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Effects of Alginate Coating and Neutral Electrolyzed Water on *E. coli* O_{157}:H_7 Contamination of Salmon Fillets

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ABSTRACT

Background and objectives: Neutralized electrolyzed water (NEW) is a novel natural disinfectant. It has been suggested that application of NEW can improve the shelf life of fish. This study aimed to investigate effect of NEW incorporated in alginate coating on growth of *Escherichia coli* O_{157}: H_7 on salmon fillets over a period of 12 days.

Methods: Fish fillets were inoculated with *E. coli* O_{157}:H_7 and divided into six different treatment groups: control (no coating), distilled water, alginate, EW, EW & alginate (Samples coated with alginate solution prepared by EW), and EW+ alginate (samples immersed in EW, 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-hoctest at statistical significance of 0.05.

Results: Treatment with alginate coating and EW alone could significantly reduce *E. coli* O_{157}: H_7 count on the salmon fillets. However, maximum reduction (1.27 log CFU/g) of bacteria was achieved when using alginate coating combined with EW.

Conclusion: According to the results, the combination of alginate coating with EW can be applied as a natural antimicrobial for increasing safety of food products, especially fish, against pathogenic bacteria such as *E. coli* O157: H7.

Keywords: Alginates, Escherichia coli O157, Salmon.

INTRODUCTION

Recently, consuming seafood as a rich, high-quality source of proteins and essential vitamins has been growing. However, fresh fish products tend to get spoiled in a shorter period than other food products due to high water activity(aw), neutral pH, slightly high levels of free amino acids, autolytic enzymes, and high levels of unsaturated fatty acids (1).

In 1982, *Escherichia coli* was first recognized as a human pathogen. *E. coli* O_{157}: H_7 is a highly recognized strain because it represents 157 somatic antigens (O) and seven flagellate antigens (H). *E. coli* O_{157}: H_7 is transmitted through contaminated food and water. The bacterium is mainly found in meat, especially beef, fish and raw milk (2, 3). Human infections caused by *E. coli* O_{157}:H_7 can present a broad range of clinical spectrum ranging from asymptomatic cases to death. Most cases initially present with non-bloody diarrhea that self-resolves without further complication. However, some patients progress to bloody diarrhea or hemorrhagic colitis in 1–3 days. In 5–10% of HC patients, the disease can progress to the life-threatening sequelae, hemolytic-uremic syndrome or thrombocytopenic purpura. Children and the elderly are at higher risk of severe clinical symptoms, such as hemolytic-uremic syndrome (4).

Many companies have found interest in using edible coatings in various food products such as fish since they can function as carriers for many food additives such as antibrowning agents, coloring agents, flavors, nutrients, spices, and antimicrobial substances that can improve shelf-life. Edible coatings contain proteins, polysaccharides, and lipids as the principal elements (5, 6). Alginate is a salt of alginic acid obtained from brown seaweed, but it can also be synthesized by microorganisms (1). Due to its good coating properties such as strength, thickness, emulsion stability, and gel/film formation, it is considered an attractive material for food coating (7, 8). Alginates are also more available, biodegradable, and cost-effective compared to natural shells (9). Electrolyzed water (EW) is another suitable approach for storing fresh products due to its favorable antioxidant and antimicrobial properties (10). It is obtained by adding sodium chloride to tap water or RO-generated water in a container with a separated polyester membrane (11). Compared with chlorine-containing compounds, EW offers many benefits including biocompatibility and strong antimicrobial activity against many foodborne pathogens (12, 13). Therefore, EW has been identified as a safe (GRAS), cost-effective, and convenient antimicrobial substance (14). Several researchers have been conducted on the factors associated with EW's sterilization processes, including pH, available chlorine concentration, and oxidation-reduction potential against a wide range of microorganisms. The oxidation-reduction potential was found as the most important factor in microbial inactivation research due to its ability to attack and damage the outer and inner membranes of bacteria (15). Although many studies have been conducted on the use of alginate-based edible coatings, no study has yet evaluated the antimicrobial effects of alginate coating combined with EW. Therefore, the aim of this study was to investigate antimicrobial effect of alginate coating prepared by EW on *E. coli* O_{157}: H_7 in salmons stored at 4 ˚C.

MATERIALS AND METHODS

The Department of Food Hygiene, Faculty of Veterinary Medicine at the Ferdowsi University of Mashhad (Iran) provided the *E. coli* O_{157}:H_7 NCTC 12900 lyophilized cultures. The reagents used in the study were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (USA).

Electrolyzed water was produced using an EW generator (Model #P30HST44T, EAU, GA, USA) (14, 16, 17). A 12% salt solution and softened tap water were consistently poured into the EW generator. To achieve equilibration, the generator operated at 19A and 10V for 15 min. Then, the required EW amounts were collected and dispensed at 1.5 l/min. The generated EW had neutral pH (6.5) and a free chlorine level of 200 ppm. The researchers heated the EW to the desired temperature using a hot container and placed it in a preheated water container at the predetermined temperature (18).

Fresh salmon fish weighing 300 ± 50 g was purchased from a local farm in Mashhad in summer 2018. The fish was immediately transported to the laboratory after deboning. The fillets were rinsed for cleaning the bloodstains and slime. The fillets were thoroughly dried and then chopped into pieces (10 ± 1g). Next, the surface of fillets were sprayed with ethanol (70% v/v), burnt, and trimmed. Then, 100 µl aliquots of *E. coli* O_{157}:H_7 (~10^7 CFU/ml) suspension were inoculated onto each fillet (10 g) to achieve a final dilution of ~10^5 CFU/g (17).

Alginate solutions were prepared by dissolving alginate powder (3% by weight, Sigma-Aldrich, USA) into sterile distilled water/EW (Table 1) containing 2% glycerol (Merck, Germany) as a plasticizer under a controlled environment (45° C) and stirring continuously for 15 minutes until obtaining a transparent solution. Calcium chloride (2% w/v, Merck, Germany)) was dissolved in distilled water and sterilized by autoclave at 121 °C for 15 minutes. Inoculated salmon fillets were divided into six treatment groups (Table 1). Then, they were immersed in the desired treatments (1 minute), drained, and immersed again in $CaCl_2$ solution. Finally, the inoculated salmon fillets were analyzed on days 0, 2, 4, 8, and 12 (17).

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 three minutes to obtain a homogeneous suspension (dilution: 10^{-1}). Then, one ml of the supernatant was collected into a tube containing nine ml of sterilized peptone water to obtain a 10^{-2} dilution. After the preparation of decimal dilutions, 10 µl (drops method) of serial dilutions of homogenates were transferred onto Cefixime Tellurite Sorbitol MacConkey agar (Merck, Germany) and incubated at 37 °C for 24 hours. All tests were performed in pentaplicate.

Statistical analysis of data was carried out using SPSS 21 software (SPSS, Inc. Chicago, IL, USA). Data were analyzed using repeated measure analysis of variance (ANOVA), followed by Bonferroni post-hoc test or Dunnette T3 test. A p-value of less than 0.05 indicated statistically significant difference.

Table 1- List of treatments performed on salmon fillets

Treatment	Description
Control	Samples without any coating solution
DW	DW-coated samples
Alg	Alg solution-coated samples
EW	EW-coated samples
EW & Alg	Samples coated with alginate solution prepared by EW
EW + Alg	Samples immersed in EW, then coated with alginate solution

RESULTS

In the present study, the primary count of *E. coli* O_{157}: H_7 in fish samples was 5.8 log CFU/g in the control and DW samples, 5.9 log CFU/g in Alg samples, 5.3 log CFU/g in EW, EW and Alg samples, and 5.2 log CFU /g in EW + Alg samples. As the storage time came to an end, the bacteria count was reduced to 5.1,5, 3.9, 3.6, 3.5 and 3.4 log CFU /g, respectively (Figure 1).

According to the results, the number of bacteria in all groups decreased because the studied strains were mesophilic. The highest average reduction rate of *E. coli* O_{157}: H_7 count (1.27 log CFU/g) was observed in the EW + Alg and control samples when compared with other samples (Table 2).

Table 2- Mean reduction rate of the *E. coli* O_{157}: H_7 counts (log CFU/g) regarding treatments when compared to each other during storage time (days: 0-12)

Mean Difference I-J / Group (I)	Group (J)	Distilled water	Alginate	Electrolysis water	EW & Alginate	EW+ Alginate
Control		.081	.31	.93*	1.08*	1.27*
Distilled water			.23	.85*	.99*	1.18*
Alginate				.62*	.76*	.95*
Electrolyzed water					.14	.33
EW & Alginate						.19

* $p<0.05$

DISCUSSION

Several studies have investigated the antimicrobial effect of EW on food quality. Abadias et al. (2007) reported that EW could be used instead of sodium hypochlorite as an effective disinfectant for eliminating some foodborne pathogens on vegetables, such as *Salmonella*, *E. coli* O_{157}:H_7 and *L. monocytogenes* (19). Al-Holy and Rasco (2015) evaluated the bactericidal activity of EW against three pathogens including *E. coli* O_{157}: H_7 on fish, poultry, and beef. They reported that treated samples showed significant antimicrobial activity against *E. coli* O_{157}: H_7 and decreased by about 1 to 1.5 times after 5-10 minutes. They concluded that EW could be a suitable option for reducing or eliminating bacterial contamination in fish, poultry, and beef, which is in line with our findings (13). In addition, Deza et al. (2003) assessed the antimicrobial effect of EW on four bacterial strains, including *E. coli* O_{157}: H_7. They reported that EW could effectively reduce the population of *E. coli* O157: H_7 on the surface of tomatoes without affecting their organoleptic properties (20). These findings highlight the great potential of EW as an antimicrobial for use in the food industry. Similar to our results, Liu et al. (2019) investigated the kinetics of *E. coli* O157: H7 and *Salmonella typhimurium* inactivation on organic carrots (*Daucus carota* L.) after treatment with low concentration EW and short-term heat treatment. They concluded that the application of EW combined with short-time heating improved safety of organic carrot, without negatively affecting the sensory properties (21).

To our knowledge, the present study is the first to investigate the in vitro antimicrobial activity of alginate coating combined with EW. Our findings revealed that the combined use of alginate coating and EW had stronger antimicrobial effect against *E. coli* O157: H7 compared to alginate coating and EW alone. Similar findings have been reported by studies that have applied coating solutions together with other natural antimicrobials (17, 22, 23).

CONCLUSION

The results showed that EW and alginate coating each can affect growth of *E. coli* O157: H7 on salmon fillets. However, the combined use of alginate coating and EW has significantly stronger antimicrobial effect.

Given the general tendency to use natural antimicrobials in food products, it is recommended to use an alginate coating solution with EW in salmon fillets to achieve higher safety against pathogenic bacteria such as *E. coli*.

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DECLARATIONS

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Ethics approvals and consent to participate

Not applicable.

Conflict of interest

The authors declare that there is no conflict of interest regarding publication of this article.

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