Golestan University of Medical Sciences
Jorjani Biomedicine Journal
Online ISSN: 2645-3509

Antimicrobial Effect of Chitosan Coating Prepared by Neutral Electrolyzed Water against Inoculated *Escherichia coli* O_{157}:H_7 on Rainbow trout fillets

Fatemeh Mohajer[1], Saeid Khanzadi [1*], Mohammad Hashemi[2,3], Mohammad Azizzadeh[4]

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

Article Type:

Original article

Article History:

Received: 29 May 2022
Revised: 18 Jul 2022
Accepted: 20 Jul 2022
Published: 6 Aug 2022

****Correspondence:***

Saeid Khanzadi,

Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

khanzadi@um.ac.ir

DOI: 10.29252/jorjanibiomedj.10.3.26

Abstract

Background and Objectives: Nowadays, to avoid synthetic preservatives, which do more harm than good, numerous studies are currently focused on using natural ingredients to enhance food product quality and shelf life. Since no study has been conducted on combining coatings with electrolyzed water containing natural antimicrobial compounds, the present study has innovation and priority. In the present study, the effect of chitosan coating prepared through Neutral Electrolyzed Water (NEW) on inhibiting the growth of *Escherichia coli* O157:H7 inoculated in rainbow trout fillet over 12 days at four ˚C was examined.

Material and Methods: Fish samples were allocated into six groups following inoculation with *E. coli* O_{157}:H_7 (final concentration: ~ 10^5 CFU/g). Treatments included control (CON), distilled water (DW), neutral electrolyzed water (NEW), chitosan 2% (CH), chitosan coating prepared by neutral electrolyzed water (CH/NEW), and neutral electrolyzed water followed by chitosan (NEW+CH). Treatments were kept at a low temperature (refrigerator), and counting bacteria was done on 0, 1st, 3rd, 6th, 9th, and 12th days. Data analyses were done through repeated measure ANVOA and Bonferroni post hoc tests.

Results: As indicated by the findings, the reduction rate of EW, CH, CH+WE, and CH/EW samples were 1.04, 1.45, 2.01, and 2.02 log CFU/g compared with the CON, respectively. The CH/NEW sample observed the highest reduction rate due to the antimicrobial activity of chitosan and neutral electrolyzed water.

Conclusion: Chitosan coating can be combined with NEW in fish to increase the safety against pathogenic bacteria and *E. coli* O_{157}:H_7. Therefore, it can suggest using these natural antimicrobial compounds in the food industry.

Keywords: Keywords: Food Preservation [MeSH]; *Escherichia coli* O157 [MeSH]; Oncorhynchus mykiss [MeSH]; Chitosan [MeSH]; Food Safety [MeSH]

 Publisher: Golestan University of Medical Sciences.

Highlights

- Nowadays, the application of antimicrobial agents is a good approach to increasing the shelf life of food products.
- Chitosan coating can be combined with Neutral Electrolyzed Water (NEW) in fish to increase the safety against pathogenic bacteria and E. coli O_{157}:H_7.

Introduction

Rainbow trout is among the most consumed fish species globally due to the nutrients it contains, fast growth rate, and easy cultivation (1). However, it is a quickly perishable product because it has a high content of polyunsaturated fatty acids, high water activity, enzymatic, and microbiological activities (2).

After contaminating fish and its products, the pathogenic bacteria proliferate rapidly, among which, *E. coli* O_{157}:H_7 is a bacterial agent to infecting the fish and its products (3). It has evolved from a clinical novelty to a worldwide health public concern during the past decade (4). It produces enterohemorrhagic toxins and is responsible for hemorrhagic colitis, hemolytic uremic syndrome, and death (5). *E. coli* O_{157}:H_7 is transmitted by food and water because of contamination of the surface by post-processing phases, in particular, it may be found in fish products due to wastewater contamination (6).

Using some methods is necessary to improve the shelf life and loss of quality of foods (7). Edible film and coating have been researched as beneficial for enhancing food safety (8). These provide an excellent oxygen barrier, possess appropriate mechanical attributes, and prevent biochemical decay and flavor loss (9). Edible coatings are made of lipids, proteins, and polysaccharides (10). Chitosan is a natural biopolymer made from chitin (11). The use of chitosan in biomedical, food, and chemical industries has recently increased since it offers several advantages over non-antigenic, nontoxic, bioactivity, and biofunctional (12). Chitosan coating has attracted considerable interest to its antimicrobial, antifungal, and antioxidant functions as a packaging material for food safety (13). Chitosan has offered a versatile and promising biodegradable polymer for food packaging. In addition, chitosan possesses immense potential as an antimicrobial packaging material owing to its antimicrobial activity and non-toxicity. To the best of our knowledge, there have been no reports of chitosan consumption side effects on human health (14).

Electrolyzed Water (EW) is a promising sanitizer for food products (15). It is produced from the electrolysis of dilute NaCl by introducing an electric current in water with dissolved NaCl (16). Electrolyzed water has been used widely in various sectors such as the food and medical industries due to being regarded as eco-friendly, low-cost, and easily implementable. The ability for on-site production is the main advantage of electrolyzed water. Electrolyzed water, as applied to food, has shown no adverse effects on humans or the environment (17). Three different kinds of EW can be found determined by the anode or cathode side where the product is formed (18). The produced product from the anode and cathode side is known as Acidic Electrolyzed Water (AEW) and Basic Electrolyzed Water (BEW), respectively (18). Neutral Electrolyzed Water (NEW) is made by combining the cathodic and anodic solution or with a single-cell chamber (19). NEW has shown better features than others. Because of its neutral pH, it doesn't damage human health and also doesn't cause corrosion of processing equipment; however, it is the same at reducing the microbial load (20). Several studies have investigated the use of electrolyzed water as a disinfected method in the food industry (20-22). Cap et al. (2020) reported reductions of 4 log CFU/g for *Salmonella* spp. when lettuce was dipped in electrolyzed water for 45 s (23). In another investigation, Hernández-Pimentel et al. (2020) applied neutral electrolyzed water, observing the reduced TVC of stored chicken meat (24).

Recent studies have examined using chitosan coating for meat products such as chicken meat

(Yaghoubi et al., 2021, Afshar Mehrabi et al., 2021; (25, 26)), turkey meat (Sayadi et al., 2021; (27)), roast duck (Chen et al., 2021; (28)), fish product (Yang et al., 2021, Chaparro-Hernandez et al., 2015; (29, 30)). Limited research has used electrolyzed water followed by chitosan coating to improve the shelf life of meat products (Xu et al., 2014, Luan et al., 2017, Zhou et al., 2011; (31-33)). To the best of our knowledge, no studies have expressed the protective effects of chitosan coating prepared by electrolyzed water against inoculating bacteria in meat products. Therefore, this study attempts to obtain an edible coating with chitosan and electrolyzed water and examine the antibacterial effects on inoculated *E. coli* O_{157}:H_7.

Materials and Methods

Experimental Materials

Low molecular weight (LMW; 1.03×105) chitosan was supplied by Sigma-Aldrich Company (St. Louis, MO, USA). The media cultures were obtained from Merck (Darmstadt, Germany). *E. coli* O_{157}:H_7 (NCTC 12900) was provided by the Department of Food Hygiene at the Faculty of Veterinary Medicine of Ferdowsi University of Mashhad, Iran.

Production of NEW

A NEW generator (Aquastel Balti OU, Tallinn, Estonia) was used to generate NEW. At 32 A, tap water and 25% of salt solution were pumped constantly into the generator. The pH of the NEW was equal to 6.5, and the free chlorine level was 100 ppm. To measure the pH, a pH meter was utilized (34).

Fish sample preparation and inoculation of the bacteria

The fresh rainbow trout fillets (*Oncorhynchus mykiss*) were purchased from a local market in Mashhad (Iran) and immediately transferred to the laboratory. The blood and slime of fish samples were removed by washing, and then dried. The fillets were sectioned into pieces (10±1 grams), sprayed by ethanol (70% v/v), and burnt to remove the surface microorganisms. Each sample side was separately inoculated by 50µL of bacterial suspension (final concentration: ~ 10^5 CFU/g). To count the inoculated bacteria, the volume of 10 grams of the samples was increased to 90 milliliters using 0.1% sterile peptone water. Afterward, samples were homogenized in a stomacher (Seward Medical, UK) for 2 minutes. Finally, 0.1 ml of serial dilutions of homogenates were cultured on Cefixime Tellurite Sorbitol-MacConkey agar and incubated at 37°C for 24 hours (35).

Preparation of coating solutions and treatments

Chitosan (2% w/v) was dissolved in distilled water/electrolyzed water for 10 min, followed by adding 1 mL of glacial acetic acid (1% v/v) to the mixture. Subsequently, glycerol was added to the chitosan solution at 0.75 mL/g concentration as a plasticizer and was stirred for 10 min (7).

The inoculated fish fillets were allocated to 6 categories, as shown in Table 1. The CON was kept in zipped polyethylene bags, and the treated samples were kept in the different coating solutions for 3min. The samples of all groups were drained for 30 minutes, placed in polyethylene bags, and kept at 4°C. Finally, the inoculated fillets were counted for *E. coli* O157:H7 on the 0th, 1st, 3rd, 6th, 9, and 12th days.

Table 1. Various treatments performed on rainbow trout fillets

Treatment	Description
Con	Untreated fish fillets.
DW	Fish fillets were immersed in distilled water.
NEW	Fish fillets immersed in neutral electrolyzed water.
CH	Fish fillets immersed in chitosan coating.
NEW/CH	Fish fillets immersed in chitosan coating prepared by neutral electrolyzed water.
NEW+CH	Fish fillets immersed in neutral electrolyzed water followed by chitosan coating solution.

Statistical Analysis

The experiments were conducted three times, and the collected data were analyzed in SPSS (v.21). To examine the significant differences at P<0.05, ANOVA was carried out repeatedly along with Bonferroni’s posthoc test.

Results

The *E. coli* O_{157}:H_7 growth as influenced by the treatments over the 12 days is pictured in Figure 1. The *E. coli* O_{157}:H_7 was counted at 5.50 log CFU/g in CON samples at the beginning of storage time. According to the results, the growth of *E. coli* O_{157}:H_7 decreased during the storage time in all treatments. The same findings were achieved in a previous study (36, 37).

The mean decline rate of *E. coli* O_{157}:H_7 count in different treatments is listed in Table 2. All the groups caused a significant difference in *E. coli* O_{157}:H_7 count to mean rate compared to CON ($P < 0.001$). *E. coli* O_{157}:H_7 counts reduced to 1.04, 1.45, 2.01, and 2.02 log in NEW, CH, CH+EW, and CH/EW treatments compared to CON, respectively. The maximum reduction rate belonged to the NEW/CH (2.02 log CFU/g) and NEW + CH treatments (2.01 log CFU/g) compared to the control group.

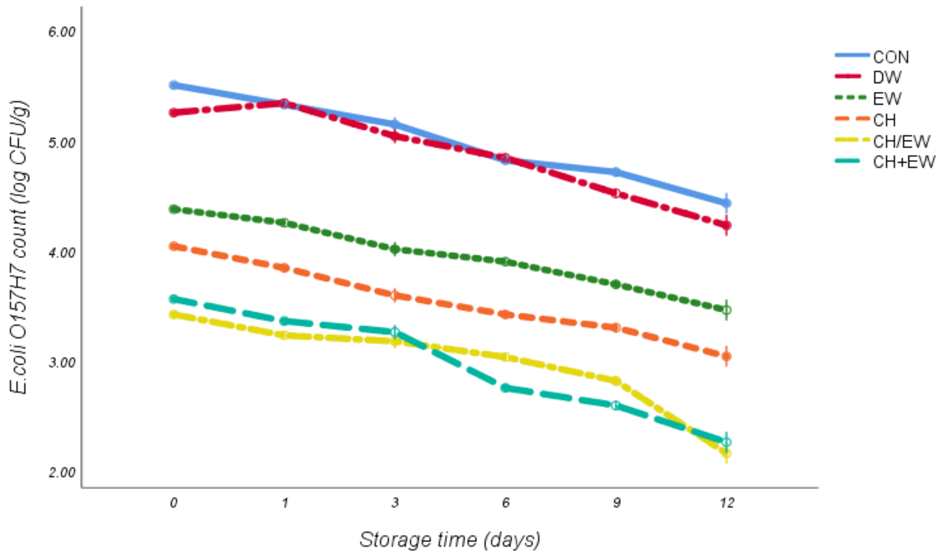

Figure 1. Changes in *E. coli* O_{157}:H_7 count (log CFU/g) of rainbow trout fillet in different treatments over 12 days at four ˚C. Data are expressed as mean ± SD (n = 3).

Table 2. The mean rate of reduction of *E. Coli* O_{157}:H_7 counts in treatments compared together in the study1.

Mean Difference (I-J)		Group (J)				
		CH/EW	EW+CH	CH	EW	DW
Group (I)	Control	2.02*	2.01*	1.45*	1.04*	0.11*
	DW	1.90*	1.89*	1.33*	0.92*	
	EW	0.98*	0.97*	0.41*		
	CH	0.57*	0.56*			
	CH + EW	0.08*				

1 Data are expressed as mean ± SD (n = 3).
*Indicates a statistically significant difference ($p < 0.05$).

Discussion

Many studies have investigated the antibacterial effect of electrolyzed water and chitosan on food products. Ogunniyi et al. (2021) found that neutral electrolyzed water could decrease microbial contamination of fresh spinach leaves (38). Ogunniyi et al. also (2021) indicated that neutral electrolyzed water proved to be an effective sanitizer for pre-harvest disinfection of minimally processed vegetables (39). In another study, Al-Nabulsi et al. (2020) reported that chitosan coating with or without ZnO nanoparticles significantly reduced the initial numbers of *E. coli* O_{157}:H_7 in white brined cheese by 2.5 and 2.8 log CFU/g, respectively, when stored at 4 °C, compared with the control group (40).

The primary bacterial count in CON exceeded those of all treated samples due to the primary effect of treatments on the growth of *E. Coli* O_{157}:H_7. This result is consistent with that of Zhou et al. (2011) who reported that combined treatment of electrolyzed water and chitosan reduced the initial total viable count significantly compared to control samples in puffer fish during refrigerated storage (33).

According to figure 1, the reduction in bacterial growth in other treatments occurs with a greater slope than CON due to the antimicrobial activity of chitosan and neutral electrolyzed water.

In the same vein, Shimamura et al. (2016) found that combination treatment with alkaline electrolyzed water and strong acidic electrolyzed water on fresh chicken breasts and beef liver significantly reduced the *Salmonella* Enteritidis, *Escherichia coli*, *Staphylococcus aureus*, and *S. aureus*, and reduction of more than 1 log colony-forming units (CFU)/g was achieved (41). Kanatt et al. (2013) also observed about 1-3 log CFU/g reduction of gram-positive and gram-negative bacteria in ready-to-cook meat products treated by chitosan coating (42).

NEW induced a bactericidal effect on most pathogenic bacteria known to man as it has active oxidizers such as HOCl, ClO−, HO2, and O2 (17). CH is affected on the outer surface of the bacterial cell, and it's positively charged interacts with the bacterial cell membranes with a negative charge. The protein leakage and constituents of the bacteria occur and finally cell agglutination happens (43). Our result shows that using combinational antimicrobial agents yielded better results in microbial growth than their separate use. Similarly, Shiroodi et al. (2021) found that NEW could decrease the *Vibrio parahaemolyticus*, *Escherichia coli* O_{157}: H_7, and *Listeria innocua* Biofilms mainly when it uses with other antimicrobial agents (44). Meanwhile, Al-Holy et al. (2015) studied the effect of acidic electrolyzed oxidizing water as a disinfectant for raw fish, chicken, and beef surfaces, and the findings indicated that EOW could decrease pathogenic microorganisms such as *L. monocytogenes* and *Salmonella Typhimurium* significantly on the surface of meat product with no effect on organoleptic properties (45). Rahman et al. (2013) also observed by about 3 logs CFU/g reduction of *Escherichia coli* O_{157}:H_7 and *Listeria monocytogenes* in fresh pork treated by low concentration electrolyzed water and calcium lactate combinedly, without changing in color (46).

Conclusion

NEW and chitosan coating could inhibit the growth rate of *E. Coli* O_{157}:H_7 by about 2 logs CFU/g at the end of storage. Fillets treated by NEW or CH in combination and individually showed significantly reduced *E. Coli* O_{157}:H_7 count. Hence, the high efficiency of the combined use of CH and NEW makes this an attractive alternative to other preservatives in the food industry. Notably, the treatment cannot guarantee the safety of refrigerated trout fillets after contamination with *E. Coli* O_{157}:H_7 at a high dose. Therefore, it is recommended to be employed in combination with other antimicrobial agents to ensure safety.

Funding source

Ferdowsi University of Mashhad, Mashhad, Iran. Grant code: 3/51856

Ethics approvals

Not applicable

Conflict of interest

The authors declare that they have no competing interests.

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How to cite:

Mohajer F, Khanzadi S, Hashemi M, Azizzadeh M. Antimicrobial Effect of Chitosan Coating Prepared by Neutral Electrolyzed Water against Inoculated *Escherichia coli* O157:H7 on Rainbow trout fillets. *Jorjani Biomedicine Journal*. 2022; 10(3):26-34.