Effect of Chitosan Coating Enriched with Peppermint Essential Oil Emulsion on the Microbiological Quality of Fish Meatballs

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Abstract

In this study, the effects of chitosan coating incorporated with peppermint essential oil (PMO) emulsion on the microbiological quality of fish meatballs were determined. For this purpose, chitosan coating solutions were prepared with (PMO) and without (CF) 1% concentration of peppermint essential oil emulsion. Fish meatballs were coated with these chitosan solutions and stored at 4±1°C for 18 days. One group left as control (C) without chitosan coating. Total psychrophilic bacteria (TPB), total mesophilic bacteria (TMB), total Enterobacteriaceae and total lactic acid bacteria (LAB) were evaluated. According to the results of study, the TPB count of the C and CF groups were reported as 7.59 and 7.15 log CFU/g at the end of storage, while it was found as 6.14 log CFU/g in the PMO group. Total mesophilic bacteria count of fish meatballs coated with only chitosan was determined as 5.93 log CFU/g at the end of the storage and the lowest (5.18 log CFU/g) TMB count was observed in the fish meatballs treated with chitosan coating enriched with peppermint oil emulsion. The highest LAB counts were determined in C and CF groups throughout the storage period. At the end of the storage, PMO group showed the lowest LAB count as 4.54 log CFU/g. The results of the study revealed that the usage of peppermint essential oil emulsion in the chitosan coating is an effective way to inhibit microbial growth in the fish meatballs during the cold storage.

Keywords: chitosan coating, fish meatball, microbial quality, mackerel, peppermint oil, LAB

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INTRODUCTION

In recent years, the increase in the number of working women and people living alone in search of durable and easy-to-serve food causes ready-made food technology to gain increasing importance (Kılınççeker et al., 2009). The production and consumption of products such as fish burgers, fish balls and fish croquettes are spreading rapidly in the ready-to-eat food sector, and these products are among the ready-to-eat foods that are loved and consumed. Along with the technological developments in the world, important technological developments have been recorded in the fish processing sector in our country as well. Studies show that storage at temperatures below -12°C inhibits microbial growth and slows down enzymatic activity (Rodriguez-Turienzo et al., 2011). However, cooling or freezing alone is not sufficient to completely inhibit lipid oxidation, protein denaturation and microbial activity. For this reason, it has become common today to use more than one processing and packaging technology together.

Packaging technology is widely used to extend shelf life, maintain hygiene and maintain quality, especially in foods sensitive to microbial and oxidative spoilage (Ahmad et al., 2012).

Due to the increasing consumer awareness and sensitivity to the environment recently, there is pressure to avoid the use of synthetic substances and there is an increasing trend towards the use of substances obtained from natural sources. Many studies show that edible coatings made of protein, polysaccharide and lipid-containing materials help extend the shelf life of foods and maintain their edible quality qualifications (Kılınççeker et al., 2009). The most widely used biopolymers are protein and polysaccharides. Chitosan is a natural and cationic polyaminosaccharide obtained from different degrees of alkaline deacetylation of chitin. It is one of the most abundant polysaccharides in nature, and its use as a coating material is very convenient and common.

The use of edible coatings with plant extracts, essential oils and antioxidants can provide some benefits such as improving the organoleptic and nutritional properties of the product they are applied to (Bourtoom, 2008; Falguera et al., 2011). 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. While many studies have been conducted on the effects of edible films and coatings on the maintenance of quality of fish and fish products (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), there is no enough information about the chitosan coating supplemented with peppermint essential oil on the quality of mackerel meat balls. Therefore, in this study, it is aimed to create emulsions with peppermint essential oil and to apply the chitosan coating prepared with this emulsion to fish meatballs. It is aimed to preserve the microbiological quality of fish meatballs coated with chitosan during cold storage.

MATERIALS AND METHODS

Materials

Mackerel fillets were supplied fresh from the fishermen in the Niğde region. They were placed in styrofoam boxes together with ice and delivered to the laboratory as soon as possible. The fish were cleaned quickly; the internal organs and bones were removed and grounded into minced meat. Peppermint oil was supplied commercially from a local market in Niğde.

Method

Fish meatballs preparation

Minced fish was placed in a bowl and mixed in a homogeneous way by adding weighed substances in certain sizes. Each meatball was prepared as 30 gr and consist of 83.75% minced fish meat and 16.25% other additives (10% breadcrumbs, 2% salt, 0.5% cumin, 0.5% sweet red pepper, 0.5% allspice, 0.5% ground black pepper, % 1 sunflower oil, 1.25% garlic powder) (Keser, 2019).

Preparation of chitosan coatings

Chitosan coating solution was prepared acording to the method of Ojagh et al. (2010). One of the chitosan coating solution was prepared without adding the essential oil emulsion.

Fish meatballs were immersed in the prepared chitosan solutions for 30 seconds and left for 2 minutes, then the fish meatballs 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. The control group was not coated with any chitosan solution. All samples were stored at 4^{o}C±1 for 18 days and quality analyzes were carried out periodically every 3 days.

Microbiological 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^{o}C for 7 days for total psychrophilic bacteria counts and at 37^{o}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^{o}C for 36-48 h. For the enumeration of lactic acid bacteria, spread plate method was used on Man Rogosa and Shape (MRS Agar) agar. Petri dishes were then incubated in anaerobic jars at 30^{o}C for 48 hours.

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

One of the main causes of spoilage of fish and fish products is bacterial growth. The effect of chitosan coating on the microbial quality of fish meatballs is given in Fig. 1. Total psychrophilic bacteria (TPB) count was determined as 2,94 log CFU/g at the beginning of storage and increased in all groups during the storage period. It was observed that the number of TPB in fish meatballs coated with chitosan supplemented with 1% peppermint oil emulsion was significantly ($P<0.05$) lower than the C and CF groups during storage.

While the TPB count of the C and CF groups were 7.59 and 7.15 log CFU/g on the 18th day of storage, it was 6.14 log CFU/g in the PMO group. The lowest TPB count values during storage were observed in fish meatballs samples coated with chitosan supplemented with 1% peppermint oil emulsion. In a study in which rosemary extract was added at different concentrations (0.4% and 0.8%) to mackerel burgers, it was reported that the total number of bacteria during storage was lower than the control group (Ucak et al., 2011). Keser and Izci (2020) found the initial psychrophilic bacteria count as 4.22 log CFU/g in trout meat. In the study where the number of psychrophilic bacteria in fish burgers prepared with different antioxidants (thyme, sage, laurel, green tea) was found to be 4.90 log CFU/g at the beginning, it was reported that the number of TPB in the groups to which antioxidant was added during the frozen storage was lower than the control group (Özoğul and Uçar, 2013).

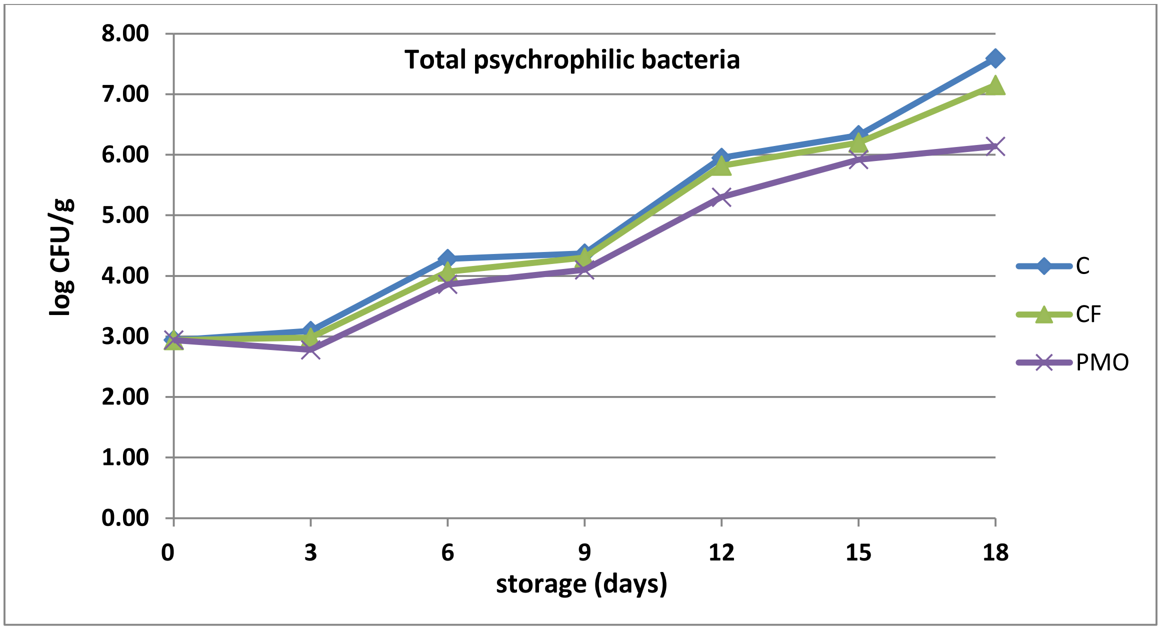

Figure 1. Changes in total psychrophilic bacteria count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion during storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

The changes of total mesophilic bacteria (TMB) count of mackerel meatballs coated with chitosan are presented in Fig. 2. At the beginning of the storage TMB count of mackerel was found to be 2.32 log CFU/g. This value was increased in all groups throughout the storage period and showed the highest value as 6.24 log CFU/g in control group at the end of the storage.

Total mesophilic bacteria count of fish meatballs coated with only chitosan was determined as 5.93 log CFU/g at the end of the storage period, whereas the lowest (5.18 log CFU/g) TMB count was observed in the fish meatballs treated with chitosan coating enriched with peppermint oil emulsion.

In the study conducted by Keser and İzci (2020), the total bacterial count of the trout meatballs prepared with laurel and rosemary essential oils was found to be considerably higher (5.24 log CFU/g) than the current study. They also reported that essential oils of laurel and rosemary slowed the growth of bacteria. Ucak (2020) reported the initial viable count of trout burgers as 2.92 log CFU/g.

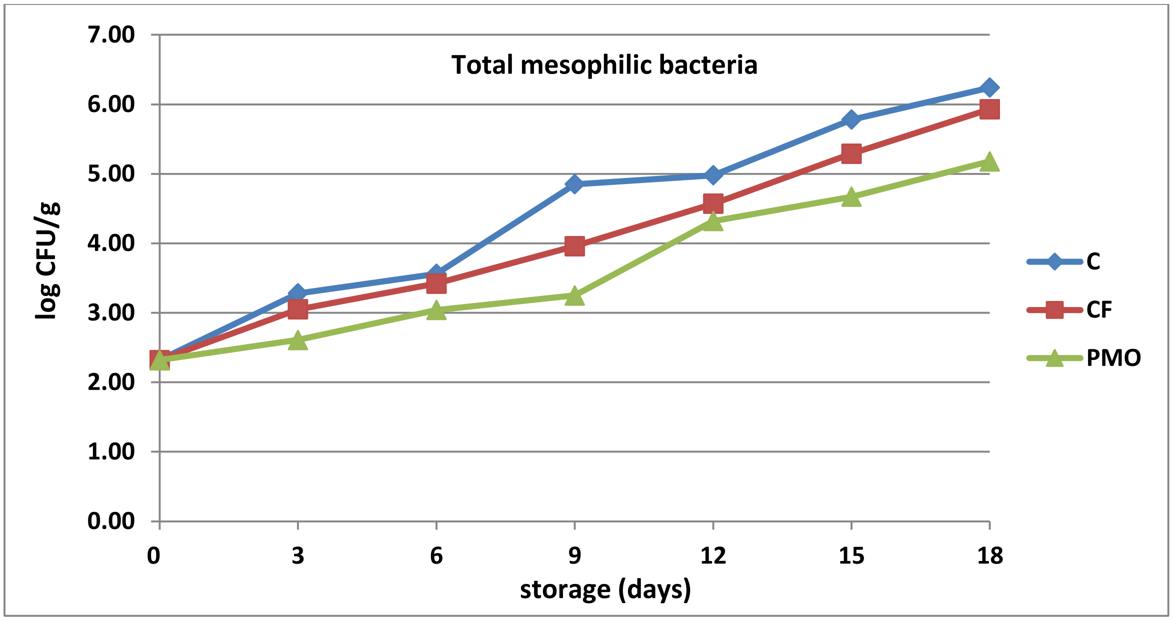

Figure 2. Changes in total mesophilic bacteria count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

Lactic acid bacteria (LAB), which are optional anaerobes, were reported as the part of the natural microbiota of fish fillets. The impacts of chitosan coating on the lactic acid bacteria (LAB) count of fish meatballs were given in the Fig. 3.

The initial LAB count of mackerel was determined as 2.11 log CFU/g and increased in all groups until at the end of the storage. The highest values were observed in control and CF groups, respectively during the storage period. Lactic acid bacteria count of control group was 5.64 log CFU/g at the end of the storage, while this value was 5.49 log CFU/g in the CF group. At the end of the storage, PMO group showed the lowest LAB count as 4.54 log CFU/g. According to Fernandez et al. (2012) LAB, which are gram-positive bacteria, are susceptible to essential oils.

Cai et al. (2014) reported that the LAB counts increased in the sea bass fillets during the storage period, but was low since LAB group grow slowly at refrigeration temperatures. In another study, the initial LAB value of rainbow trout fillets were found as 3.08 log CFU/g and increased throughout the storage period (Agdar GhareAghaji et al., 2021).

Control group showed higher LAB values than the fillets treated with edible coating containing orange peel essential oil. In the present study, control and chitosan coating treated groups also showed higher values than the group coated with chitosan containing peppermint essential oil emulsion.

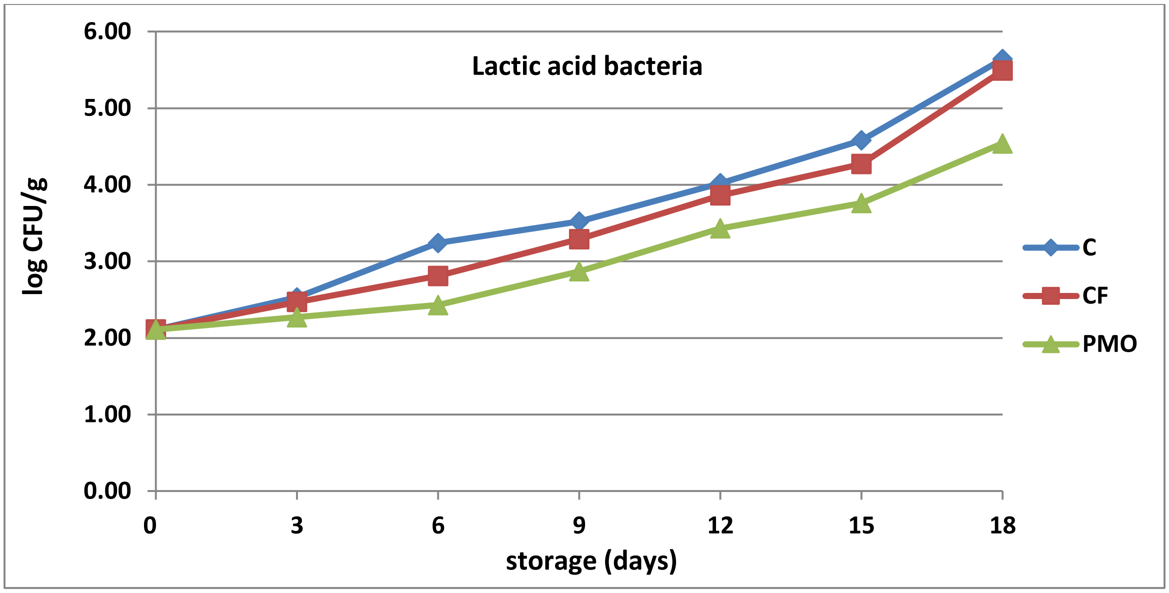

Figure 3. Changes in total lactic acid bacteria count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion during storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

Enterobacteriaceae group is considered as an indicator microorganism which is a part of aquaculture microflora and marine products Total Enterobacteriaceae changes of fish meatballs coated with chitosan solution enriched with peppermint oil were presented in Fig. 4. The initial total Enterobacteriaceae value was found as 2.35 log CFU/g in mackerel.

Total Enterobacteriaceae is considered the indicator of hygiene in the fish and fish products. The highest values were determined in control group during the storage period, while CF group showed lower values than the control.

Fish meatballs coated with chitosan enriched with peppermint oil emulsion had lowest total total Enterobacteriaceae values during the storage period. At the end of the storage, total Enterobacteriaceae values were 5.67, 5.46 and 4.34 log CFU/g in C, CF and PMO groups, respectively.

Rezaeifar et al. (2020) reported that the chitosan edible coating enriched with lemon verbena extract and essential oil inhibited the total Enterobacteriaceae growth in trout fillets. Similarly, Chamanara et al. (2012) found that total Enterobacteriaceae in the control sample are higher than other treated samples with chitosan incorporated with jujube extract.

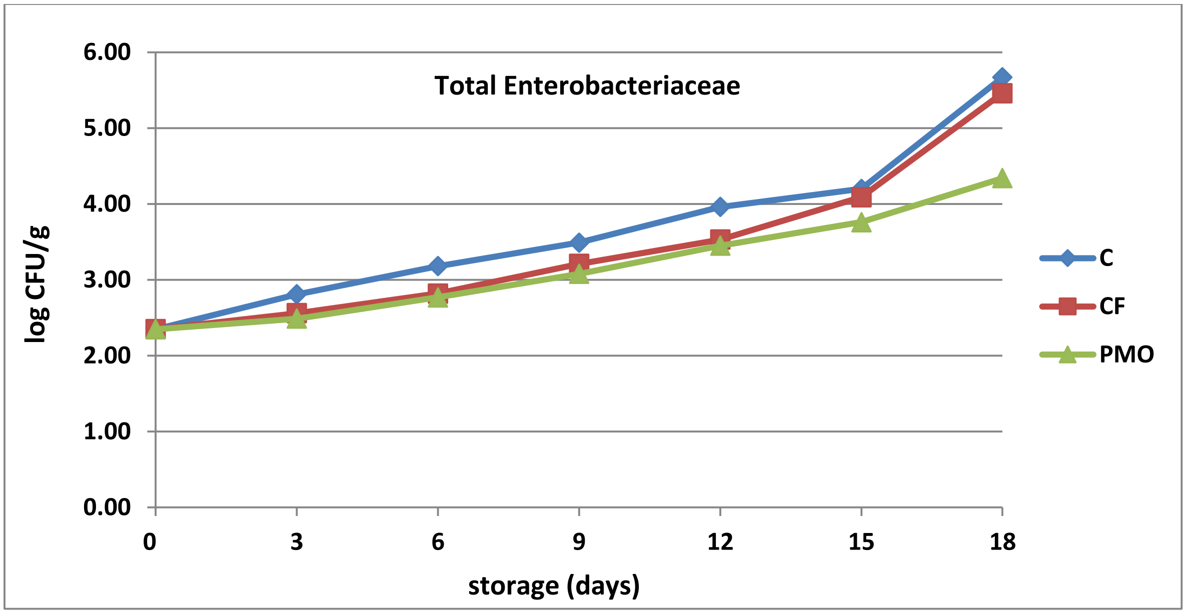

Figure 4. Changes in total Enterobacteriaceae count of fish meatballs coated with chitosan enriched with peppermint essential oil emulsion during storage at 4°C. C: Control without gelatin film, CF: fish meatballs coated with chitosan, PMO: fish meatballs coated with chitosan enriched with 1% peppermint essential oil emulsion.

CONCLUSION

After evaluation of the impact of the incorporation of peppermint essential oil emulsion with chitosan coating for the maintenance of microbiological quality of fish meatballs it can be concluded that the addition of peppermint essential oil emulsion in the chitosan coating inhibited the bacteria growth in the mackerel meatballs.

During the 18 days storage period, fish meatballs coated with chitosan enriched with peppermint essential oil emulsion showed the lowest total psychrophilic bacteria, total mesophilic bacteria, total lactic acid bacteria and total Enterobacteriaceae counts. Besides, these values did not excided the limit values in PMO group throughout the storage period.

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