JOURNAL OF NUTRITION FASTING AND HEALTH

Alginate Coating and Electrolyzed Oxidizing Water: Antimicrobial Effects against Inoculated *Listeria Monocytogenes* on Salmon Fillets

Romina Saei Hamedani[1], Saeid Khanzadi[1*], Mohammad Hashemi[2], Mohammad Azizzadeh[3]

1. Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran

ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 12 May 2021
Accepted: 01 Jun 2021
Published: 27 Dec 2021

Keywords:
Alginate coatin
Electrolyzed water
Fish fillets
Listeria monocytogenes

ABSTRACT

Introduction: The present study aimed to evaluate the inhibitory effects of alginate coating and electrolyzed oxidizing water (EOW) on *Listeria monocytogenes* in fish samples during 12 days in cold storage condition (4˚C).

Methods: Initially, fish fillets were inoculated with *L.* monocytogenes and divided into different groups. Following that, treated samples including controls (no coating), distilled water, alginate, EOW, and alginate coating with EOW were stored at refrigeration temperature. The fillets were preserved at the temperature of 4°C, and the bacterial count was performed on days zero, two, four, eight, and 12.

Results: The separate and combined use of alginate and EOW could significantly inhibit the growth of inoculated *L. monocytogenes* compared to the control samples, and the maximum reduction was observed in the EOW and alginate treatment (1.37 log CFU/g).

Conclusion: It is recommended that alginate coating combined with EOW in fish improved safety against *L. monocytogenes* infection.

► Please cite this paper as:
Saei Hamedani R, Khanzadi S, Hashemi M, Azizzadeh M. Alginate Coating and Electrolyzed Oxidizing Water: Antimicrobial Effects against Inoculated Listeria Monocytogenes on Salmon Fillets. J Nutr Fast Health. 2021; 9(4): 272-276. DOI: 10.22038/JNFH.2021.57724.1334.

Introduction

Seafood has a high nutritional value and is an abundant source of proteins, minerals, vitamins, and omega-3 polyunsaturated fatty acids (n-3 PUFAs) (1). Seafood safety is of paramount importance, especially in the countries with a high consumption rate of raw seafood (2). It is acknowledged that fish consumption has nutritional and health benefits for humans (3). However, fresh fish is prone to deteriorate primarily due to biological reactions such as protein degradation/decomposition following the activity of endogenous/microbial enzymes (4). On the other hand, fish and its products have been classified as major causes of foodborne diseases.

Listeria monocytogenes is considered to be a prominent foodborne pathogen, posing public health risks such as increased hospitalization and mortality rates due to listeriosis (5). *L. monocytogenes* is also known to cause seafood-borne listeriosis (6), which is a grave concern due to its severe pathogenicity (7). The intake of foods containing this pathogen at infectious levels could cause various diseases in immunocompromised patients and pregnant women (8). To mitigate the health-related risks and financial damage caused by foodborne microorganisms, natural products serving as antibacterial substances could be used for controlling the propagation of pathogenic bacteria and prolonging the lifespan of processed foods (9). Antimicrobial coatings or films act as a protective barrier and are able to delay microbial growth (10).

Hydrophilic edible coatings are excellent barriers against oxygen and carbon dioxide with considerable mechanical properties at low relative humidity. Multiple experiments have

* *Corresponding author:* Saeid Khanzadi, Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. Tel: +985138763852; Email: khanzadi@um.ac.ir.

demonstrated that edible films filled with proteins, polysaccharides, and oil-containing compounds could increase the lifespan of fish and maintain its quality. Furthermore, these coatings might act as antimicrobial agents and antioxidant vectors, thereby leading to the presence of preservatives on the top layer of food at high levels. Alginate is a brown algae-derived linear anionic polysaccharide comprised of two building blocks, which are α 1-4 hyaluronic acid and β-D-mannuronic acid. Alginate forms solid gels or non-soluble polymers through its distinct colloidal properties. Such biopolymer-based films increase the water barrier, prevent microbial contamination, and preserve flavor, which will result in the high quality of food and its extended shelf life (11). In addition, they are capable of forming biologically active alginate-based films for seafood conservation (12).Provision of healthy food sources is a viable option to minimize the incidence of foodborne diseases. The antimicrobial effects of electrolyzed oxidizing water (EOW) have been confirmed on a wide range of microorganisms, such as *L. monocytogenes* (13). Recently, EOW has been introduced for application as a sanitizer (14). EOW is a newly-made antimicrobial agent in Japan, which was produced following dilute salt water exposure to an electric current inside a sealed container. EOW is remarkably cost-efficient and environmentally safe compared to conventional disinfectant solutions. Furthermore, the use of EOW has shown no harmful effects on the organoleptic properties of various food products (color, smell, taste, or texture) (15). The disinfectant impact of EOW has been confirmed on the pathogenic bacteria found in fish and the sanitization of seafood product surfaces (15). Several experiments have been performed using EOW to prevent microbial contamination in various seafood products (15-17).

To date, several studies have investigated the use of alginate as a coating material for food, as well as the antimicrobial effects of EOW. However, no studies have been focused on the antimicrobial effects of the combinational use of alginate coating and EOW.

The present study aimed to evaluate the antimicrobial effects of *L. monocytogenes* on an alginate coating prepared by EOW in salmon stored at the temperature of 4˚C.

Materials and Methods

Microorganisms and Materials

Four strains of lyophilized *L. monocytogenes* (ATCC: 7644, 7834, 10671, 82119) were supplied from the Iranian Biological Resource Center in Tehran, Iran. The culture media were obtained from Merck (Merck, Darmstadt, Germany), and the reagents (analytical grade) were purchased from Sigma (Sigma-Aldrich Chemical Co. St. Louis, USA). The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences in Mashhad, Iran (IR.MUMS.fm.REC.1395.155).

EOW Preparation

An EW generator (model: P30HST44T, EAU, GA, USA) was used to generate EOW (16). At 19A and 10 V, 12% of a salt solution and tap water were constantly pumped into the EOW generator. Prior to collecting EOW, the generator was operated for 15 minutes to enable the machine to equilibrate, and water was dispensed at 1.5 l/min. The pH of the neutral EW (NEW) was 6.5, and the free chlorine level was 200 ppm. A pH meter was utilized to measure the pH of the solution. The EOW was heated on a hot plate before immersion in a preheated water bath at the targeted temperature (17).

Preparation of Bacteria

Four strains of *L. monocytogenes* were cultured on PALCAM agar at the temperature of 37˚C for 24 hours, and biochemical tests were performed to confirm the typical colonies of *L. monocytogenes*.

Preparation of Salmon Fillets and Bacterial Inoculation

Fresh salmon fish (mean weight: 300±50 g) was supplied from a local fish farm in Mashhad, Iran in summer 2018, filleted, and quickly transferred to the laboratory. Following that, the fillets were rinsed to remove blood and slime and dried. The fillets were cut into pieces weighing 10±1 grams, sprayed with 70% ethanol (v/v), burnt, and trimmed to eliminate surface microorganisms (7). One strain of *L. monocytogenes* (50 µl of ~10^7 CFU/ml dilution) was inoculated using adjustable volume micropipettes on each side of separate fillets (final concentration: ~ 10^5 CFU/g).

Preparation of Coating Solutions and Treatments

Alginate solutions were prepared by dissolving alginate powder (3% by weight) into sterile distilled water/EW (Table 1) containing 2% glycerol as a plasticizer in a controlled environment (45°C) and stirring continuously for 15 minutes until achieving transparency. Afterwards, 2% calcium chloride (v/v) was dissolved in distilled water and sterilized using an autoclave at the temperature of 121°C for 15 minutes. The inoculated salmon fillets were divided into six treatment groups (Table 1), immersed in the desired treatments for one minute, drained, and immersed again in a $CaCl_2$ solution. Finally, the inoculated salmon filets were analyzed on days zero, two, four, eight, and 12 (16).

Table 1. List of Treatments in Study

Treatment	Description for Samples
Control	with no coating solution
DW	coated with distilled water
Alg	coated with the alginate solution
EOW	coated with electrolyzed oxidizing water
EOW & Alg	coated with alginate solution prepared by electrolyzed oxidizing water
EOW + Alg	immersed in electrolyzed oxidizing water, then coated with alginate solution

Enumeration of L. monocytogenes

At this stage, the fish fillets (10 g) were homogenized in a stomacher (Seward Medical, UK) for three minutes at a total volume of 90 milliliters of sterile buffered peptone water (0.1% w/v). Serial dilutions (1:10) were also prepared for each sample, and 10 microliters of each sample was cultured on PALCAM agar. PALCAM agar plates were incubated at the temperature of 30°C for 48 hours (16).

Statistical Analysis

All experiments were carried out in triplicate. Data analysis was performed in SPSS version 21 (SPSS, Inc. Chicago, IL, USA). To assess significant differences at $P<0.05$, the analysis of variance (ANOVA) was repeatedly performed, followed by Bonferroni post-hoc test.

Results and Discussion

Figure 1 shows the impact of the treatments on *L. monocytogenes* growth after a 12-day storage period. Throughout the storage time, the initial count of *L. monocytogenes* increased in the control, distilled water, and alginate groups, while decreasing in the other three groups (i.e., EOW, EOW & alginate, EOW + alginate). In a study in this regard, Hudaa Neetoo et al. (2010) investigated the effectiveness of using alginate-based coatings in limiting *L. monocytogenes* growth to increase the microbial safety of filleted salmon, concluding that antimicrobial coatings could improve salmon safety and inhibit the growth of *L. monocytogenes*. Furthermore, the mentioned study indicated that microbial growth may be slightly slower in the products that are naturally contaminated, which is consistent with the findings of the current research (2). According to our findings, the lowest final count of *L. monocytogenes* was observed in the EOW + alginate (3.92 log CFU/g) and EOW and alginate (3.98 log CFU/g).

Table 2 shows the mean reduction rate of *L. monocytogenes* count in various treatments. Accordingly, the maximum reduction rate belonged to the EOW & alginate (1.37 log CFU/g) and EOW + alginate groups (1.34 log CFU/g) compared to the control group. According to the information in Table 2, using combinational antimicrobial agents was more effective against microbial growth compared to the separate use. In another study, Andres Rivera-Garcia et al. (2019) analyzed the impact of neutral electrolyzed water as a disinfectant for eggshell artificially contaminated with *L. monocytogenes,* reporting that NEW is a viable option for use as a disinfectant solution to reduce/eliminate *L. monocytogenes* without affecting the quality or mineral content of eggshell.

Our findings are consistent with the previous research conducted in this regard (17). For instance, Desa et al. (2003) investigated the effectiveness of NEW as a disinfectant for tomato surface, and the obtained results indicated that using NEW could significantly reduce pathogenic microorganisms such as *L. monocytogenes* and *E. coli* O_{157}: H_7 on the surface of tomatoes without affecting their organoleptic properties. Therefore, it could be concluded that NEW has a potential application to decontaminate the

contact surface of new products, which is consistent with the results of the present study (18).

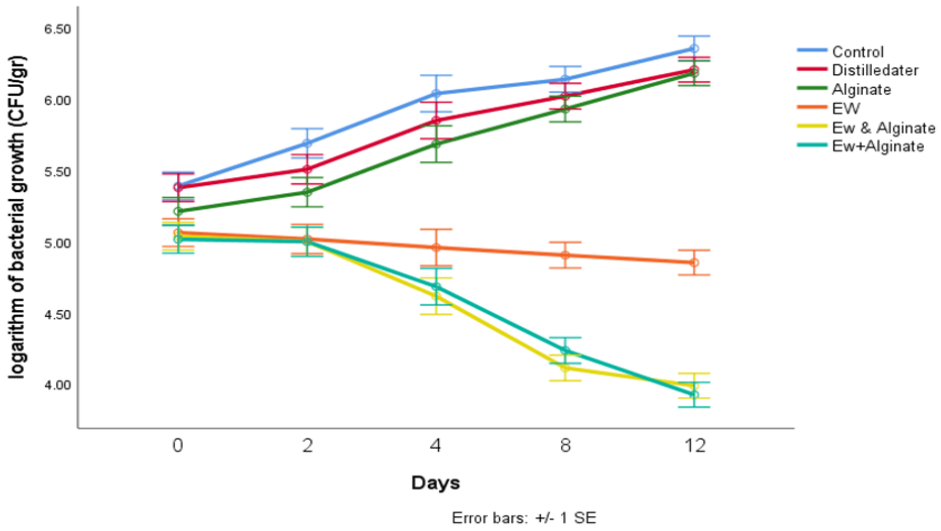

Figure 1. Evolution in Bacterial Count (log CFU/g) of Fish Fillet Samples Inoculated with *L. monocytogenes* during Storage

Table 2. Comparison of Mean Reduction Rate of *L. monocytogenes* Count in Different Treatments during Study Period A

Mean Difference I-J Group (I)	Group (J)	DW	Alg	EOW	EOW & Alg	EOW + Alg
Control		0.12	0.25	0.96*	1.37*	1.34*
DW			0.12	0.83*	1.24*	1.21*
Alg				0.71*	1.11*	1.09*
EOW					0.40*	0.38
EOW & Alg						0.02

**Significant difference (P<0.05)*

Conclusion

According to the results, EOW and alginate coating could significantly inhibit the growth of *L. monocytogenes.* Furthermore, the EOW & Alg had the most significant effect on the inhibition of *L. monocytogenes* growth in the salmon fillet samples. Due to the priority of using natural antimicrobials in food products by producers and consumers, it is recommended that an alginate coating solution be employed in combination with EOW in salmon fillet to ensure higher safety against pathogenic bacteria such as *L. monocytogenes.*

Acknowledgments

Hereby, we extend our gratitude to Ferdowsi University of Mashhad in Mashhad, Iran for the financial support of this study (grant No. 3/50510). We would also like to thank Mrs. S. Khajenasiri for assisting us in this research project.

Conflicts of Interest

None declared.

References

1. Ozer NP, Demirci A. Electrolyzed oxidizing water treatment for decontamination of raw salmon inoculated with Escherichia coli O157: H7 and Listeria monocytogenes Scott A and response surface modeling. J Food Eng. 2006;72(3):234-41.
2. Neetoo H, Ye M, Chen H. Bioactive alginate coatings to control Listeria monocytogenes on cold-smoked salmon slices and fillets. *Int J* Food Microbiol. 2010;136(3):326-31.
3. Min S, Harris LJ, Krochta JM. Listeria monocytogenes inhibition by whey protein films and coatings incorporating the lactoperoxidase system. J Food Sci. 2005;70(7):m317-24.
4. Hosseini SF, Rezaei M, Zandi M, Ghavi FF. Effect of fish gelatin coating enriched with oregano essential oil on the quality of refrigerated rainbow trout fillet. J Aquat Food Prod Technol. 2016;25(6):835-42.
5. Possas A, Pérez-Rodríguez F, Tarlak F, García-Gimeno RM. Quantifying and modelling the

inactivation of Listeria monocytogenes by electrolyzed water on food contact surfaces. J Food Eng. 2021;290:110287.
6. Ozer NP, Demirci A. Inactivation of Escherichia coli O157: H7 and Listeria monocytogenes inoculated on raw salmon fillets by pulsed UV-light treatment. *J Food* Sci Technol. 2006;41(4):354-60.
7. Sharifi F, Khanzadi S, Hashemi M, Azizzadeh M. Control of Listeria monocytogenes and Escherichia coli O157: H7 inoculated on fish fillets using alginate coating containing lactoperoxidase system and Zataria multiflora boiss essential oil. J Aquat Food Prod Technol. 2017;26(9):1014-21.
8. Tocmo R, Krizman K, Khoo WJ, Phua LK, Kim M, Yuk HG. Listeria monocytogenes in vacuum-packed smoked fish products: occurrence, routes of contamination, and potential intervention measures. Compr Rev Food Sci Food Saf. 2014;13(2):172-89.
9. Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157: H7, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes. *Food Control*. 2007;18(5):414-20.
10. Datta S, Janes M, Xue QG, Losso J, La Peyre J. Control of Listeria monocytogenes and Salmonella anatum on the surface of smoked salmon coated with calcium alginate coating containing oyster lysozyme and nisin. J Food Sci. 2008;73(2):M67-M71.
11. Song Y, Liu L, Shen H, You J, Luo Y. Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (Megalobrama amblycephala). Food Control. 2011;22(3-4):608-15.
12. Li P, Zhou Q, Chu Y, Lan W, Mei J, Xie J. Effects of chitosan and sodium alginate active coatings containing ε-polysine on qualities of cultured pufferfish (Takifugu obscurus) during cold storage. Int J Biol Macromol. 2020;160:418-28.
13. Huang Y-R, Hsieh H-S, Lin S-Y, Lin S-J, Hung Y-C, Hwang D-F. Application of electrolyzed oxidizing water on the reduction of bacterial contamination for seafood. Food Control. 2006;17(12):987-93.
14. Yang H, Swem B, Li Y. The effect of pH on inactivation of pathogenic bacteria on fresh-cut lettuce by dipping treatment with electrolyzed water. J Food Sci. 2003;68(3):1013-7.
15. Phuvasate S, Su Y-C. Effects of electrolyzed oxidizing water and ice treatments on reducing histamine-producing bacteria on fish skin and food contact surface. Food Control. 2010;21(3):286-91.
16. Raeisi M, Tabaraei A, Hashemi M, Behnampour N. Effect of sodium alginate coating incorporated with nisin, Cinnamomum zeylanicum, and rosemary essential oils on microbial quality of chicken meat and fate of Listeria monocytogenes during refrigeration. *Int J* Food Microbiol. 2016;238:139-45.
17. Rivera-Garcia A, Santos-Ferro L, Ramirez-Orejel JC, Agredano-Moreno LT, Jimenez-Garcia LF, Paez-Esquiliano D, et al. The effect of neutral electrolyzed water as a disinfectant of eggshells artificially contaminated with Listeria monocytogenes. Nutr Food Sci. 2019;7(7):2252-60.
18. Deza M, Araujo M, Garrido M. Inactivation of Escherichia coli O157: H7, Salmonella enteritidis and Listeria monocytogenes on the surface of tomatoes by neutral electrolyzed water. Lett Appl Microbiol. 2003;37(6):482-7.