



Antibacterial Effects of Alginate Coating Prepared by Electrolyzed Water on Pseudomonas aeruginosa Inoculated on Salmon Fillets



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ARTICLE INFO

Article type: Original article

Article history: Received: 25 September 2021 Revised: 23 October 2021 Accepted: 28 November 2021

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DOI: 10.52547/jhehp.7.4.221

Keywords:

Alginate coating Electrolyzed water Pseudomonas aeruginosa

ABSTRACT

Background: Electrolyzed oxidizing water (EOW) is a novel natural disinfectant. It has been suggested that applying EOW can improve the shelf life of fish. The purpose of this research was to compare the effect of electrolyzed water with a solution of alginate coating prepared/followed by EOW and examine their impact on pseudomonas aeruginosa growth inhibition in salmon fillets kept under refrigeration.

Methods: Fish fillets were inoculated with pseudomonas aeruginosa and divided into six different treatment groups: control (no coating), distilled water, alginate, EOW, EOW & alginate (samples coated with alginate solution prepared by EOW), and EOW+alginate (samples immersed in EOW, then coated with alginate solution). The fillets were kept at 4 °C, and the bacterial count was determined on days: 0, 2, 4, 8, and 12. Data analysis was performed using repeated ANOVA and Bonferroni post-hoc test at statistical significance of 0.05.

Results: Additionally, both the solo and combined application of electrolyzed water and alginate coating have been shown to substantially limit developing Pseudomonas aeruginosa. However, their combined usage had a greater impact than control sample. When coating solution was applied followed by electrolyzed water, the greatest decrease rate (1.27 logs CFU/g) was found in comparison to control samples.

Conclusion: According to the findings, applying alginate coating in conjunction with electrolyzed water may be used realistically in food systems, particularly seafood.

1. Introduction

Today, seafood diet, particularly fish products is encouraged due to their high nutritional value. However, these products are very susceptible to contamination by microorganisms, which may quickly multiply in fish. Numerous outbreaks of food-borne illnesses have been recorded as a result of eating contaminated seafood [1].

Fish products may potentially pose a danger and result in food-borne illness, which is often caused by psychrotolerant bacterial infections. Pseudomonas aeruginosa is often identified from seafood (up to 90% of bacteria in spoiled fish) and may spoil them. The presence of this bacteria in food causes digestive (gastrointestinal) problems in people, particularly those who have immune system defects [2].



Electrolyzed oxidizing water (EOW) is a common disinfectant that has captured considerable attention recently due to its antibacterial properties [3]. EOW, obtained by adding sodium chloride to tap water or RO-generated water in a container with a separated polyester membrane. Compared with chlorine-containing compounds, EOW offers many benefits as it is robustly antimicrobial against many foodborne pathogens, while it is more environmentally friendly than others. Electrolyzed oxidizing water can be cost-efficient, affordable, and environmentally friendlier than conventional disinfection treatments. Additionally, applying EOW on a variety of food items had no adverse effect on the organoleptic characteristics of color, odor, taste, or texture [4, 5]. EOW is widely regarded as safe and its ease of manufacture on-site and low cost make it a viable antimicrobial treatment option [6]. The greatest advantage of EOW for the inactivating pathogenic microorganisms relies on its less adverse impact on the environment as well as users' health because of no hazardous chemicals added in its production. Moreover, it has been clarified that EOW is harmless to the human body. Two types of water are produced simultaneously. EOW, with low pH (2.3–2.7), high oxidation-reduction potential (ORP, >1000 mV), high dissolved oxygen and contains free chlorine (concentration depends on the EW machine setting), is produced from the anode side. However, electrolyzed reduced (ER) water, with high pH (10.0–11.5), high dissolved hydrogen, and low ORP (800 to 900 mV), is produced from the cathode side [4].

Numerous studies have been conducted to determine the antibacterial capabilities of EW against a range of microorganisms [7–9]; however, no study has evaluated the antimicrobial effects of alginate coating combined with EOW. Therefore, this study aimed to investigate the antimicrobial effect of alginate coating prepared by EOW on *Pseudomonas aeruginosa* in salmon stored at 4 °C.

2. Materials and Methods

The Department of Food Hygiene, Faculty of Veterinary Medicine at the Ferdowsi University of Mashhad (Iran) provided the *Pseudomonas aeruginosa* lyophilized cultures. All the culture media were purchased from Merck (Merck, Darmstadt, Germany). All used reagents were of analytical grade and purchased from Sigma (Sigma-Aldrich Chemical Co. St. Louis, USA).

2.1. Electrolyzed oxidizing water preparation

EOW was generated by using an electrolyzed oxidizing water generator (P30HST44T, EAU, GA, USA). Softened tap water and a 12 percent salt solution were continuously fed into the generator which was set at 10 V and 19 A. The generator was operated for 15 min before collecting EOW to let the system equilibrate; then, water was supplied at a rate

of 1.5 L/min. The neutral electrolyzed water had a pH of 6.5 and a free chlorine concentration of 200 parts per million. A pH meter (8601 AZ pH, Chia, Taiwan) was used to determine the pH of the liquids [10].

2.2. Salmon fillets preparation and inoculation of the *Pseudomonas aeruginosa*

Fresh salmon fish (*Salmo salar*) (were bought fresh from a local fish store in summer 2019 (Mashhad County), filleted and cleaned to remove slime and blood, and afterward they were dried. The fillets were then sliced (10 ± 1 g), sprayed with ethanol (70 v/v), and then burned and trimmed to remove any remaining germs on the surface [1, 11]. Then, 100 μ L aliquots of *pseudomonas aeruginosa* ($\sim 10^7$ CFU/mL) suspension were inoculated onto fillet samples (10 g) to achieve a final dilution of $\sim 10^5$ CFU/g [1].

2.3. Fish fillet treatments preparation

Alginate solutions were prepared by dissolving alginate powder (3% by weight, Sigma-Aldrich, USA) into sterile distilled water/EOW (Table 1) containing 2% glycerol (Merck, Germany) as a plasticizer under a controlled environment (45 °C) and stirring continuously for 15 min until a transparent solution was obtained. Calcium chloride (2% w/v, Merck, Germany) was dissolved in distilled water and sterilized by autoclave at 121 °C for 15 min. Inoculated salmon fillets were divided into six treatment groups. Then, they were immersed in the desired treatments (1 min) according to Table 1, drained, and immersed again in CaCl₂ solution. Finally, the inoculated salmon fillets were analyzed on days 0, 2, 4, 8, and 12 [1].

2.4. *Pseudomonas aeruginosa* enumeration

Initially, the 10-gram fish samples were mixed with 90 mL of sterilized peptone water in zipper packs and placed in a bag mixer for 3 min to obtain a homogeneous suspension (dilution: 10⁻¹). Then, 1 mL of the supernatant was collected into a tub containing 9 mL of sterilized peptone water to obtain a 10⁻² dilution. After preparing decimal dilutions, 10 μ l (drops method) of serial dilutions of homogenates were transferred onto pseudomonas base agar supplement and

Table 1: List of treatments in the current study

Treatment	Description
CON	Salmon fillets with no coating solution
DW	Salmon filets immersed in distilled water
Alg	Salmon fillets coated with the alginate solution without using electrolyzed oxidizing water
EW	Salmon fillets immersed in electrolyzed oxidizing water
EW & Alg	Salmon fillets coated with alginate solution prepared by electrolyzed oxidizing water
EW + Alg	Salmon fillets immersed in electrolyzed oxidizing water, then coated with alginate solution

incubated at 37 °C for 24 h. All the tests were performed in pentaplicate [11].

2.5. Statistical analysis

SPSS ver. 21 software was used for statistical analysis (SPSS, Inc., USA). To detect significant differences at the $P < 0.05$ level, repeated measure analysis of variance and one-way ANOVA followed by the Bonferroni post-hoc test were utilized. Each test was conducted in triplicate.

3. Results and Discussion

The results in Figure 1 demonstrate the effect of several treatments on developing *pseudomonas aeruginosa* after storing it for 12 days at 4 °C. The findings indicate that the number of *pseudomonas aeruginosa* was raised in DW, Alg, and control samples over storage time, but decreased in the three treatments of EW, EW & Alg, and EW + Alg. The results indicated that when EW and alginate coating were used, growth rate of *pseudomonas aeruginosa* was significantly reduced in comparison to control samples. Similar findings have been reported before for antibacterial effects of EW and alginate coating in food models applications [12, 13]. Additionally, results showed that treatments with a combination of EW and alginate coating had greater antibacterial effects with ultimate bacterial counts of 4.97 ± 0.14 and 4.92 ± 0.51 logs CFU/g, respectively, at the end of storage period. This is the first study to examine EW and coating solutions combination, although; previous research has shown that the combination use of antimicrobial agents is more effective than their separate use against microbial growth [14].

Loris Pinto *et al.* (2015) evaluated the antimicrobial

activity of neutral (new) electrolysis water against 14 strains of *Pseudomonas* spoilage of freshly cut vegetables in cold storage. The antimicrobial effect of neutral electrolysis water against different bacterial strains at 105 cells mL, with different combinations of free chlorine concentration/contact time was investigated. All the concentrations above 100 mg / L, regardless of the salt used, were antibacterial after 2 min. When chicory and lettuce leaves were immersed in dilute neutral electrolysis water for 5 min, the microbial loads of mesophilic bacteria and Enterobacteriaceae decreased by an average of 1.7 logs CFU / g. In addition, when lettuce leaves were immersed in a cell suspension of *Pseudomonas chicorii* I3C, diluted neutral electrolysis water was able to reduce the *Pseudomonas* population by about 1.0 logs CFU / g. Due to its high antimicrobial activity against spoilage microorganisms and low cost, electrolysis cycles in washing water seemed to be an effective tool in controlling the microbial contamination of fresh vegetables in the washing process [15]. These findings are consistent with those of the current research.

The average decrease rate of *Pseudomonas aeruginosa* numbers in various treatments is shown in Table 2. The greatest decrease rates of *pseudomonas aeruginosa* (1.39 logs CFU/g and 1.32 logs CFU/g, respectively) were found in EW+ Alg and EW & Alg samples respectively, when compared to the control sample. In general, three germicidal variables contribute to electrolyzed water's antibacterial mechanisms: available chlorine concentration, pH, and oxidation-reduction potential [1]. The decreased rate of *Pseudomonas aeruginosa* count in samples treated by alginate coating (Alg) was significantly lower than the control samples as well, which may be due to the alginate coating's ability to act as an oxygen absorption barrier.

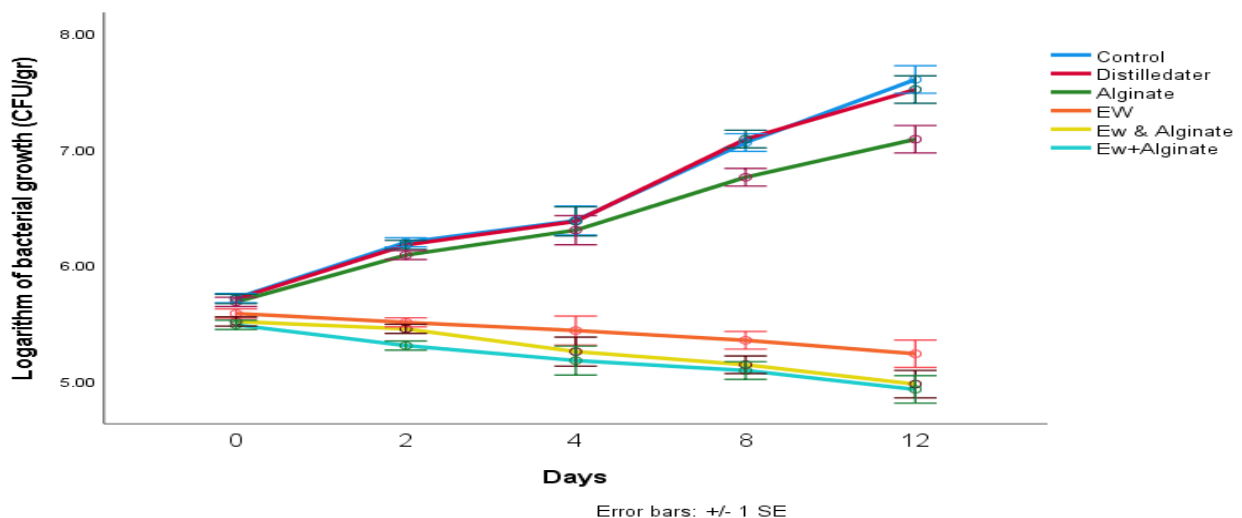


Figure 1: Evolution in the bacterial count (Logs CFU/g) of fish fillet samples inoculated with *pseudomonas aeruginosa* throughout 12-day storage (4 °C)

Table 2 :Average reduction rate of the *pseudomonas aeruginosa* counts (Logs CFU/g) among fish fillet samples when compared together during 12-day storage (4 °C)

Mean Difference I-J Group (I)	Group (J)	DW	Alg	EOW	EOW & Alg	EOW + Alg
Control		0.18	0.20	1.016*	1.32*	1.39*
DW			0.18	1.15*	1.30*	1.37*
Alg				0.96*	1.11*	1.18*
EOW					0.15*	0.22*
EOW & Alg						0.069

*The mean difference is significant at the 0.05 level.

4. Conclusion

As the current research has shown, electrolysis water and alginate coating can be applied in *pseudomonas aeruginosa* in salmon fillets that can considerably hinder *pseudomonas aeruginosa* enhancement in salmon fillets. However, the combined use of alginate coating and EW has significantly stronger antimicrobial effect. The results indicated that the strongest inhibitory effects against *pseudomonas aeruginosa* growth were seen in EW+ Alg and EW & Alg samples, which were capable of reducing *pseudomonas aeruginosa* counts by approximately 1.39 logs CFU/g and 1.32 logs CFU/g, respectively. Due to the priority of using natural antimicrobials in food products by producers and consumers, it is recommended to use alginate coating solution in combination with EOW in salmon fillets to ensure higher safety against pathogenic bacteria.

Authors' Contributions

Romina Saei: Writing-original draft; Investigation; Methodology. **Saeid Khanzadi:** Conceptualization; Funding acquisition; Project; Resources; Supervision; Validation. **Mohammad Hashemi:** Writing-review and Editing; Administration, Methodology. **Mohammad Azizzadeh:** Data curation; Formal analysis.

Conflicts of Interest

The authors affirm that there is no conflicts of interest that may have influenced the preparation of this manuscript.

Acknowledgements

We would like to express our gratitude to the Research Deputy of Ferdowsi University of Mashhad in Mashhad, Iran, for their financial support (Grant Number. 3/46031), as well as to Mrs. S. Khajenasiri for her invaluable help with this research.

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