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## Effects of electrolyzed oxidizing water and ice treatments on reducing histamine-producing bacteria on fish skin and food contact surface

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## ABSTRACT

This study investigated efficacy of electrolyzed oxidizing water (EO water) and ice (EO ice) treatments in reducing histamine-producing bacteria (*Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Morganella morganii* and *Proteus hauseri*) on food contact surfaces (ceramic tile and stainless steel) and fish skin (Atlantic salmon and yellowfin tuna). Soaking ceramic tile and stainless steel in EO water (50 ppm chlorine) for 5 min inactivated inoculated bacteria on the surface (>0.92 to >5.4 log CFU/cm<sup>2</sup> reductions). *E. cloacae*, *K. pneumoniae* and *P. hauseri* did not survive well on fish skin. Soaking salmon skin in EO water (100 ppm chlorine) for 120 min resulted in 1.3 and 2.2 log CFU/cm<sup>2</sup> reductions of *E. aerogenes* and *M. morganii*, respectively. A treatment of EO ice (100 ppm chlorine) for 24 h was capable of reducing *E. aerogenes* and *M. morganii* on tuna skin by 2.4 and 3.5 log CFU/cm<sup>2</sup>, respectively. EO water and EO ice can be used as post-harvest treatments for reducing histamine-producing bacteria on food contact surfaces and fish skin.

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## 1. Introduction

Scombroid poisoning is a common illness resulted from consumption of fish containing histamine, a toxic chemical produced by enzymatic decarboxylation of histidine. Several bacterial species, such as *Morganella morganii*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*, are known to be capable of producing histidine decarboxylase and can convert free histidine in fish muscle to histamine in improperly handled fish (Rawles, Flick, & Martin, 1996). These bacteria are recognized as major histamine producers associated with fish and have been isolated from skin and gill of fresh fish (Kim, Field, Change, Wei, & An, 2001; Kim, Price, Morrissey, Field, Wei, & An, 2001). Scombroid fish, including tuna, mackerel, and bonito, that contain high levels of free histidine in muscles have been frequently implicated in scombroid poisoning. However, some nonscombroid fish, such as mahi-mahi, bluefish, herring, sardine, and anchovy, that were not properly stored have also been linked to scombroid outbreaks (Rawles et al., 1996; Taylor, 1986).

The formation of histamine in fish is mainly related to the growth of bacteria which produce histidine decarboxylase. Improper storage of fish at temperatures higher than 7.2 °C (45 °F) allows growth of histamine-producing bacteria (HPB) and production of histidine decarboxylase (FDA, 2001; López-Sabater, Rodríguez-

Jerez, Hernández-Herrero, Roig-Sagués, & Mora-Ventura, 1996). Once histamine is produced in fish, it will not be destroyed by cooking, freezing or smoking processes (Bremer, Osborne, Kemp, van Veghl, & Fletcher, 1998). Storing fish at refrigeration temperatures from harvest to consumption has been recognized as the most effective means to prevent scombroid poisoning. Sensory examination commonly used to evaluate freshness of fish cannot be used to predict the presence of histamine in fish because histamine is odorless and tasteless.

To reduce incidence of scombroid poisoning associated with fish consumption, the United States Food and Drug Administration (FDA) recommend that fish should be chilled immediately upon death and established an advisory level that limits histamine at a level of <50 ppm in fish for consumption (FDA, 2001). However, exposure of fish to elevated temperatures (>8 °C) during retail storage cannot be totally avoided. Exposure of fish to temperatures above 8 °C could allow HPB to grow and produce histidine decarboxylase in fish. Therefore, development of post-harvest treatments for reducing HPB in fish upon harvest is an important intervention strategy to prevent histamine formation in fish and control scombroid poisoning.

Electrolyzed oxidizing (EO) water, produced through electrolysis of a dilute salt solution (0.05–0.2% NaCl), was recently introduced as a new sanitizer with strong antibacterial activities against many foodborne pathogens including *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Listeria monocytogenes* and *Bacillus cereus* (Kim, Hung, & Brackett, 2000b; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999a). Application of EO water as a disinfectant

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for reducing microbial contaminations has been reported for fresh fruits and vegetables (Izumii, 1999; Koseki, Yoshida, Isobe, & Itoh, 2001, 2004), poultry carcasses (Fabrizio, Sharma, Demirci, & Cutter, 2002; Park, Hung, & Brackett, 2002), cutting boards (Venkitanarayanan, Ezeike, Hung, & Doyle, 1999b; Chiu, Duan, Liu, & Su, 2006), and food or non-food contact surfaces (Liu & Su, 2006; Park, Hung, & Kim, 2002).

Mahmoud et al. (2004) reported that soaking whole carp in EO water (40.8 ppm chlorine) at 25 °C for 15 min could reduce total bacterial populations on skin by 2.8 log CFU/cm<sup>2</sup>. Huang et al. (2006) studied EO water for reducing microbial population on tilapia and reported that EO water (120 ppm chlorine) treatments reduced *Vibrio parahaemolyticus* by 1.5 and 2.6 log CFU/cm<sup>2</sup> after 5 and 10 min, respectively. In addition, Kim et al. (2006) studied effects of tap water ice and ice prepared from EO water (47 ppm chlorine) on preserving freshness on Pacific saury (mackerel pike) and reported that EO ice storage significantly retarded growth of aerobic and psychrotrophic bacteria on the fish. Sensory and microbiological analyses showed that the saury stored in EO ice had a shelf life that was 4–5 days longer than fish stored in regular ice. These results indicate that EO water might be used as a disinfecting agent for reducing HPB in fish and seafood processing environments. Our preliminary study confirmed that EO water (pH: 2.74, chlorine: 30 ppm, ORP: 1211 mV) was effective in inactivating HPB (*E. aerogenes*, *Enterobacter cloacae*, *K. pneumoniae*, *M. morgani* and *Proteus hauseri*). All these HPB were reduced by greater than 5.0 log CFU/ml after 30 s in the EO water (unpublished data). This study was conducted to determine the effectiveness of EO water and EO ice on inactivating HPB on food-contact surface and fish skin.

## 2. Materials and methods

### 2.1. Bacterial cultures

Five histamine-producing bacteria (HPB), including *E. aerogenes* (ATCC 13048), *E. cloacae* (ATCC 23355), *K. pneumoniae* (ATCC 13883), *M. morgani* (ATCC 25830), and *P. hauseri* (ATCC 13315), obtained from the American Type Culture Collection (ATCC, Manassas, VA) were used. Each strain was grown in 10 ml of tryptic soy broth (TSB; Difco, BD, Sparks, MD) at 37 °C for 18–24 h. Enriched culture was centrifuged in a sterile centrifuge tube (3000g Sorvall RC-5B, Kendro Laboratory products, Newtown, CT) at 5 °C for 15 min. Pellets were re-suspended in phosphate-buffered saline (PBS) to obtain a suspension of approximately 10<sup>8–9</sup> CFU/ml.

### 2.2. Material preparation

Two types of materials, glazed ceramic tiles (FC30, Wanke Cascade, Portland, OR) and stainless steel sheet (SS304, K-Manufacturing, Astoria, OR), commonly used in food processing environments as either non-food or food contact surface were used. The materials were cut into small chips (5 × 5 cm<sup>2</sup>), washed with cleaning detergent, and autoclaved. Each chip was inoculated with one strain of HPB by spreading 50 µl of the culture suspension over surface with a sterile colony spreader. Inoculated chips (10<sup>5–6</sup> CFU/cm<sup>2</sup>) were held inside a biological safety cabinet (NuAire, Plymouth, MN) for 45 min at room temperature to allow HPB to attach to surface.

### 2.3. Fish skin preparation

Skins of Atlantic salmon (*Salmo salar*) and yellowfin tuna (*Thunnus albacares*) were used. Fresh Atlantic salmon fillet was purchased from a local supermarket and frozen yellowfin tuna fillet was obtained from a local seafood company. Skin on salmon or tuna fillet was removed using a sterile knife and rinsed briefly

under tap water. The skin was then cut into small pieces (3 × 3 cm<sup>2</sup>) and allowed to dry in the biological safety cabinet at room temperature for 10 min. Each piece of skin was inoculated with one strain of HPB by spreading 100 µl of the culture suspension over the skin with a sterile colony spreader to produce a contamination level of 10<sup>6–7</sup> CFU/cm<sup>2</sup>. Inoculated skins were allowed to dry inside the biological safety cabinet at room temperature for 15 min.

### 2.4. Electrolyzed oxidizing (EO) water and ice preparation

EO water containing 50 or 100 ppm chlorine was prepared with electrolysis of 1% sodium chloride (NaCl) solution using an EO water generator (model V-500, Electric Aquagenics Unlimited, Inc., Linton, UT) with a setting of 2.2 or 3.2 Amperes (A), respectively. EO water was produced on the day of experiments, kept in a sealed container, and used within 2 h of production. Chlorine contents, pH and oxidation–reduction potential (ORP) of EO water were determined with a commercial chlorine detection kit (HACH Company, Loveland, CO), a pH meter (model 420A, Orion Research, Inc., Boston, MA) and an ORP meter (CheckmateII Systems with Redox Sensor, Corning, Inc., Corning, NY), respectively.

EO ice containing 50 or 100 ppm of chlorine was prepared by freezing EO water containing 120 or 250 ppm of chlorine in plastic ice trays immediately after production. The ice trays were sealed with adhesive plastic films and held inside a –18 °C freezer overnight. EO ice was crushed using a rubber hammer and used within 30 min. The chlorine content in EO ice was measured with ice melt after heating EO ice in a sealed container in a water bath (48 °C) for 20 min.

### 2.5. EO water treatment of ceramic tile and stainless steel

Each ceramic tile or stainless steel chip inoculated with HPB was soaked in a plastic beaker containing 200 ml of EO water (50 ppm chlorine) for 5 min at room temperature. The EO water-treated chips were transferred to individual beakers containing 200 ml of sterile Butterfield's phosphate buffer and held for 1 min to neutralize the acidity before conducting microbiological analysis. Inoculated chips soaked in distilled water followed by sterile Butterfield's phosphate buffer were used as controls. Populations of HPB on each chip were analyzed before and after treatments. All treatments were conducted in triplicate.

### 2.6. EO water treatment of fish skin

Efficacy of EO water treatment in reducing HPB on fish skin was conducted with Atlantic salmon. Each fish skin inoculated with one strain of HPB (10<sup>6–7</sup> CFU/cm<sup>2</sup>) was soaked in 200 ml EO water in a sealed plastic jar (500 ml) and held at room temperature for 120 min with a change of EO water after 60 min. Reductions of HPB on fish skin treated with EO water were compared with those treated with distilled water. Fish skin not inoculated with HPB was analyzed for presence of HPB before inoculation. To further investigate effects of chlorine contents in EO water on reducing HPB on fish skin, HPB that survived well on fish skin were inoculated to salmon skin and treated with EO water containing 50 or 100 ppm at room temperature for 120 min. Populations of HPB on the skin were analyzed after 60 and 120 min of treatments. All treatments were conducted in triplicate.

### 2.7. EO ice treatment of fish skin

Efficacy of EO ice in reducing HPB on fish skin was determined by placing salmon and tuna skin inoculated with HPB strains that survived well on fish skin in EO ice. HPB-inoculated fish skin

( $10^{6-7}$  CFU/cm<sup>2</sup>) was placed scale side up on a bed (5.5 cm from the bottom) of EO ice (50 or 100 ppm chlorine) in a 500-ml plastic jar and covered with a layer (4 cm) of EO ice. All jars were sealed and stored in polystyrene coolers (L 43 × W 30 × H 30 cm) covered with regular ice to prevent fast melting of EO ice. Populations of HPB on fish skin were determined after 6, 12 and 24 h of treatments. Inoculated skins stored in regular ice were used as controls. All tests were conducted in triplicate.

### 2.8. Microbiological analysis

Populations of HPB on surface of ceramic tile and stainless steel sheet were determined by swabbing entire surface of a chip (5 × 5 cm<sup>2</sup>) with 3–4 sterile swabs until the surface became totally dry. All swabs were placed in a sterile centrifuge tube containing 25 ml of PBS. The tube was vigorously vortexed for 1 min to allow bacterial cells to detach from swabs. For determination of HPB on fish skin, each piece of fish skin was placed in a bottle containing 90 ml of sterile PBS. The bottle was shaken for 30 s to prepare 1:10 sample suspension. Additional serial dilutions were prepared with sterile Butterfield's buffer. Presence of HPB in each sample suspension was determined by the pour-plate method using a differential plating medium designed for quantitative determination of HPB bacteria based on histidine decarboxylation activity (Niven, Jeffrey, & Corlett, 1981). The plates were incubated at 35 °C for 48 h. Colonies formed on plates with purple halo were considered as HPB. Results were reported as means of triplicate determinations.

### 2.9. Statistical analysis

Results of microbiological tests were transformed into log values and analyzed with two-sample t test and ANOVA using S-PLUS (Insightful Corp., Seattle, WA). Significant differences between treatments were established at a level of  $P = 0.05$ .

## 3. Results and discussion

### 3.1. Survival of HPB on food-contact surfaces

All the HPB bacteria, except *P. hauseri*, were able to survive on stainless steel (SS) to some degrees (Table 1). Among them, *E. aerogenes* and *M. morganii* appeared to be more resistant to dry condition than others. Populations of *E. aerogenes* and *M. morganii* decreased by about 1 log CFU/cm<sup>2</sup> on SS after 45 min at room temperature while greater reductions of 1.9 and 4.0 log CFU/cm<sup>2</sup> were observed for *E. cloacae* and *K. pneumoniae*, respectively. No vegeta-

tive cells of *P. hauseri* were recovered from the SS after 45 min at room temperature.

When the HPB were inoculated to CT, only *E. aerogenes*, *E. cloacae*, and *K. pneumoniae* were recovered after 45 min at room temperature (Table 1). No vegetative cells of *M. morganii* or *P. hauseri* were recovered from the CT and reductions of *E. aerogenes* (2.1 log CFU/cm<sup>2</sup>), *E. cloacae* (3.1 log CFU/cm<sup>2</sup>), and *K. pneumoniae* (4.8 log CFU/cm<sup>2</sup>) on CT were all greater than those observed for SS. These results indicate that certain HPB, such as *E. aerogenes*, *E. cloacae*, and *M. morganii*, could survive well on food contact surfaces even after 45 min at room temperature. This is similar to a previous report showing that populations of *E. aerogenes* (6.10 log CFU/cm<sup>2</sup>) on glazed ceramic tile and stainless steel remained almost unchanged after 30 min of drying at 30 °C (Park, Hung, & Kim, 2002). Therefore, food contact surface should be sanitized immediately after handling raw seafood to avoid transfer of HPB to subsequently handled products.

### 3.2. Survival of HPB on fish skin

In addition to their abilities to survive on CT and SS, *E. aerogenes* and *M. morganii* also survived well on salmon skin. Populations of both HPB remained almost unchanged on salmon skin after 15 min at room temperature (Table 2). However, *E. cloacae*, *K. pneumoniae* and *P. hauseri* did not survive well on salmon skin. Populations of *E. cloacae* and *K. pneumoniae* on the salmon skin decreased rapidly by 3.5 and 4.9 log CFU/cm<sup>2</sup>, respectively, after drying at room temperature for 15 min. *Proteus hauseri* was once again confirmed very sensitive to dry conditions. No vegetative cells of *P. hauseri* were recovered from the skin after 15 min. These results suggested that both *E. aerogenes* and *M. morganii* would be the major species of concern for causing scombroid poisoning because of their abilities to survive on food contact surface and fish skin. Many studies have reported isolating *E. aerogenes* and *M. morganii* from a variety of fish with *M. morganii* capable of producing large amounts of histamine (>1000 ppm) in fish when exposed to elevated temperatures (Kim, Field, et al., 2001; Kim, Price, et al., 2001; Kim et al., 2002; López-Sabater et al., 1996; Middlebrooks, Toom, Douglas, Harrison, & McDowell, 1988).

### 3.3. Efficacy of EO water treatment in inactivating HPB on food-contact surface

Soaking HPB-inoculated CT and SS in DI water for 5 min at room temperature resulted in some reductions of *E. aerogenes*, *E. cloacae*, and *K. pneumoniae* on CT (0.14–0.93 log CFU/cm<sup>2</sup>) as well as *E. aerogenes*, *E. cloacae*, *K. pneumoniae*, and *M. morganii* on SS (0.87–1.37 log CFU/cm<sup>2</sup>) (Table 1). These results indicate that rinsing

**Table 1**  
Efficacy of EO water (50 ppm chlorine) treatment (5 min) in reducing histamine-producing bacteria on ceramic tile and stainless steel.

Treatments	<i>Enterobacter aerogenes</i>	<i>Enterobacter cloacae</i>	<i>Klebsiella pneumoniae</i>	<i>Morganella morganii</i>	<i>Proteus hauseri</i>
<b>Glazed ceramic tile</b>					
Initial inoculation	6.41 <sup>a</sup>	5.12	5.70	4.58	4.72
After drying	4.29 ± 0.11 <sup>b</sup> A	2.03 ± 0.12 A	0.92 ± 0.48	ND	ND
Distilled water	3.36 ± 0.06 B (0.93) <sup>c</sup>	1.89 ± 0.09 A (0.14)	ND (>0.92)	ND	ND
EO water	ND <sup>d</sup> (>4.29)	ND (>2.03)	ND (>0.92)	ND	ND
<b>Stainless steel</b>					
Initial inoculation	6.41	5.12	5.70	4.58	4.72
After drying	5.41 ± 0.03 A	3.19 ± 0.05 A	1.74 ± 0.15 A	3.47 ± 0.04 A	ND
Distilled water	4.54 ± 0.03 B (0.87)	2.23 ± 0.04 A (0.96)	0.37 ± 0.10 B (1.37)	2.39 ± 0.05 B (1.08)	ND
EO water	ND (>5.41)	ND (>3.19)	ND (>1.74)	ND (>3.47)	ND

<sup>a</sup> Bacterial populations (log CFU/cm<sup>2</sup>).

<sup>b</sup> Data are means of three determinations ± standard deviation. Means with the same letter in the same column are not significantly different ( $P > 0.05$ ).

<sup>c</sup> Reductions of bacterial populations (log CFU/cm<sup>2</sup>) after treatments.

<sup>d</sup> Not detectable by plate count method with a detection limit of <1 CFU/cm<sup>2</sup>.



**Table 2**  
Effects of EO water (50 ppm chlorine, 120 min) treatments on reducing histamine-producing bacteria on salmon skin.

Treatments	<i>Enterobacter aerogenes</i>	<i>Enterobacter cloacae</i>	<i>Klebsiella pneumoniae</i>	<i>Morganella morganii</i>	<i>Proteus hauseri</i>
Initial inoculation	6.75	5.16	6.29	5.88	5.58
After drying	6.52 ± 0.02 <sup>a</sup> A	1.70 ± 0.09 A	1.37 ± 0.30	5.93 ± 0.18 A	ND
Distilled water	6.31 ± 0.08 B (0.21) <sup>b</sup>	1.60 ± 0.11 A (0.10)	ND <sup>c</sup>	5.74 ± 0.11 AB (0.19)	ND
EO water	5.58 ± 0.07 C (0.94)	ND (>0.70)	ND	5.36 ± 0.28B (0.57)	ND

<sup>a</sup> Data are means of three determinations ± standard deviation. Means with the same letter in the same column are not significantly different ( $P > 0.05$ ).

<sup>b</sup> Reductions of bacterial populations (log CFU/cm<sup>2</sup>) after treatments.

<sup>c</sup> Not detectable by plate count method with a detection limit of <10 CFU/cm<sup>2</sup>.

food contact surface with water would not be an effective means for eliminating HPB from contaminated surface. The reductions of HBP on food contact surface after soaking in distilled water were probably due to a washing off effect of HPB cells that were loosely attached to surface. However, soaking HPB-inoculated CT and SS in EO water (pH: 2.7, chlorine: 50 ppm, ORP: 1160 mV) for 5 min eliminated HPB on both materials (Table 1). The reductions of HPB ranged from >0.92 log CFU/cm<sup>2</sup> for *K. pneumoniae* on CT to >5.41 log CFU/cm<sup>2</sup> for *E. aerogenes* on SS depending on the populations survived on chips after inoculation. These results demonstrated that EO water could be used as a surface sanitizer to eliminate HPB contamination.

#### 3.4. Effects of EO water treatment on reducing HPB on fish skin

Soaking fish skin inoculated with HPB in distilled water for 120 min at room temperature resulted in little reductions (0.10–0.37 log CFU/cm<sup>2</sup>) of the bacteria (Table 2). Treatments of fish skin with EO water (50 ppm chlorine) for 120 min resulted in slightly greater reductions (0.57–0.94 log CFU/cm<sup>2</sup>) of HPB when compared with distilled water treatments. However, the reductions were limited to <1.0 log CFU/cm<sup>2</sup>. These results indicate that treatment of fish with EO water containing 50 ppm of chlorine would not be effective on reducing HPB on the skin.

The antibacterial activity of EO water is known to relate to its low pH, available chlorine (Cl<sub>2</sub>, HOCl, OCl<sup>-</sup>), and high ORP (Kim, Hung, & Bracket, 2000a; Oomori, Oka, Inuta, & Arata, 2000). Therefore, the antibacterial activity of EO water is expected to decrease when organic compounds are present because of interaction between organic substances and available chlorine. Liu, Duan, and Su (2006) reported that a 5-min EO water (50 ppm chlorine) treatment was much more effective in reducing *L. monocytogenes* contamination on clean food-contact surface (3.73–4.24 log CFU/cm<sup>2</sup>) than on crabmeat-containing surface (2.3 log CFU/cm<sup>2</sup>). Since fish skin contain large numbers of organic materials such as glycoproteins from mucus (Ebran, Julien, Orange, Auperin, & Molle, 2000), EO water treatment will be less effective on inactivating bacteria on the skin unless the chlorine concentration in the water is increased. Su and Morrissey (2003) reported that dipping whole sal-

mon in acidified sodium chlorine solution containing 50 ppm of chlorine for 1 min could only reduce total bacterial populations on skin by 0.43 log CFU/cm<sup>2</sup>.

To evaluate the effect of EO water with higher chlorine content on inactivating HPB on fish skin, salmon skin was inoculated with *E. aerogenes* or *M. morganii* that survived well on food-contact surface and fish skin and treated with EO water containing 100 ppm of chlorine (pH: 2.5, ORP: 1173 mV). Results showed that treatments of EO water containing 100 ppm of chlorine for up to 120 min did not result in increased reductions of *E. aerogenes* on salmon skin when compared with EO water treatments containing 50 ppm of chlorine (Table 3). However, the treatment did increase the reduction of *M. morganii* on salmon skin by 0.93 log CFU/cm<sup>2</sup> after 120 min when compared with the treatment containing 50 ppm of chlorine. These results indicate that a treatment of EO water (50–100 ppm chlorine) for 120 min could reduce HPB contamination on fish skin by 1–2 log units, which can be an intervention strategy for preventing histamine formation in fish contaminated with low levels of HPB.

#### 3.5. Effects of EO ice treatment on reducing HPB on fish skin

Holding salmon skin inoculated with *E. aerogenes* and *M. morganii* in regular ice for 24 h resulted in small reductions of *E. aerogenes* (0.56 log CFU/cm<sup>2</sup>) and *M. morganii* (1.17 log CFU/cm<sup>2</sup>) (Table 4). Storing inoculated salmon skin in EO ice (50 ppm chlorine) for 24 h resulted in a similar reduction of *M. morganii* (1.15 log CFU/cm<sup>2</sup>) but a greater reduction of *E. aerogenes* (1.27 log CFU/cm<sup>2</sup>) on the skin. These results showed that keeping fish in regular ice inhibited growth of HPB and chlorine in ice provided power for inactivating the bacteria. This is evidenced by greater reductions of *E. aerogenes* (1.62 log CFU/cm<sup>2</sup>) and *M. morganii* (2.02 log CFU/cm<sup>2</sup>) on the skin after 24 h of treatment with EO ice containing 100 ppm of chlorine.

To validate the effectiveness of EO ice on reducing HBP on fish skin, the study was repeated with yellowfin tuna skin because yellowfin tuna is more frequently involved in scombroid poisoning than salmon. Holding tuna skin inoculated with *E. aerogenes* and *M. morganii* in regular ice for 24 h resulted in similar reductions

**Table 3**  
Reductions of *Enterobacter aerogenes* and *Morganella morganii* on salmon skin by treatments with EO water containing different contents of chlorine (50 and 100 ppm) for 60 and 120 min.

Treatments	Time (min)	Bacterial populations (log CFU/cm <sup>2</sup> )	
		<i>Enterobacter aerogenes</i>	<i>Morganella morganii</i>
Initial populations		7.00 ± 0.03 <sup>a</sup> A	6.86 ± 0.03 A
EO water (50 ppm chlorine)	60	6.07 ± 0.08 B (0.93) <sup>b</sup>	5.88 ± 0.09 B (0.98)
	120	5.72 ± 0.06 C (1.28)	5.62 ± 0.12 B (1.24)
EO water (100 ppm chlorine)	60	5.83 ± 0.12 BC (1.17)	5.68 ± 0.21 B (1.18)
	120	5.68 ± 0.16 C (1.32)	4.69 ± 0.17 C (2.17)

<sup>a</sup> Data are means of three determinations ± standard deviation. Means with the same letter in the same column are not significantly different ( $P > 0.05$ ).

<sup>b</sup> Reductions of bacterial populations (log CFU/cm<sup>2</sup>) after treatments.

**Table 4**  
Reductions of *Enterobacter aerogenes* and *Morganella morganii* on salmon skin stored in EO ice.

Bacteria	Time (h)	Bacterial populations (log CFU/cm <sup>2</sup> )		
		Regular ice	EO ice (50 ppm chlorine)	EO ice (100 ppm chlorine)
<i>Enterobacter aerogenes</i>	0	7.06 ± 0.03 <sup>a</sup> A	7.18 ± 0.02 A	7.25 ± 0.09 A
	6	6.79 ± 0.02 B (0.37) <sup>b</sup>	6.59 ± 0.15 B (0.59)	6.34 ± 0.35 B (0.91)
	12	6.76 ± 0.18 B (0.40)	5.82 ± 0.36 C (1.36)	6.30 ± 0.35 B (0.95)
	24	6.60 ± 0.07 B (0.56)	5.91 ± 0.25 C (1.27)	5.63 ± 0.12 C (1.62)
<i>Morganella morganii</i>	0	7.00 ± 0.11 A	7.15 ± 0.02 A	7.15 ± 0.02 A
	6	6.54 ± 0.09 B (0.46)	6.24 ± 0.28 B (0.91)	5.75 ± 0.33 B (1.40)
	12	6.34 ± 0.28 B (0.66)	5.88 ± 0.30 B (1.27)	5.84 ± 0.33 B (1.31)
	24	5.83 ± 0.17 B (1.17)	6.00 ± 0.28 B (1.15)	5.13 ± 0.12 C (2.02)

<sup>a</sup> Data are means of three determinations ± standard deviation. Means with the same letter in the same column are not significantly different ( $P > 0.05$ ).

<sup>b</sup> Reductions of bacterial populations (log CFU/cm<sup>2</sup>) after treatments.

**Table 5**  
Reductions of *Enterobacter aerogenes* and *Morganella morganii* on yellowfin tuna skin stored in EO ice.

Bacteria	Time (h)	Bacterial populations (log CFU/cm <sup>2</sup> )		
		Regular ice	EO ice (50 ppm chlorine)	EO ice (100 ppm chlorine)
<i>Enterobacter aerogenes</i>	0	6.08 ± 0.18 <sup>a</sup> A	6.08 ± 0.18 A	6.20 ± 0.06 A
	6	5.40 ± 0.08 B (0.68) <sup>b</sup>	4.54 ± 0.38 B (1.54)	4.17 ± 0.18 B (2.03)
	12	5.46 ± 0.18 B (0.62)	3.86 ± 0.11 B (2.22)	3.64 ± 0.13 C (2.56)
	24	5.37 ± 0.25 B (0.71)	4.02 ± 0.14 B (2.06)	3.77 ± 0.16 C (2.43)
<i>Morganella morganii</i>	0	6.32 ± 0.02 A	6.32 ± 0.02 A	6.16 ± 0.03 A
	6	5.37 ± 0.14 B (0.95)	5.00 ± 0.09 B (1.32)	3.99 ± 0.42 B (2.17)
	12	5.18 ± 0.19 B (1.14)	4.72 ± 0.19 B (1.60)	3.93 ± 0.47 B (2.23)
	24	4.94 ± 0.09 B (1.38)	4.69 ± 0.40 B (1.63)	2.66 ± 0.27 C (3.50)

<sup>a</sup> Data are means of three determinations ± standard deviation. Means with the same letter in the same column are not significantly different ( $P > 0.05$ ).

<sup>b</sup> Reductions of bacterial populations (log CFU/cm<sup>2</sup>) after treatments.

of *E. aerogenes* (0.71 log CFU/cm<sup>2</sup>) and *M. morganii* (1.38 CFU/cm<sup>2</sup>) when compared with the salmon skin study (Table 5). Treatments of inoculated tuna skin with EO ice (50 ppm chlorine) for 24 h increased reductions of *E. aerogenes* and *M. morganii* to 2.06 and 1.63 log CFU/cm<sup>2</sup>, respectively. The reductions of *E. aerogenes* and *M. morganii* on tuna skin increased to 2.43 and 3.50 log CFU/cm<sup>2</sup> after keeping the skin in EO ice containing 100 ppm of chlorine after 24 h. No apparent change in fish skin color was observed during the EO ice (100 ppm chlorine) treatment except a slight discoloration was noted for both salmon and tuna skin after 24 h. These results demonstrated that storing fish in EO ice containing 100 ppm of chlorine could be used as a post-harvest treatment for reducing HPB on yellowfin tuna.

Holding fish in ice immediately after catch is a common practice to prevent rapid growth of bacteria on fish. Although keeping fish in ice retards growth of bacteria, the practice generally does not inactivate bacteria. Once fish is removed from ice and exposed to temperature-abused environments before consumption, the bacteria including HPB can multiply and cause spoilage or produce histamine if fish is contaminated with high levels of HPB. Kim et al. (2002) studied histamine formation in mackerel, mahi-mahi and albacore tuna inoculated with *M. morganii* (10<sup>6</sup> CFU/g) and stored at 4 °C. The investigators reported a gradual increase of histamine in the fish from <10 ppm after 6 days of storage to 78 (mackerel), 54 (mahi-mahi), and 46 ppm (albacore tuna) after 14 days of storage.

While fish flesh is generally free of bacteria before fish dies, contamination with HPB can occur through contact with skin or processing surface during filleting. Therefore, reducing HPB on fish skin after catch will reduce the possibility of cross-contamination when fish fillet is prepared. This study demonstrated that EO ice (100 ppm of chlorine) could be used to store fish and reduce HPB on skin. Even a short 6-h of holding fish in the EO ice was capable of reducing HPB on the fish by 2-log units.

In conclusion, EO water containing 50 ppm of chlorine could be used as a sanitizer to eliminate HPB contamination on food-contact surface. Soaking fish in EO water (100 ppm chlorine) for 120 min reduced *E. aerogenes* and *M. morganii* on salmon skin by 1.3 and 2.2 log CFU/cm<sup>2</sup>, respectively. Storing fish in EO ice containing 100 ppm of chlorine reduced *E. aerogenes* and *M. morganii* on yellowfin tuna skin by 2.4 and 3.5 log CFU/cm<sup>2</sup>, respectively, after 24 h. EO ice (100 ppm chlorine) can be used as a post-harvest treatment to reduce histamine-producing bacteria on fish skin and minimize histamine formation in fish during storage.

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