The Influence of Neutral Electrolyzed Water in Combination with Temperature on *Listeria monocytogenes* Inactivation on Salmon Fillet

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Material and Methods

Fresh Atlantic salmon (*Salmo salar*) fillets were purchased from a local grocery store in Pullman, WA and transferred to the lab within 30 min on ice.

Microbial Analysis

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 5,000 rpm (3,380 x *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 (3×10^9 CFU/mL). *L. monocytogenes* were used for inoculation of salmon fillets. Samples were inoculated by spreading 0.1 ml of the cell suspension on the surface of the 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 6×10^7 CFU/g of fish fillets. Each experiment was conducted in triplicate.

Preparation of EO water

Acidic 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. Neutral electrolyzed water was received as gift from AQUAOX (Fontana, CA) and its free chlorine adjusted by deionized water to obtain a neutral electrolyzed water with 60 ppm free chlorine.

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.

Treatment of salmon fillets with EO water

Inoculated samples were subjected to different treatments including Neutral electrolyzed water, Acidic electrolyzed water and sterile deionized water at different temperatures (20, 50 and 60 °C) and different times (2, 6 and 10 min). Clean plastic beakers with solutions were placed into the

water bath at given temperatures. All samples were immersed into the solutions at different temperatures with different exposure times.

Recovery of L. monocytogenes and culture media

To recover *L. monocytogenes*, at the end of each experiment, 100 g salmon fillets were removed from beakers and placed in sterile strainer-filter bags and homogenized with of sterile 0.1% peptone water (Bacto, BD, Sparks, MD) using a Stomacher 400 (Seward Ltd., London, UK). One mL of the homogenized suspension was then serially diluted (dilution range, 10^0 to 10^{-6}) in 9 mL of sterile 0.1% peptone (Bacto, BD, Sparks, MD). A 1-mL aliquot of each dilution was divided into 5 aliquots of 0.2 mL and cultured using spread plate technique on Listeria Palcam agar (CM0877, Oxoid Ltd., Basingstoke, United Kingdom) (Al-Qadiri et al., 2015). Listeria agar plates were aerobically incubated at 37 °C for 24 to 48 h, and the numbers of viable cells were determined as CFU per gram of salmon fillet (N=3).

Results

The results of the microbial inactivation and the efficacy of different solutions at different temperatures and time are presented in Table 1. The results showed that the neutral electrolyzed water (NEW) could decrease the *L. monocytogenes* significantly compared to acidic electrolyzed water (AEW) and deionized water (DW) at 20, 50 and 60 °C. The log reduction was higher in the fish which were exposed and treated by NEW. In addition, at higher temperatures the efficacy of the electrolyzed waters (NEW and AEW) was increased. For example, at 20 °C, the log reduction after 10 min for NEW was 0.82 log, while for the same solution the log reduction after 10 min at 60 °C was 5 log. After 10 min at 60 °C the bacteria decreased 1.8 log in AEW treatment however,

for NEW it was 5 log. The control group which was sterile deionized water only showed 1.3 log reduction at 60 °C after 10 min.

Table 1: The *L. monocytogenes* reduction on Salmon fillet exposed to different solutions at different times and temperatures[1,2].

Solution	20 °C			50 °C			60 °C		
	2 min	6 min	10 min	2 min	6 min	10 min	2 min	6 min	10 min
NEW	6.60	6.30	6.17	6.60	6.04	5.60	5.70	3.00	2.00
	(0.39)	(0.69)	(0.82)	(0.39)	(1)	(1.4)	(1.3)	(4)	(5)
AEW	6.61	6.47	6.25	6.59	6.07	5.59	6.07	5.95	5.20
	(0.38)	(0.52)	(0.74)	(0.4)	(0.92)	(1.04)	(0.92)	(1.04)	(1.8)
DW	6.90	6.87	6.84	6.38	6.14	6.07	6.27	6.17	5.7
	(0.09)	(0.12)	(0.15)	(0.61)	(0.79)	(0.92)	(0.72)	(0.82)	(1.3)

[1] Values are the CFU/g salmon fillet.

[2] Values in parenthesis are log reduction.