0.6% NaCl; pegs were also incubated in 1/3 TSBYE or 1/3 TSBYE + 0.6% NaCl. Incubation was carried out for 18 h at 30 °C. Survival was determined by assessing turbidity of the growth medium.

2.4. Conveyor belt and fish inocula preparation

The efficacy of EO water was determined against *L. monocytogenes* strains Lm 422, Lm 424, Lm 3325, and Lm 3649, and *M. morganii* strain Mm 301 attached to conveyor belt coupons and fish fillets (see Sections 2.6 and 2.8). Cells (1 ml) from 18-h cultures were collected by centrifugation at 4000× *g* for 10 min; the pellets were washed twice with 1 ml of PBS and resuspended in 1 ml of PBS. Cell suspensions were diluted 1:10 in PBS (fish inocula) or in 1% fish slurry prepared with PBS (conveyor belt inocula). Cell counts were determined by plate count on TSAYE or TSAYE + 1% NaCl after incubation at 30 °C for 18 h.

2.5. Preparation of conveyor belt coupons

New polyethylene conveyor belt samples were obtained from Intralox (Harahan, LA). Polyethylene was chosen because it is chemically resistant and can be used at low temperatures. The belts were cut into 5 cm × 5 cm coupons. One-half of the coupons were sanded to simulate wear. Coupons were soaked in 200 ppm chlorine overnight. They were then rinsed twice with municipal water, twice with sterile distilled water, and once with ethanol, and allowed to dry in a laminar flow hood under UV light at room temperature.

2.6. Inoculation of conveyor belt coupons

Individual unsanded ("new") and sanded ("worn") conveyor belt coupons were inoculated with each strain of *L. monocytogenes* or *M. morganii* in 1% fish slurry by spreading 100 μl of the culture suspension over the coupon surface with a pipette tip. Inocula levels, based on the average of three experiments, are provided in Table 2. Inoculated coupons were held inside a laminar flow hood for 2 h at room temperature to allow attachment of cells.

2.7. Treatment of conveyor belt coupons

A spray bottle was used to saturate inoculated coupons with PBS (control) or EO water. The coupons were allowed to stand at room temperature for 5 min in a laminar flow hood. Treated coupons were dipped in 150 ml of neutralizing buffer in 250-ml beakers for 10 s. Individual coupons were then transferred to Whirl-Pak® bags (Nasco, Modesto, CA) containing 25 ml of wash buffer. Attached cells on coupons were released by sonication for 10 min. Treatments were done in triplicate.

2.8. Inoculation of fish

Fresh salmon (*Salmo salar*) and mahi-mahi (*Coryphaena hippurus*) fillets were purchased from a local grocer and transferred to the laboratory in ice. Fillets were sprayed with ethanol, aseptically cut into 25-g portions, and frozen at −20 °C in Whirl-Pak® bags until use. The muscle side of thawed salmon or mahi-mahi fillet portions was inoculated with individual strains of *L. monocytogenes* (Lm 422) or *M. morganii*, respectively, by spotting 100 μl of culture suspensions onto the surface. Inocula levels, based on the average of three experiments, are given in Table 3. Cells were allowed to attach to fillets for 20 min at room temperature in a laminar flow hood followed by attachment for 2 h at 4 °C.

2.9. Treatment of fish

Raw fish portions inoculated with *L. monocytogenes* or *M. morganii* were immersed in 100 ml EO water or PBS in Whirl-

Table 2
Effects of sterile deionized water and EO water on inoculated conveyor belt coupons.

Strain	Ave. inoculum/cm^2 (log_{10}CFU ± SD)	Ave. number of surviving cells/cm^2 recovered (log_{10}CFU ± SD)			
		Sterile deionized water		EO water	
		unsanded	sanded	unsanded	sanded
Lm[a] 422	6.41 ± 0.49	2.12 ± 0.48	1.66 ± 0.49	2.30 ± 0.94	1.04 ± 0.70
Lm 424	5.45 ± 0.57	2.53 ± 0.86	1.43 ± 0.82	0 (+)[c]	0 (+)
Lm 3325	5.96 ± 0.26	2.24 ± 0.60	1.42 ± 1.08	0 (+)	0 (+)
Lm 3649	6.04 ± 0.12	1.00 ± 0.00	1.48 ± 0.48	0 (+)	0 (+)
Mm[b] 301	5.37 ± 0.65	1.31 ± 1.30	1.36 ± 1.34	0 (+)	0 (+)

[a] Lm, *Listeria monocytogenes*.
[b] Mm, *Morganella morganii*.
[c] (+), stressed cells recovered with enrichment.

Table 3
Effect of cold storage (4 °C) on inoculated raw salmon (Lm[a]) and mahi-mahi (Mm[b]) fillets treated with sterile deionized water and EO water.

Inoculated strain and storage time	Ave. number of surviving cells/g ($\log_{10}$CFU/g ± SD)	
	Sterile distilled water	EO water
Lm 422 inoculum	4.47 ± 0.14	4.47 ± 0.14
Day 0	2.55 ± 0.33	2.33 ± 0.14
Day 2	3.51 ± 0.42	3.52 ± 0.22
Day 6	5.66 ± 0.17	5.46 ± 0.37
Day 10	7.91 ± 0.09	7.62 ± 0.21
Mm 301 inoculum	4.02 ± 0.29	4.02 ± 0.29
Day 0	4.28 ± 0.48	2.48 ± 0.14
Day 2	5.50 ± 0.31	5.64 ± 0.24
Day 6	5.14 ± 0.20	5.46 ± 0.32
Day 10	8.65 ± 0.28	8.69 ± 0.17

[a] Lm, *Listeria monocytogenes*.
[b] Mm, *Morganella morganii*.

Pak® bags and agitated on a platform shaker for 5 min at room temperature. Uninoculated fish portions were also assayed for the presence of *L. monocytogenes* or *M. morganii*. Treated fish were rinsed in neutralizing buffer for 15 s and drained in a petri dish. The fish portions were incubated at 4 °C in stomacher 400 filter bags (Seward Laboratory Systems, Bohemia, NY) for 4 h ($T = 0$) to 10 d. The fish were then homogenized in 75 ml of wash buffer for 3 min using a Pulsifier (Microbiology International, Frederick, MD). Treatments were performed in triplicate.

2.10. Determination of numbers of surviving cells

Numbers of *L. monocytogenes* and *M. morganii* cells recovered in the wash buffer were determined by spread plating serial dilutions onto R & F® Listeria monocytogenes chromogenic plating medium (R & F Products, Inc., Downers Grove, IL) or modified Niven's agar (5 g tryptone [Difco], 5 g yeast extract, 5 g NaCl, 27 g histidine [MP Biomedicals, Salon, OH], 1 g Ca CO_3 [Acros Organics, Geel, Belgium], 30 g Oxoid agar [Oxoid, Hampshire, England], 0.06 g bromcresol purple [Acros], 1 L deionized water, pH 5.3–5.5), respectively. Colony counts were recorded after 24–48 h incubation at 30 °C. 1 ml of wash buffer was added to 9 ml of TSBYE or TSBYE + 1% NaCl for enrichment (recovery of *L. monocytogenes* or *M. morganii* cells, respectively) at 30 °C for 18 h. Turbid broths were streaked to R & F® Listeria monocytogenes chromogenic plating medium or modified Niven's agar with incubation at 30 °C for 24–48 h. This step was conducted in order to recover cells whose numbers fell below the standard plate count limit of detection (25 CFU/ml of wash buffer or 25 CFU/cm^2).

2.11. Data analysis

Results of triplicate experiments were transformed into log values and reported as means. Mean values and standard deviations were determined for results of independent triplicate trials for sterile deionized water (SDW) and EO water treatments of conveyor belt coupons and fish fillets. With respect to calculation of means and standard deviations, a value of 0.99 was assigned to sample outcomes below the lower limit of detection (10 CFU/ml) of the plating method. Data from the three trials were combined for statistical analyses. Where sample outcomes were frequently below the limit of detection, significance of differences between paired treatments within the trials were assessed using the nonparametric Wilcoxon signed rank test. Where outcomes were not censored, the paired sample *t*-test was used. Thus, the nonparametric test was used to determine significance of differences in numbers of Lm 422, Lm 424, Lm 3325, Lm 3649, and Mm 301 recovered (1) from unsanded and sanded conveyor belt coupons (data was pooled across strains and SDW or EO water treatment) and (2) after SDW vs. EO water treatment for each individual strain and pooled across strains (pooled over unsanded and sanded coupons). Means obtained for SDW- and EO water-treated fish fillets were compared using the paired sample *t*-test to determine significant differences in numbers of Lm 422 and Mm 301 recovered during time in cold storage. Data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, NC). An alpha level of 0.05 was considered the threshold for significance.

3. Results and discussion

3.1. MBEC™ Assay

All bacterial isolates used in this study (Table 1) were susceptible to treatment with EO water based on results obtained using the MBEC™ P & G Assay System. Approximately 7 $\log_{10}$CFU/ml of *L. monocytogenes* and *M. morganii* cells were recovered from untreated (control) biofilms following sonication of pegs. No *L. monocytogenes* or *M. morganii* cells were recovered from sonication buffer (biofilm cells) or wash buffer (loosely attached cells) following EO water treatment of inoculated pegs for 5 min. Four *L. monocytogenes* strains (Lm 422, Lm 424, Lm 3325, Lm 3649) and one *M. morganii* strain (Mm 301) were subsequently selected from this group of isolates to examine the effects of EO water on cells attached to unsanded ("new") and sanded ("worn") conveyor belt coupons and raw fish fillets.

3.2. EO water-treated conveyor belt surfaces

L. monocytogenes and *M. morganii* cells attached to sanded polyethylene conveyor belt surfaces were no more resistant to treatment with EO water than were cells attached to unsanded belt surfaces (Table 2). Although there was marginal evidence ($P \leq 0.10$) of a difference between numbers of bacterial cells recovered from sanded and unsanded coupons, the difference was not statistically significant at an alpha level of 0.05 based on the number of samples analyzed. The attachment period chosen was 4 h to represent typical processing plant conditions. Longer attachment times would likely result in more firmly attached cells and a heavier, more resistant biofilm.

Based on data pooled over all strains, there was a highly significant difference ($P \leq 0.0001$) between numbers of bacterial cells recovered from conveyor belt coupons treated with SDW versus EO water. No Lm 424, Lm 3325, Lm 3649, or Mm 301 cells were recovered from the wash buffer of EO water-treated conveyor belt coupons. The presence of fish protein did not appear to affect the efficacy of the EO water treatment against these isolates. However, all strains were recovered following enrichment in broth culture. As 25 CFU/ml of wash buffer or 25 CFU/cm^2 would be required for detection by standard plate count, it was calculated that 2.5–25 CFU/coupon or 0.1–1 CFU/cm^2 remained on treated coupons. Although elimination of cells was incomplete in this case, continued intermittent disinfection of conveyor belts with removal of food residue might prevent establishment and growth of certain strains of *L. monocytogenes* and *M. morganii*. Numbers of Lm 422 cells attached to conveyor belt material were not reduced by EO water exposure indicating strain to strain variability in susceptibility to EO water. This variability might also have been observed for *M. morganii* had more strains been used in this study; however, additional investigation is needed. Variability in resistance needs to be taken into account when testing specific sanitizers and exposure conditions for efficacy against different strains of *L. monocytogenes*.

The alternating use of sanitizers in food processing environments could aid in addressing this problem (Clavero, 2004).

EO water has been used as a disinfectant for food processing equipment and for inactivating bacteria in raw seafood. Huang et al. (2006) reported that EO water was effective for cleaning fish contact surfaces in grocery stores and fish markets. Liu et al. (2006) noted that immersion in EO water (50 mg/L chlorine) for 5 min reduced *L. monocytogenes* by 3.73 $\log_{10}$CFU/25 cm^2 on stainless steel, 4.24 $\log_{10}$CFU/25 cm^2 on ceramic tile, and 1.52 $\log_{10}$CFU/25 cm^2 on floor tile. Soaking ceramic tile and stainless steel in EO water (50 ppm chlorine) for 5 min reduced (>0.92 to >5.4 $\log_{10}$CFU/cm^2) HPB on food contact surfaces (Phuvasate & Su, 2010). Venkitanarayanan, Ezeke, Hung, and Doyle (1999) reported that immersion of smooth, plastic cutting boards in EO water could inactivate *L. monocytogenes* and *E. coli* O157-H7. However, like conveyor belts, used plastic cutting boards have scarred surfaces that can protect bacteria from cleaning and sanitation processes. Gloves used in handling food can become contaminated by contact with raw products or contaminated surfaces. Although food residue on processing gloves reduced the efficacy of EO water against attached *L. monocytogenes*, Liu and Su (2006) reported that soaking inoculated gloves in EO water at room temperature for 5 min eliminated >4.46 $\log_{10}$CFU *L. monocytogenes*/cm^2. EO water treatment reduced numbers of *L. monocytogenes* on crabmeat-soiled floor tile by 1.52 $\log_{10}$ and on stainless steel and ceramic tile by 2.33 $\log_{10}$; however, its effectiveness was greatly reduced by the crabmeat residue (Liu et al., 2006). As a result, Liu et al. (2006) stated that EO water is not satisfactory for sanitation in the presence of food residue.

3.3. EO water-treated fish surfaces

In the present study, exposure of Lm 422 cells attached to raw salmon to SDW and EO water for 5 min resulted in an initial reduction of 2 $\log_{10}$CFU/g (Table 3). This reduction was likely due to removal of loosely attached cells during the treatment and rinse steps. Subsequent growth of Lm 422 was not inhibited by EO water treatment or cold storage as Lm 422 cell counts increased by 1 $\log_{10}$CFU/g during storage for 2 d at 4 °C. The increase in numbers on EO water-treated fish was the same as for SDW-treated fish through refrigerated storage for 10 d. Initial treatment with SDW of mahi-mahi inoculated with 4 $\log_{10}$CFU/g Mm 301 resulted in no reduction in numbers of inoculated cells, while treatment with EO water resulted in removal of 2 $\log_{10}$CFU/g (Table 3). Storage of SDW- and EO water-treated fish fillets at 4 °C did not prevent growth of Mm 301 as numbers increased to 5 $\log_{10}$CFU/g after 2 d. There was no significant difference (at an alpha level of 0.05) in numbers of Lm 422 and Mm 301 recovered from SDW- versus EO water-treated fish fillets during cold storage except for Mm 301 immediately after treatment. The efficacy of the EO water was likely reduced by organic compounds associated with the fish (Liu et al., 2006). These results do not support the use of EO water for inactivation of *L. monocytogenes* and *M. morganii* on raw fish under the conditions used in this study. Longer exposure times were reported to reduce numbers of *L. monocytogenes* and *M. morganii* on salmon and tuna (Ozer & Demirci, 2006; Phuvasate & Su, 2010). However, lengthy treatment periods might not be feasible for use during food processing.

Mahmoud et al. (2004) reported that dipping whole and filleted carp for 15 min at 25 °C in electrolyzed NaCl solutions reduced aerobic bacteria by 2.8 and 2.0 $\log_{10}$, respectively, and could prevent spoilage and extend shelf life. Huang et al. (2006) reported that EO water was effective for reducing numbers of *E. coli* and *Vibrio parahaemolyticus* on tilapia skin surfaces. Ozer and Demirci (2006) showed that EO water reduced *L. monocytogenes* by 1.1 $\log_{10}$CFU/g on fresh salmon fillets and suggested that EO water could potentially be used to decontaminate raw fish. Phuvasate and Su (2010) used EO water and EO ice, respectively, to reduce *M. morganii* by 2.2 $\log_{10}$CFU/cm^2 on salmon skin and by 3.5 $\log_{10}$CFU/cm^2 on tuna skin.

L. monocytogenes is ubiquitous in smoked salmon processing plants (Dauphin, Ragimbeau, & Malle, 2001). It was suggested that contamination of cold-smoked salmon was due to contamination during processing although raw salmon was also considered a potential source (Vogel et al., 2001). Specific persistent types of *L. monocytogenes* that are resistant to cleaning and disinfection can colonize and remain on processing equipment and contaminate the salmon during processing (Wulff et al., 2006). Chou et al. (2006) reported the presence of *L. monocytogenes* in raw catfish products and in processed catfish fillets, suggesting that inadequate sanitation procedures were employed by the plant examined in their study or that the isolates came from the catfish habitat. Kim et al. (2003) reported that gills and skin of fresh fish were the source of *M. morganii* contamination and that raw fish was responsible for contamination of processing plant equipment with HPB. On-board handling was recognized as a critical control point to prevent growth of HPB and subsequent histamine formation in fish. Reducing HPB on fish skin after catch could reduce the possibility of cross-contamination during preparation of fish fillets (Phuvasate & Su, 2010). Prevention of contamination of fish is required from catch to final product (Destro, Leitao, & Farber, 1996).

4. Conclusion

In conclusion, EO water can be used to reduce numbers of attached bacterial cells on food processing surfaces. However, variability in strain resistance must be considered when developing HACCP protocols to control contamination. Results of the present study do not support the use of EO water for removing bacteria on raw fish. There remains a need for safe, affordable antimicrobials that are effective against bacterial flora of public health concern on fish to prevent entry and spread of these organisms in processing plants, especially those that produce RTE products.

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