

## **Antimicrobial Efficiency of Chitosan Coatings Containing Garlic Essential Oil on The Preservation of Rainbow Trout Fillets**

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### **Abstract**

This study objected to evaluate the antimicrobial effects of chitosan coatings enriched with garlic essential oil (GEO) in the rainbow trout fillets during cold storage. Rainbow trout fillets were treated with chitosan coating solutions with (GEO) and without (CF) garlic essential oil emulsion. One group left as control (C) without chitosan coating. All samples were stored at refrigeration for 15 days and during the storage period total psychrophilic bacteria, total mesophilic bacteria and total Enterobacteriaceae counts were determined. During the storage period the highest bacteria growth was reported in the control group and the group coated with chitosan solution. At the end of the storage, total psychrophilic bacteria count was found as 5.34, 4.71 and 4.16 log CFU/g in C, CF and GEO groups, respectively. Control group showed the highest total mesophilic bacteria count at 15th day (5.91 log CFU/g), while this value was 5.24 log CFU/g and 4.83 log CFU/g in the CF and GEO, respectively. The group coated with chitosan solutions containing garlic essential oil emulsion showed the lowest Total Enterobacteriaceae count during the storage and remained as 3.82 log CFU/g at the end of the storage. The results showed that the addition of garlic essential oil emulsion in the chitosan solutions as coating material is showed antimicrobial effect in the rainbow trout fillets during refrigerated storage.

**Keywords:** Chitosan, Rainbow trout, Garlic essential oil, Antimicrobial effect

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### **INTRODUCTION**

Fish has a very important place in human nutrition because it contains antioxidants such as proteins, vitamins, carotenoids and tocopherols, beneficial effects on human health, and long-chain omega-3 polyunsaturated fatty acids (PUFA), which are essential nutrients for growth and development. Especially eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are responsible for many beneficial effects on human health. Fish meat is also known as a high quality protein source. However, fish meat spoils very quickly due to its high moisture content, near-neutral pH value, weak connective tissue structure, and high content of unsaturated fatty acids. Especially lipid oxidation and microbial spoilage are the most important causes of spoilage during the storage of fish.

However, today, where reliability comes to the fore in the production of foodstuffs, the demands for plastic packaging materials made of synthetic materials are gradually decreasing and the use of natural additives is gaining importance both in daily life and industrially.

Recently, essential oils have been used as natural antioxidant and antimicrobial agents in edible coatings to increase the shelf life of perishable foods such as fish. However, the hydrophobic and volatile properties of essential oils and their sensitivity to oxygen and light reduce their stability during processing and storage. In addition, since essential oils can cause organoleptic deterioration in foods, effective application doses are limited in direct applications. Therefore, the use of essential oils with edible films or coatings is an alternative way. Forming emulsions with essential oils and adding them to edible coatings increase their stability and effectiveness. There are some studies on the effects of edible films and coatings on the maintenance of quality of fish and fish products (Ucak and Afreen, 2022; Renur et al., 2016; Ebadi et al., 2019; Ucak et al., 2021; Ucak, 2020; Hosseini et al., 2016; Alsaggaf et al., 2017; Ucak, 2019; Ucak et al., 2019; Shahbazi et al., 2018), however, about the chitosan coating combined with garlic essential oil on the rainbow trout fillets there is not enough study. Therefore, the main objective of this study is to prepare emulsions with garlic essential oil and to apply the chitosan coating prepared with this emulsion to fish fillets. It is objected to inhibit microbial growth in fish fillets during cold storage.

## **MATERIALS AND METHODS**

### **Materials**

Rainbow trout (*Oncorhynchus mykiss*) fillets were provided freshly from a fish market in Niğde region. Garlic essential oil was supplied commercially from a local market in Niğde.

### **Method**

#### **Chitosan coatings preparation application to fish fillets**

Chitosan coating solution was prepared according to the method of Ojagh et al. (2010). Emulsions were formed by adding the 1% concentration of garlic essential oil and the same amount of Tween 80. One of the chitosan coating solution was prepared without adding the essential oil emulsion. One group left as control without chitosan coating. Fish fillets were immersed in the prepared chitosan solutions for 30 seconds and left for 2 minutes, and then the fish fillets were immersed in the solution for a second time for 30 seconds and allowed to dry for 2 minutes (Ojagh et al., 2010). Then the samples were taken into styrofoam plates and covered with stretch film. All samples were stored at  $4^{\circ}\text{C}\pm 1$  for 15 days and microbiological analyzes were carried out at 0, 3, 5, 8, 12, and 15th days of storage period.

### **Analyzes**

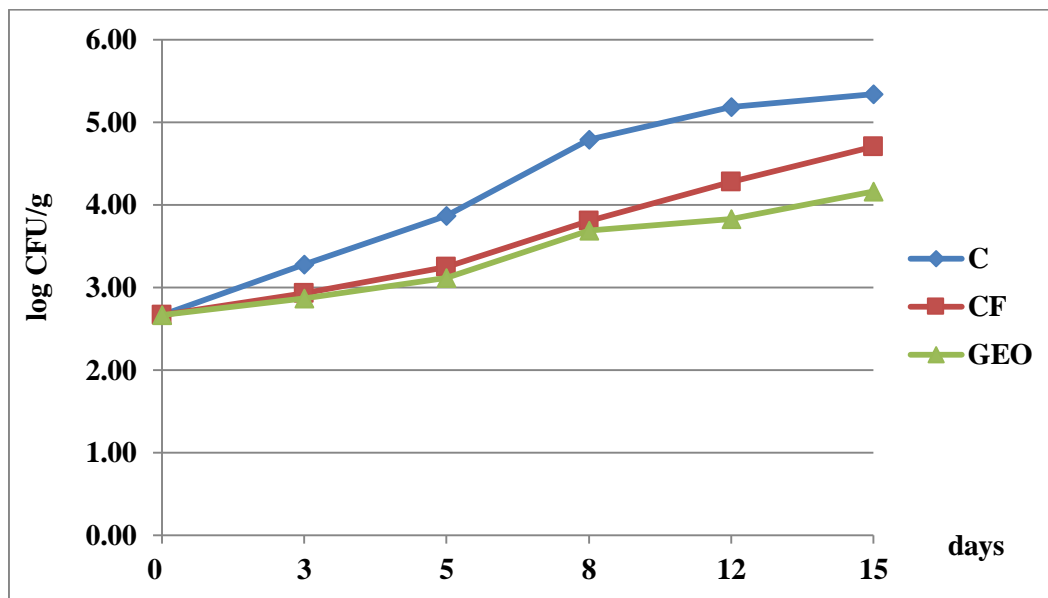
For the determination of total mesophilic and total psychrophilic bacteria counts Plate Count Agar (PCA) was used by the spread plate method (ICMSF, 1982). The plates were incubated at  $8^{\circ}\text{C}$  for 7 days for total psychrophilic bacteria counts and at  $37^{\circ}\text{C}$  for 24-48 h for total mesophilic bacteria counts, respectively. Total Enterobacteriaceae were enumerated according to the method of FDA (1998) by the use of Violet Red Bile Agar (VRBA). Pour plating method was performed incubating at  $37^{\circ}\text{C}$  for 36-48 h.

## Statistical analysis

Statistical analyzes were performed with SPSS software (Statistical Analysis System, Cary, NC, USA) and Duncan multiple comparison test (One-way Anova at  $P < 0.05$  significance level) were applied to compare the data obtained.

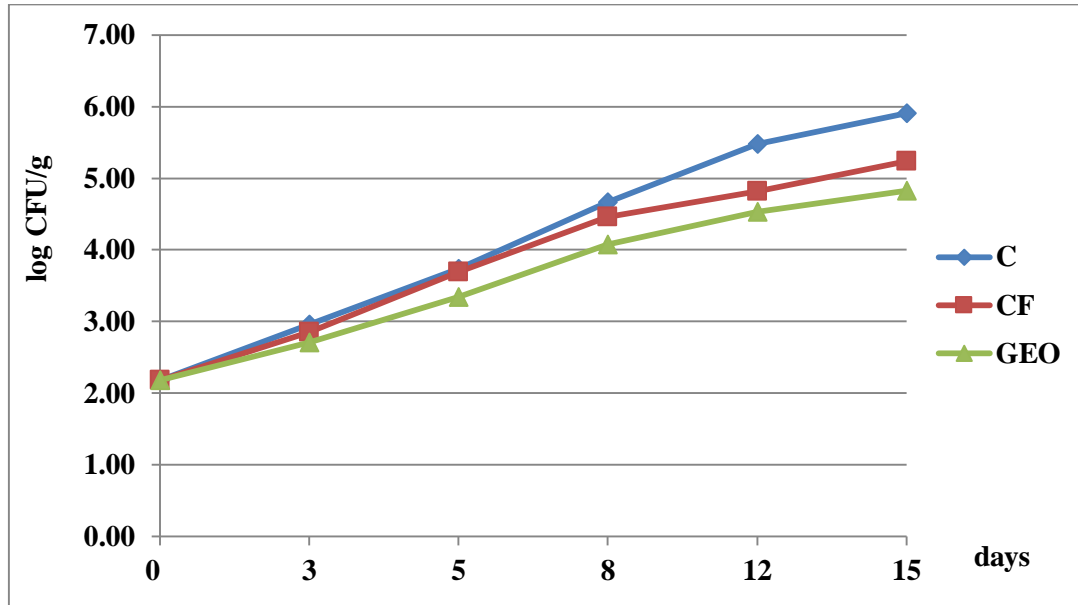
## RESULTS AND DISCUSSION

Bacterial growth is one of the main causes of spoilage of fish and fish products. The effect of chitosan coating enriched with garlic essential oil emulsion on the total psychrophilic bacteria growth in the rainbow trout fillets is presented in Fig. 1. The initial total psychrophilic bacteria count was determined as 2.67 log CFU/g in the trout fillets. During the storage period, this value showed increase in all samples while reached the highest value in the control group at the end of the storage (5.34 log CFU/g). The group coated with chitosan solutions incorporated with garlic essential oil emulsion showed the lowest values along the storage and found as 4.16 log CFU/g at the end of the storage.



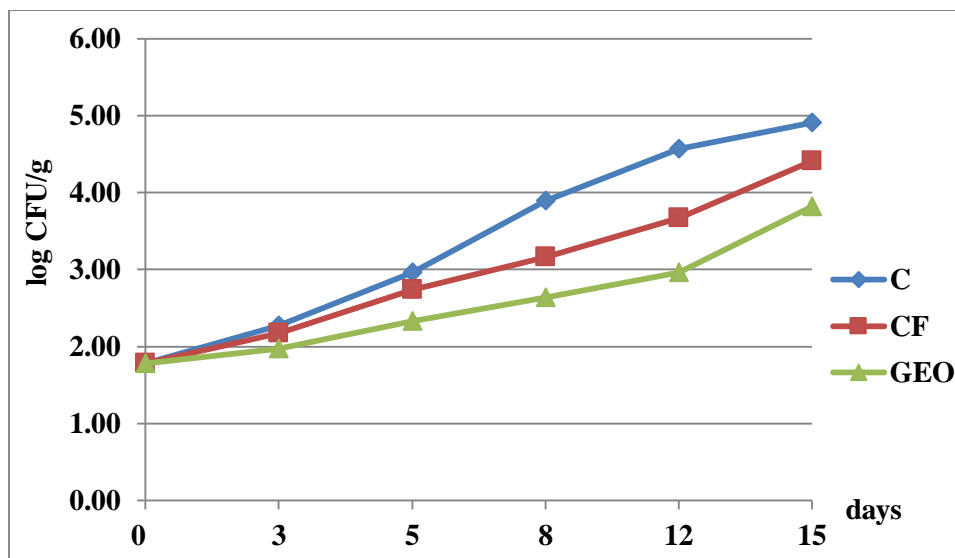
**Figure 1.** Changes in total psychrophilic bacteria count of rainbow trout fillets during storage. C: Control without chitosan coating, CF: Control coated with chitosan coating, GEO: fillets coated with chitosan coating enriched with garlic essential oil emulsion.

Total mesophilic bacteria count was 2.18 log CFU/g in the rainbow trout fillets at the beginning. This value increased in all groups until at the end of the storage. The highest total mesophilic bacteria count was found in the control group at 15<sup>th</sup> day as 5.91 log CFU/g, while this value was observed as 5.24 log CFU/g and 4.83 log CFU/g in the group coated with only chitosan and the group coated with chitosan containing garlic essential oil, respectively.



**Figure 2.** Changes in total mesophilic bacteria count of rainbow trout fillets during storage. C: Control without chitosan coating, CF: Control coated with chitosan coating, GEO: fillets coated with chitosan coating enriched with garlic essential oil emulsion.

The total number of coliform bacteria is accepted as an indicator of hygiene in fish. Total Enterobacteriaceae count of the rainbow trout fillets was found as 1.78 log CFU/g at the beginning of the storage (Fig. 3). In the control group this value reached at 4.91 log CFU/g, while it was determined as lower (4.41 log CFU/g) in the group coated only with chitosan coating. The group coated with chitosan solutions containing garlic essential oil emulsion showed the lowest total Enterobacteriaceae count during the storage and remained as 3.82 log CFU/g at the end of the storage.



**Figure 3.** Changes in total Enterobacteriaceae count of rainbow trout fillets during storage. C: Control without chitosan coating, CF: Control coated with chitosan coating, GEO: fillets coated with chitosan coating enriched with garlic essential oil emulsion.

Natural extracts are effective on total coliform bacteria growth in fish and fish products (Uçak et al., 2018; Frangos et al., 2010; Mexis et al., 2009). According to the study by Ucak et al. (2018), the initial total viable count of trout fillets were found to be 1.48 log CFU/g, similarly, Öz (2018) found the total viable count of trout fillets as 2.80 log CFU/g. In another study the total bacteria count of the trout meatballs prepared with laurel and rosemary essential oils was found higher (5.24 log CFU/g) than the present study (Keser and İzci, 2020). They also reported that essential oils of laurel and rosemary inhibited the bacteria growth. Keser and İzci (2020) found the initial psychrophilic bacteria count of trout meat as 4.22 log CFU/g.

Ucak (2019) found the total viable count as 2.27 log CFU/g in trout fillets coated with gelatin films prepared with garlic peel extract and reported that bacterial growth was slower in the fillets coated with films containing garlic peel extract. Similarly, Jouki et al. (2014) reported that the microbial growth was lower in trout fillets coated with chitosan films prepared with thyme essential oil compared to the control group.

## **CONCLUSION**

Based on these findings of this study, it can be concluded that the addition of garlic essential oil emulsion in the chitosan coating can inhibit the microbial growth in the rainbow trout fillets during refrigerated storage for 15 days. Therefore, garlic essential oil can be recommended as natural antimicrobial additives with chitosan coating to enhance the microbiological quality of rainbow trout fillets.

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