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Efficacy of electrolyzed oxidizing water as a pretreatment method for reducing *Listeria monocytogenes* contamination in cold-smoked Atlantic salmon (*Salmo salar*)

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ABSTRACT

Listeria monocytogenes contamination in ready-to-eat (RTE) fish products, in particular in cold-smoked salmon is an important food safety concern. This study evaluated the antimicrobial activity of electrolyzed oxidizing (EO) water as a pretreatment method during the process of cold-smoked salmon to inactivate *L. monocytogenes*. In addition, the effect of EO water treatment on the sensory and textural quality of the final product was also evaluated. Raw Atlantic salmon (*Salmo salar*) fillets were inoculated with *L. monocytogenes* (with an approximately cell number of 6×10^5 CFU/g *L. monocytogenes* ATCC 19114) and treated with EO water at three different temperatures (20, 30, and 40 °C) and at three different exposure time of 2, 6, and 10 min before the cold-smoking process. A combination of EO water and a mild temperature (40 °C) had reduced *L. monocytogenes* populations by 2.85 $\log_{10}$ CFU/g. The sensory as evaluated by a consumer panel (N = 71) and texture, which was measured by texture analysis showed no significant changes between EO and mild temperature treated samples and the control.

1. Introduction

Listeria monocytogenes, a psychotropic, Gram-positive and facultative anaerobe, is a food-borne pathogen capable of causing serious illness in vulnerable groups such as elderly, newborns, and individuals with weakened immune systems (Gombas, Chen, Clavero, & Scott, 2003; McCarthy & Burkhardt, 2012). The organism is well adapted to different environmental conditions including high salt content (10–20%), low temperature (less than 1 °C), low oxygen, and pH values below 6 (Farber & Peterkin, 1991) and it is a major concern to producers of RTE foods. Listeriosis has the third highest mortality rate among foodborne infections; approximately 2500 illnesses and 500 deaths are attributed to listeriosis in the United States annually (Levine, Rose, Green, Ransom, & Hill, 2001).

Despite all the efforts to eradicate *L. monocytogenes* from RTE foods, its contamination continues to occur. One class of the RTE products recognized as a potential risk for *L. monocytogenes* is cold-smoked salmon, because the temperature applied during the process (20–30 °C) is not sufficient to inactivate the organism, and if conditions are not properly controlled could encourage its growth. These foods are consumed without further cooking (Gudmundsdóttir et al., 2005; Rørvik, 2000).

The United States has set a zero tolerance level for the *L. monocytogenes* in RTE foods, including RTE seafood (Porsby, Vogel, Mohr, & Gram, 2008). Among all of the U.S. Food and Drug Administration regulated RTE foods recalled due to the *L. monocytogenes* risk from 1990 to 2006, seafood had the highest percentage of recalls (CSPI., 2008; Kang et al., 2012; Ozer & Demirci, 2006) and according to the Rapid Alert System for Food and Feed (RASFF) in EU, compared with other food product, seafood is second only to vegetables in the number of alerts activated between 2009 and 2012 (RASFF, 2013).

To date, several RTE products, such as smoked salmon, smoked mussel, smoked trout, cooked crawfish, and seafood salad, have been found to be contaminated with *L. monocytogenes* (McCarthy & Burkhardt, 2012). Therefore, reducing the occurrence of *L. monocytogenes* in RTE seafood is an important food safety goal.

So far application of different types of antibacterial compounds, such as nisin, hypochlorite, chlorine, chlorine dioxide, trisodium

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phosphate solution, acidified sodium chlorite, potassium lactate in combination with sodium acetate or sodium diacetate have been suggested to eliminate or minimize the occurrence of pathogens from raw and minimally processed seafood including cold-smoked salmon (Bremer & Osborne, 1998; Kim, Huang, Marshall, & Wei, 1999; Lakshmanan & Dalgaard, 2004; Lin, Huang, Cornell, Lin, & Wei, 1996; Mu, Huang, Gates, & WU, 1997; Nykänen, Lapveteläinen, Hietanen, & Kallio, 1999; Park, Rua, & Acker, 1991; Su & Morrissey, 2003; Vogel, Yin Ng, Hyldig, Mohr, & Gram, 2006; Yoon, Burnette, Abou-Zeid, & Whiting, 2004).

One of the disinfection methods which is becoming more common in seafood processing and sanitation is the use of electrolyzed oxidizing water (EO water), which its antimicrobial activity against a variety of microorganisms and food-borne pathogens has reported (Fabrizio & Cutter, 2003; Hricova, Stephan, & Zweifel, 2008; Huang, Hung, Hsu, Huang, & Hwang, 2008; McCarthy & Burkhardt, 2012; Ozer & Demirci, 2006; Rasco & Ovissipour, 2015).

EO water is a novel antimicrobial agent developed in Japan, and produced when dilute salt water is put through an electric current in a sealed chamber. Two different types of acidic and alkaline water, are produced from the process. The former is capable of killing harmful microorganisms, while the latter can be used to remove dirt and grease from items such as cutting boards and other kitchen utensils (Huang et al., 2006).

The disinfecting effect of EO water on fish pathogenic bacteria (Kasai, Ishikawa, Hori, Watanabe, & Yoshimizu, 2000; Ovissipour et al., 2015) and in the surface sanitization of seafood products (McCarthy & Burkhardt, 2012) have been reported. Several studies have been conducted using EO water to control the microbial contamination in different types of seafood products; soaking whole carp in EO water reduced the numbers of aerobic bacteria (Mahmoud et al., 2004); using ice prepared with EO water during the refrigerated storage, hindered the growth of aerobic and psychrotrophic bacteria (Kim et al., 2006); treatment of raw salmon and yellowfish tuna with EO water and/or ice, reduced the number of *Escherichia coli* O157:H7, *L. monocytogenes*, Enterobacter aerogenes and *Morganella morganii* (Ozer & Demirci, 2006; Phuvasate & Su, 2010). Soaking inoculated tilapia (with *E. coli* O157:H7 and *Vibrio parahaemolyticus*) into EO water resulted in 2.6 log CFU/cm^2 reduction of *V. parahaemolyticus* after 10 min, and an additional 0.7 log CFU/cm^2 reduction compared to tap water on *E. coli* O157:H7 after 1 min treatment (Huang et al., 2006). Raw salmon treated with EO water (pH of 2.6, ORP of 1150 mV and free chlorine of 90 mg/L) at 35 °C for 64 min resulted in a 1.07 log CFU/g (91.1%) and 1.12 log CFU/g (92.3%) reduction in *E. coli* O157:H7 and *L. monocytogenes*, respectively (Ozer & Demirci, 2006).

Compared to traditional disinfectant solutions, EO water has the potential to be more cost effective, less expensive, and environment friendly (Hricova et al., 2008; Ozer & Demirci, 2006; Rasco & Ovissipour, 2015). Moreover, using EO water on different food products did not negatively affect the organoleptic properties of color, scent, flavor, or texture in different food products (Hara, Watanuki, & Arai, 2003; Kim, Hung, Brackett, & Lin, 2003; Kobayashi, Tosa, Hara, & Horie, 1996; Mahmoud, 2007).

To date, there is no study of the effectiveness of EO water against pathogenic bacteria during the process of cold-smoked salmon production. Studying the efficacy of this disinfecting agent to eliminate or reduce the growth of *L. monocytogenes* in a high-risk seafood product, such as cold-smoked salmon, can provide useful information for seafood producers to prevent food-borne illnesses associated with *L. monocytogenes*.

The objective of this study was to evaluate the efficacy of the EO water treatment to inactive *L. monocytogenes* in cold-smoked Atlantic salmon (*Salmo salar*). Investigating the effect of EO water treatment on sensory and textural properties of cold-smoked salmon at different exposure times and temperature treatments was the second goal of this study.

2. Material and methods

Fresh Atlantic salmon (*S. salar*) fillets were purchased from a local grocery store in Pullman, WA. Fillets were stored in freezer at −20 °C for 24 h before conducting the experiments, followed by thawing at refrigerator for 6 h before the experiments. For the microbiology, fillets were cut into 50 g pieces (5 × 3.5 × 2 cm) prior to inoculation and EO water treatments.

2.1. Microbial analysis

2.1.1. Inoculum preparation, and inoculation of salmon fillets

L. monocytogenes ATCC 19114 was obtained from Microbiologics®, Inc. (St. Cloud, MN). *L. monocytogenes* was cultured on 50 mL of tryptic soy broth TSB (BactoTM) with 1% yeast extract for 18 h at 37 °C. After appropriate incubation of bacterial cultures, 10 mL broth of the *L. monocytogenes* was transferred under aseptic conditions to a sterile centrifuge tube, and then centrifuged for 15 min at 5000 rpm (3380×*g*) to harvest bacterial cells (AccuSpin™ model 400 bench top centrifuge, Fisher Thermo Scientific, Pittsburgh, PA). To eliminate any effect of broth components and bacterial metabolites, the resultant pellets were resuspended in 10 mL of sterile saline solution (0.85% (w/v) NaCl). After the second centrifugation, the supernatant was decanted, and the resulting washed pellets were then resuspended in sterile 10 mL aliquots as before. These were then used as pure cell suspensions to inoculate salmon fillets. The approximate initial cell number of (3 × 10^8 CFU/mL) *L. monocytogenes* was used for inoculation of salmon fillets. Samples were inoculated by spreading 0.1 mL of the cell suspension on the surface of the 50 g fillets. Inoculated fillets were kept in laminar flow hood at room temperature for 1.5 h before treated with EO water. The population of *L. monocytogenes* on the inoculated salmon fillets were about 6 × 10^5 CFU/g of fish fillets. Each experiment was conducted in triplicate.

2.1.2. Preparation of EO water

Electrolyzed water (pH: 2.7; ORP:1150 mV; free chlorine: 60 ppm) was generated at 9–12 V direct current (dc) for 15 min using a two-compartment batch scale electrolysis apparatus (Super Oxseed Labo, Electrolyzed Water Generator, Aoi Electronic Corp., Kannami, Shizuoka, Japan), with the anode and cathode sides of the chamber divided by an ion exchange diaphragm. The ORP and pH were measured with a pocket-sized redox meter (HI 98201, HANNA® Instruments, Ann Arbor, Michigan, USA) and a pH meter (FE20, Mettler-Toledo, Columbus, OH, USA), respectively. The free chlorine concentration of the EO water was measured with a DPD assay (Colorimeter™ Analysis System, Hach Co., Loveland, CO, USA) according to the manufacturer instructions. The acidic EO water was collected for the treatment of salmon fillets.

2.1.3. Treatment of salmon fillets with EO water

Inoculated samples were subjected to EO water with different temperatures (20, 30, and 40 °C) at different times (2, 6, and 10 min). Clean plastic beakers with EO water were placed into the water bath at given temperatures. All samples were immersed into the EO water at different temperatures with different exposure times. The sterile deionized water was used to study the influence of temperatures and times on *L. monocytogenes* reduction. However, since no reduction was observed, the data not shown here.