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Effects of Active Packaging Based on Chitosan Biopolymer Coating Enriched with *Zataria multiflora Essential* Oil on Shelf Life of Silver Carp during Chilled Storage

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ABSTRACT

Introduction: This study examined the impact of a chitosan coating with Zataria multiflora Boiss essential oil (ZMEO) as an antioxidant and antibacterial agent on the quality and shelf life of silver carp fillets when stored in the refrigerator.

Methods: The ZMEO-enriched chitosan coating was prepared at concentrations of 0%, 0.3%, 0.45% and 0.6%. After packaging the filet sample, the antioxidant and antibacterial properties were evaluated and analyzed after 0, 1, 3, 5, 7, 9, 11, 13, and 15 days at 4 ◦C temperature.

Results: At day 15, the chitosan coating + 0.6% ZMEO resulted in considerably reduced numbers of TVC (total viable counts), EBC (Enterobacteriaceae), and LAB (lactic acid bacteria) compared to other treatments ($p < 0.05$). The log CFU/g counts for TVC, EBC, and LAB were 4.78, 5.15, and 4.78, respectively. The application of chitosan coating + 0.6% ZMEO effectively suppressed the increase in thiobarbituric acid (TBA), total volatile elemental nitrogen (TVB-N), peroxide value (PV), and pH in the silver carp fillets. The findings showed that applying a chitosan coating with ZMEO can prolong the nutritional value of silver carp fillets when stored in refrigerated circumstances for a maximum of 15 days, without causing any negative changes in taste, smell, or texture.

Conclusions: In summary, our work has demonstrated that a chitosan coating enhanced with ZMEO has the potential to effectively prolong the shelf life of silver carp fillets when stored in refrigerated conditions.

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Introduction

The production and consumption of silver carp (Hypophthalmicthys molitrix) has increased in many countries in recent decades. The silver carp holds significant importance in the polyculture system of eastern countries.

Whole fresh fish and fillets are the main forms sold in the markets (1). Fish meat is highly perishable both microbiologically and chemically due to its high nutritional value, aw and pH (2, 3). The primary obstacle faced by the seafood sector is to prolong the shelf life and enhance the overall acceptability of fish meat by inhibiting lipid oxidation and avoiding microbial proliferation (4). Edible coatings in active packaging are a potential technology that integrates food, packaging, and preservation into a unified idea. This method utilizes biopolymers obtained from by-products of the food industry or underutilized sources of lipids, polysaccharides, or proteins to develop an efficient system that maintains food quality during its storage period. Edible coatings are thin films, either in solid or liquid form that are applied to food. They serve to prolong the shelf life and enhance the quality of food by influencing characteristics such as diffusibility, permeability, and trait retention (5).

Numerous studies have demonstrated the antibacterial, antioxidant, antiviral and antifungal properties of essential oils extracted from various parts of plants. The properties of an essential oil are determined by the number of phenolic chemicals it contains, including eugenol, thymol, and carvacrol (6).

Zataria multiflora, sometimes known as Shirazi thyme, belongs to the Lamiaceae family.

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It is widely used in Iran as a flavoring ingredient in many types of food and for some medicinal purposes. The essential oil of this plant is rich in carvacrol and thymol. Carvacrol and thymol are the primary phenolic molecules that contribute to the antibacterial and antioxidant properties of ZMEO (7). The US FDA classifies phenolic chemicals, including carvacrol and thymol, as GRAS (generally regarded as safe) (6).

Some studies have shown that edible coatings of seafood with chitosan infused with antimicrobial or antioxidant agents help prolong the duration of freshness for these products (1).

The purpose of this study was to investigate the impact of combining chitosan with ZMEO on the longevity of fresh silver carp fillets while stored at a temperature of 4ºC.

Materials and Methods

Zataria Multiflora Boiss. Extraction of the Essential Oil

ZMEO was obtained from Fars Province, Iran, and authenticated by the Institute of Medicinal Plants, Tehran University of Medical Sciences, Iran. The plant portions that had been dried in the air were subjected to steam distillation for a duration of three hours using a Clevenger-type equipment (7). The components of the ZMEO were analyzed using gas chromatography-mass spectrometry [GC-MS], following the method published by Moosavy et al (8).

Preparation of the Chitosan Coating Solution

The chitosan solution with a high molecular weight was made in the following manner: A solution was prepared by dissolving 2 grams of chitosan in 100 cubic centimeters of 1% acetic acid (volume/volume) and stirring it for three hours at room temperature. A plasticizer, glycerol, was introduced to the solution at a rate of 0.75 ml per gram of chitosan weight. The mixture was then agitated for a duration of 10 minutes. In order to exclude solid particles that had not dissolved, the chitosan coating solution was passed through a Whatman No.3 filter paper. The chitosan solution was supplemented with Tween 80 at a concentration of 0.25% v/v, along with different concentrations of ZMEO (0%, 0.3%, 0.45%, and 0.6% v/v). The solution was uniformly mixed under sterile conditions at a speed of 21600 revolutions per minute for a duration of 1 minute (9).

Preparation and Processing of the Fish Samples

Silver carp (Hypophthalmichthys molitrix) were captured in a fish farm in Tehran Province, with an average weight of 1000 grams and an average length of 350 millimeters. The fish were transported to the meat science laboratory of the Food Hygiene Department at Tehran University and subsequently divided into square pieces weighing approximately 60 grams each. The fat and protein content percentages of three fillets were analyzed using the Association of Analytical Communities (10) method.

The fillets were immersed for 30 seconds in 500 cc of different coating solutions, including A (control samples, distilled water), B (chitosan), C (chitosan enriched with 0.3% ZMEO), D (chitosan enriched with 0.45% ZMEO) and E (chitosan enriched with 0.6% ZMEO). The fish fillets were extracted and left to drain for 30 minutes at a temperature of 25°C in a microbiological hood to create the coatings. Subsequently, they were stored at a temperature of 4±1°C (9).

The shelf life of the fish was determined by conducting microbiological, chemical, and sensory investigations at eight specific time intervals (0, 1, 3, 5, 7, 9, 11, 13, and 15 days).

Bacteriological Examination

Bacteriological values were assessed by combining a 10 g sample of each treatment with 90 cc of 0.1% peptone water in sterile bags and homogenizing the mixture using a stomacher for 2 minutes. Subsequent decimal dilutions were made and subsequently plated onto the suitable medium. The TVC was calculated using the surface plate method and the plate counting agar PCA. The plates that were treated with a little amount of a substance to prevent the growth of microorganisms were thereafter placed in a controlled environment at a temperature of 30°C for a duration of three days (11, 12).

The enumeration of Enterobacteriaceae (EBC) was conducted on violet red bile glucose agar (VRBGA) using the pour plate method. The plates were subsequently infected at a temperature of 25 ºC for a duration of 48 hours. The enumeration of lactic acid bacteria (LAB) was performed on de Man Rogosa Sharpe Agar (MRS) through incubation at a temperature of 30 ºC for a period of 2-3 days (12). The microbiological data were transformed into logarithmic values

representing the number of colony-forming units (CFU/g).

Chemical Analyses

Protein and Lipid Content

The Kjeldahl technique was used to determine the total crude protein. This technique employs a three-step process to measure the amount of protein: digestion, distillation, and titration. The organic substance is digested by employing concentrated H2SO4, heat, K2SO4 (to elevate the boiling point), and a catalyst (such as selenium) to expedite the reaction. The lipid content of the fillets was evaluated using the method developed by the Fraunhofer Institute for Molecular Biology and Applied Ecology (10). The lipids were extracted from the fillets by homogenizing them in a mixture of chloroform and methanol (at a ratio of 2:1, volume to volume). The amount of lipids was then determined by measuring the weight of the extracted lipids, and this process was repeated three times for accuracy.

Measurement of the Total Amount of Nitrogen in a Sample That Exists in a Volatile Form (TVB_N)

The fish samples were analyzed for their total volatile basic nitrogen (TVB-N) content using the method previously outlined by Jouki et al (13). The fish muscles were blended in 200 cc of a 7.5% water-based solution of TCA (trichloroacetic acid). The homogenate underwent centrifugation at a force of 400 times the acceleration due to gravity for a duration of 5 minutes. The resulting liquid above the sediment was then passed through a Buchner funnel equipped with a Whatman filter paper No. 3. The process of steam distillation was conducted using a distiller of the Kjeldahl type. Twenty-five milliliters of the filtrate were introduced into the distillation tube, and then 5 ml of a 10% NaOH solution were added. A 10 ml beaker containing a 4% aqueous solution of boric acid and 0.04 ml of methyl red and bromocresol green indicator was positioned near the condenser's endpoint for the ammonia titration. The distillation process was carried out until the volume in the beaker reached a final measurement of 50 ml, with 40 ml of distillate collected. The boric acid solution underwent a color change to green when it was made alkaline by the addition of distilled TVBN. The titration was performed using a 0.01 ml microburette filled with a 0.1 N aqueous solution of sulphuric acid.

Determination of the Peroxide Value (PV)

The peroxide content was quantified in the whole lipid extracts using Pearson's technique (14). A total of 0.3 grams of the sample was carefully deposited into a stoppered flask with a capacity of 250 milliliters. The solvent was eliminated using a rotary evaporator under reduced pressure at a temperature of 40°C (equivalent to the temperature of a water bath). A volume of 10 ml of the acetic acid/chloroform combination with a ratio of 3/2 was added, and the lipids were dissolved through agitation. Next, 1 milliliter of a concentrated chlorine solution was added and the mixture was left undisturbed in a dark environment for a duration of 5 minutes. Dispense 20 milliliters of distilled water and agitate. Perform a titration of the liberated iodine using a 0.01 N sodium thiosulfate solution until the solution turns a pale yellow color. Introduce 1 milliliter of a 1.5% starch solution into the mixture as an indicator, and continue the titration process until the solution becomes colorless. Conduct blank tests in a same manner, excluding any fats. Utilize a conventional formula to denote the peroxide value.

Quantification of Thiobarbituric Acid Reactive Substances (TBARS)

The thiobarbituric acid value was measured using calorimetry, following the method described by Porkony and Dieffenbacher, as outlined by Kirk and Sawyer (15). The biological sample underwent acid digestion and was subsequently separated using targeted reflux distillation. The chemical substances present in the distillate were determined by spectrophotometry measurements at a wavelength of 538 nm, following their interaction with 2-thiobarbituric acid.

Sensory Evaluation

An acceptance test was conducted on fried fish fillet samples to analyze the sensory effects obtained from them. A panel test was conducted to evaluate the fish fillets from each treatment of chitosan enriched with varying concentrations of ZMEO (0, 0.3, 0.45, and 0.6% v/v) and a control group. The panel consisted of 50 untrained judges who were members of the Faculty of Veterinary Medicine at the University of Tehran. The panelists assessed the samples by assigning ratings on a 9-point scale, where 9 indicates a remarkable liking, 8 indicates a strong liking, 7

indicates a moderate liking, 6 indicates a slight liking, 5 indicates neither liking nor disliking, 4 indicates a slight disliking, 3 indicates a moderate disliking, 2 indicates a strong disliking, and 1 indicates an intense disliking. These ratings were given for different attributes such as appearance, color, odor, and flavor.

Statistical Analysis

The statistical analysis was conducted using SPSS 16. The data underwent analysis using one-way ANOVA, followed by Turkey's multiple comparison test. The values are presented as the mean together with their corresponding standard deviation for all outcomes. Statistical significance was determined at a p-value of less than 0.05.

Results

ZMEO's Chemical Components

The gas chromatography-mass spectrometry examination revealed that the major constituents of the ZMEO were carvacrol (73.65%), thymol (4.31%), Trans-caryophyllene (2.93%), and Gamma-terpinene (2.27%). The remaining chemicals included linalool (1.28%), thymol methyl ether (0.62%), carvacrol terpinene-4-ol (1.7%), and 1, 8-cineole (0.52%).

Protein and Lipid Content

Protein and lipid contents were measured 13.97 ± 0.59 % and 5.12 ± 0.48 %, respectively.

Bacteriological Changes

The alterations in bacterial composition (total viable count, Enterobacteriaceae count, and lactic acid bacteria count) of silver carp fillets treated with various concentrations of ZMEO (0, 0.3, 0.45, and 0.6 % v/v) throughout a 15-day period of refrigerated storage are depicted in Figure 5-7.

Figure 5 displays the variations in TVC (Total Viable Count) of silver carp fillets for each treatment. The initial total viable counts of fresh silver carp fillets were 2.97 ± 0.02 log cfu/g. The total volatile compounds (TVC) exhibited an increase in all treatments when stored at a temperature of 4ºC. The count of mesophilic bacteria in the treated samples was considerably lower ($P<0.05$) compared to the control group. The total viable count (TVC) of microorganisms in the control sample of fresh fish reached a value of 7 log cfu/g, which is the upper limit set by Salam (12), between days 13 to 15. The use of chitosan enriched with ZMEO (at concentrations of 0, 0.3, 0.45, and 0.6 %v/v) as a coating for silver carp fillets led to an increase in the fillet's shelf-life.

According to Figure 6, the initial count of Enterobacteriaceae (EBC) was 2.49 log cfu/gr. The treated samples had a significantly lower EBC count compared to the control sample, with a p-value of less than 0.5.

The initial count of lactic acid bacteria (LAB) in the silver carp fillets was 2.58 log cfu/g on the first day of the investigation (Figure 7). The application of chitosan with varying quantities of essential oils resulted in a significant reduction in the count of lactic acid bacteria ($P<0.05$).

Thiobarbituric Acid Value

As shown in Figure 1, the initial TBA value of silver carp fillets were 0.12 ± 0.20 and increased to 0.82 for control samples by the end of the storage period. By contrast, the value reached 0.43 for samples that were treated with chitosan containing 0.6% ZMEO.

Total Volatile Elemental Nitrogen (TVB-N)

Figure 2 displays the variations in TVB-N levels in silver carp fillets. The initial TVB-N value of the samples was 7 ± 0.39 mg of nitrogen per 100 grams. The TVB-N values exhibited a progressive increase throughout the investigation for all treatments, eventually reaching 41.25 and 26.87 mg nitrogen/100 g for the control group and the samples coated with chitosan integrated by 0.6% ZMEO, respectively.

The total volatile base nitrogen (TVB-N) is utilized to ascertain the existence of nitrogenous substances produced by proteolytic bacteria (16).

The TVN (Total Volatile Nitrogen) of the treated samples exhibited a decreased value compared to the control samples. The antimicrobial activity of essential oil and chitosan may be the cause. This finding is consistent with the results of prior research studies (1, 9, 13).

Peroxide Value (PV)

The PV value index is a useful tool for quantifying the extent of primary lipid oxidation in seafood. Figure 3 displays the PV values data. The peroxide value (PV) of the samples was 0.97 milliequivalents of oxygen per kilogram (meq O2/kg) on day 0. The study period revealed a decrease in PV values in samples that were coated with chitosan containing ZMEO, as shown in figure 3. The PV values exhibited a substantial rise ($P<0.05$) as the storage time at 4ºC

progressed for all treatments. There was a statistically significant decrease (P<0.05) in the PV value of the treated samples compared to the untreated samples during the storage period at 4ºC.

The study shown that the use of chitosan supplemented with ZMEO effectively delays the occurrence of primary lipid oxidation. This finding is consistent with the findings of Ojagh et al (9) and Mexis et al (18). ZMEO's substantial protective impact can be attributed to its carvacrol and thymol concentration, as well as its antioxidant properties in scavenging free radicals (18).

pH

The average initial pH of the fish samples was determined to be 6.14. Figure 4 displays the alterations in pH levels of fish fillets over the period of refrigerated storage.

The pH values of the control samples and the samples coated with chitosan integrated by 0.6 ZMEO were 8.57 and 7.17, respectively, at the end of the investigation.

Sensory Analysis

Table 1 displays the median scores for odor, taste, and acceptability of the ZEMO fish fillet samples. The fillet treated with chitosan mixed with 0.3% EO for 15 days of storage was the most favored sample. The fillet treated with chitosan combined with 0.3% EO during 15 days of storage was the most preferred sample. The evaluation of odor, color, and general acceptability of silver carp fillet samples were found in Table 1. According to the findings of this investigation, applying a coating of chitosan with 0.3% ZMEO resulted in the required sensory qualities.

Table 1. Median rating for sensory propertis of fish fillets as affected by different concentrations of ZEMO

combination	Odor	Taste	Overall acceptability
control	7	7	7
Chitosan	7	8	7
Chitosan + 0.3 % ZEMO	8	8	8
Chitosan + 0.45 % ZEMO	8	6	6
Chitosan + 0.6 % ZEMO	6	4	4

Means followed by the same letters are not significantly (P <0.05) different.
SD: standard deviation.

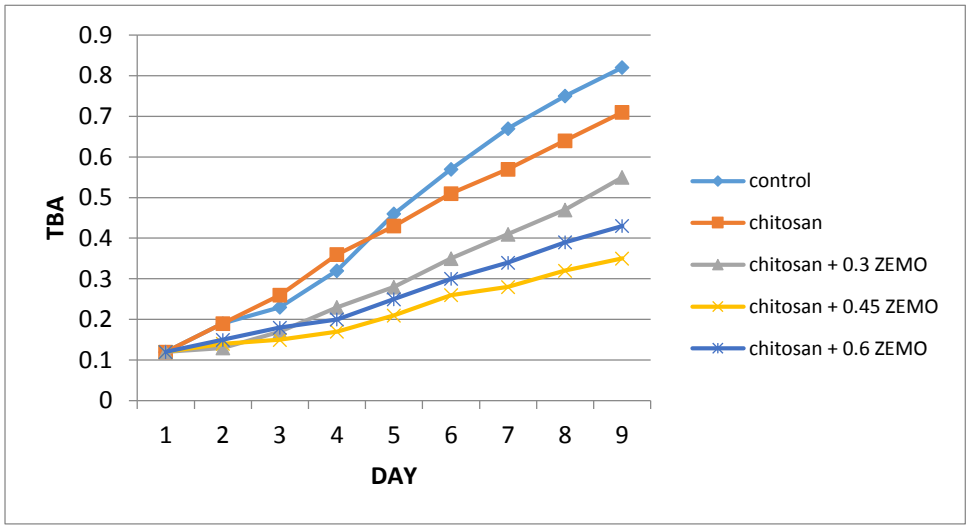

Figure 1. Changes in TBA values of the fish sample during chilled

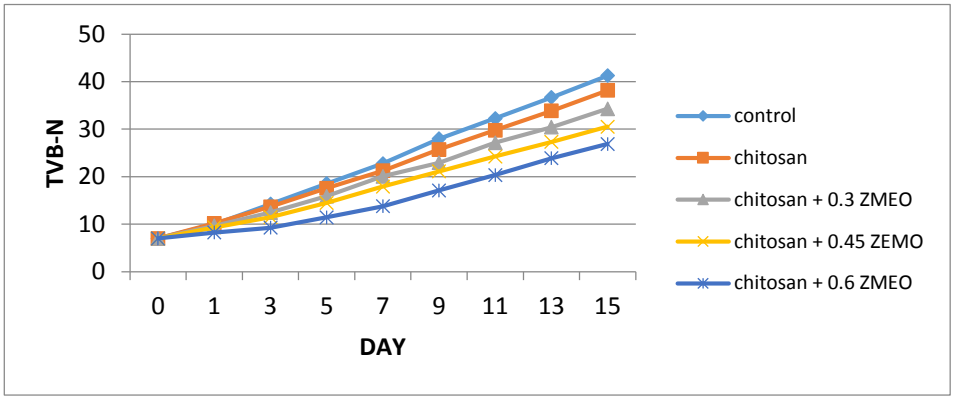

Figure 2. Changes in TVB-N values of fish samples during chilled storage.

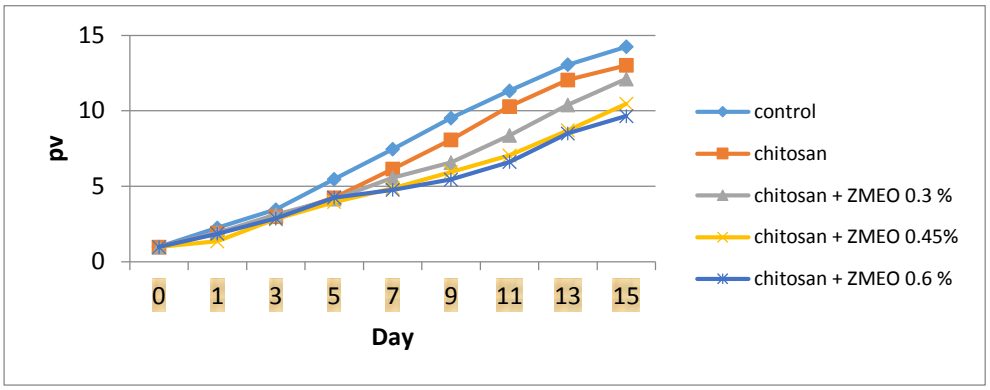

Figure 3. Changes in PV values of the fish sample during chilled storage

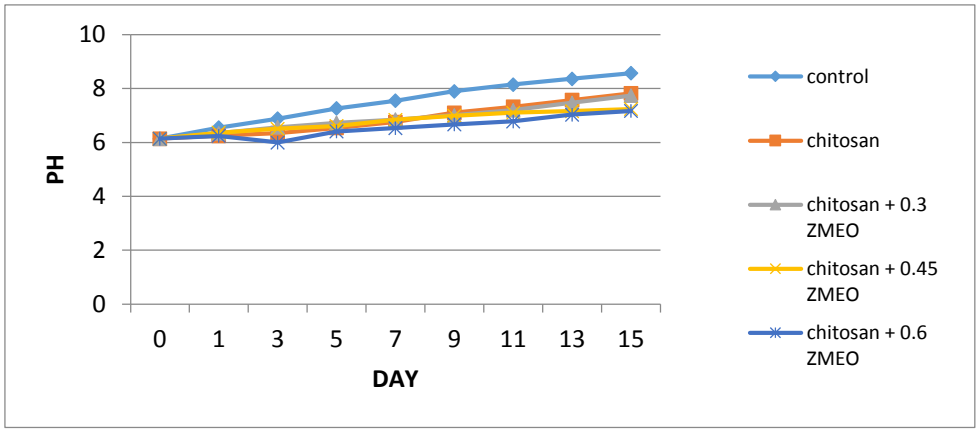

Figure 4. Changes in pH values of fish samples during chilled storage

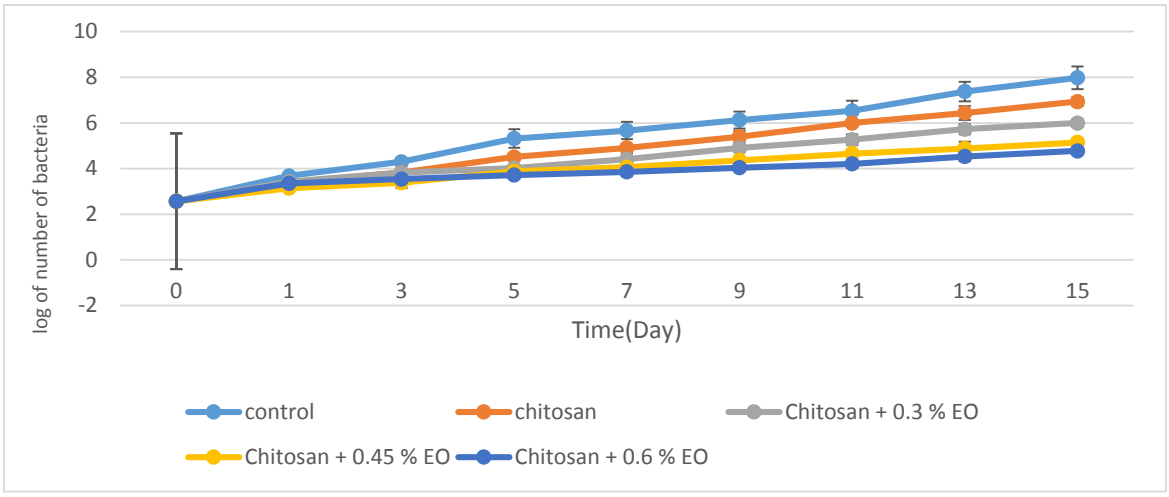

Figure 5. Changes in total viable counts (TVC) of Silver carp during chilled storage

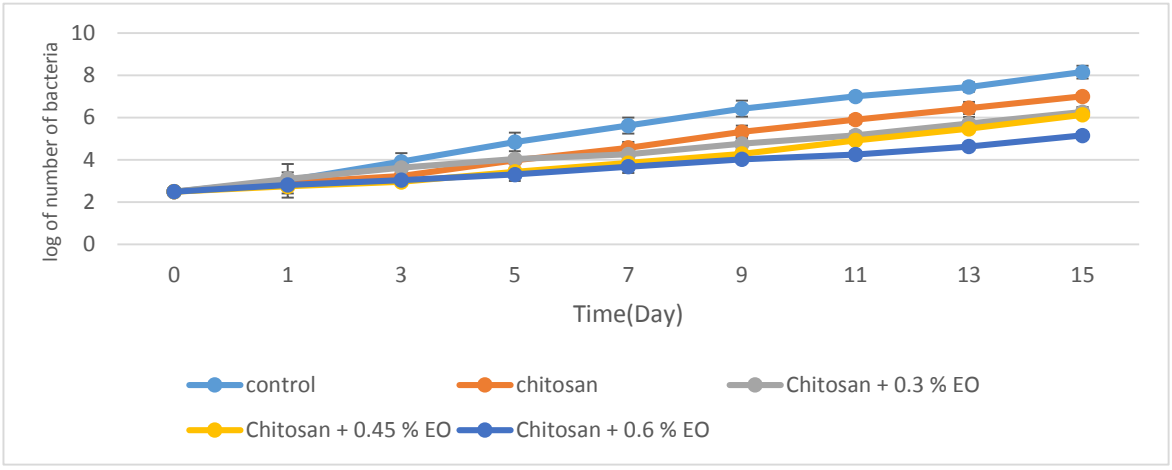

Figure 6. Changes in Enterobacteriaceae count (EBC) of silver carp during chilled storage

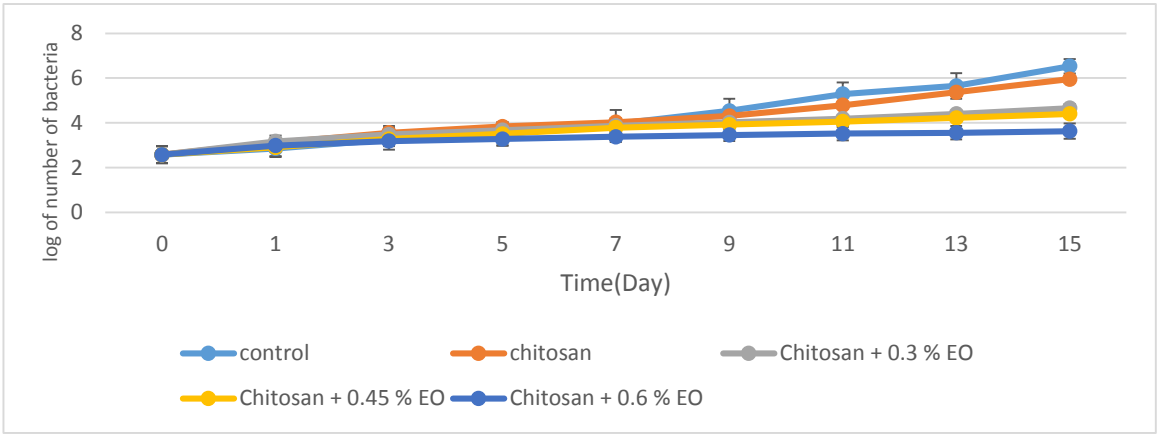

Figure 7. Changes in lactic acid bacteria count (LAB) of silver carp during chilled storage

Discussion

The chemical composition of ZMEO is influenced by various elements, including the season of harvest, the age of the plant, the soil it grows in, the geographical location, the climate conditions, the species, the method of herb drying, and the extraction method (19).

The proximate composition results are consistent with the findings reported by Asgharzadeh et al (20). In this investigation, the protein content of the control samples was found to be lower than the protein content of silver carp fillets in the Asgharzadeh et al (20) study. The presence of both report and wild silver carp can be ascribed to variations in diet.

The study utilized two primary components, chitosan and ZMEO, to treat silver carp fillets. These components exhibited antibacterial properties and effectively extended the shelf life of the fillets when stored in refrigerated conditions. Our research indicates that applying chitosan containing ZMEO to fillets extended the freshness of the fillets to 15 days. The findings align with previous research that shown a decrease in bacterial proliferation in chilled fish fillets when treated with an edible coating infused with different essential oils (9, 21).

The antioxidant activity of the coating treated with ZMEO was verified using chemical analysis, which detected the presence of substances such as thymol and carvacrol (4). Khanjari et al (17) obtained similar findings when they assessed the effects of chitosan films containing natural preservatives on refrigerated meat. According to ICMSF (22), the maximum allowable microbiological level for fish of acceptable quality is 7 log CFU/g. The observed disparities ($P<0.05$) between the control and treated samples can be attributed to the antibacterial properties of chitosan and ZMEO. This finding is consistent with other research that demonstrated the antibacterial properties of edible chitosan coating containing essential oils (9).

Several studies have reported that EBC can be extracted from fillets of trout and silver carp. The impact of EBC on the microbiota of fish should be taken into account because it has the potential to cause spoiling. Multiple researchers have documented the frequent presence of these bacteria in hog flesh (23) and trout fillet (21). In our investigation, we observed that the initial count of the sample was 2.49 log CFU/g. After treatment, the final population of the treated samples reduced dramatically by roughly 1-3 log CFU/g compared to the control sample (Figure 6). Specifically, on the 15th day, the population of Enterobacteriaceae in the control sample was measured to be 8.15 log CFU/g. However, when Chitosan + 0.6% ZMEO was present, their population was seen to be 5.15 log CFU/g (Figure 6). Prior studies have indicated that several essential oils and extracts have the ability to hinder the growth of the Enterobacteriaceae population in fish products (18).

Conclusion

According to the findings of this research, applying a layer of chitosan infused with ZMEO onto silver carp fillets. Slows down the growth of microorganisms and unwanted chemical reactions in meat, hence increasing the amount of time silver carp fillets can be stored in refrigerated conditions. The capabilities of ZMEO and chitosan can be attributed to the presence of natural components that function as antioxidants and antimicrobials. Future studies should focus on enhancing the stability and hardness of the coating's physical qualities to make it suitable for commercial application. In summary, this coating has the potential to serve as an effective antimicrobial agent that can be utilized to prevent the growth of undesirable microbes in food products, particularly those derived from fish and shellfish.

References

1. Fan W, Sun J, Chen Y, Qiu J, Zhang Y, Chi Y. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chemistry. 2009; 115(1):66-70.

2. Siddaiah D, Sagar Reddy GV, Raju CV, Chandrasekhar TC. Changes in lipids, proteins, and kamaboko forming ability of silver carp (Hypophthalmichthys molitrix) mince during frozen storage. Food Research International. 2001; 34(1): 47-53.

3. Goulas AE, Kontominas MG. The combined effect of light salting, modified atmosphere packaging, and oregano essential oil on the shelf-life of sea bream (Sparus aurata): Biochemical and sensory attributes. Food Chemistry. 2007; 100(1): 287-96.

4 .Fiore A, Park S, Volpe S, Torrieri E, Masi P. Active packaging based on PLA and chitosan-caseinate enriched rosemary essential oil coating for fresh minced chicken breast application. Food Packaging and Shelf Life. 2021; 29(4):100708.

5 .Nunes C, Silva M, Farinha D, Sales H, Pontes R, Nunes J. Edible Coatings and Future Trends in Active Food Packaging–Fruits' and Traditional Sausages' Shelf Life Increasing. Foods [serial online].
2023; 12 (17): 3308.
6 .Burt S, Essential oils: their antibacterial properties and potential applications in foods—a review. ICFMH. 2004; 94(3): 223-53.
7 .Basti AA, Misaghi A, Khaschabi D. Growth response and modeling of the effects of Zataria multiflora Boiss. essential oil, pH and temperature on Salmonella Typhimurium and Staphylococcus aureus. LWT. 2007; 40(6):973-81.
8. Moosavy M-H, Akhondzadeh Basti A, Misaghi A, Karim G, Salehi T, Mostafavi E. Effects of Zataria multiflora Boiss. essential oil and nisin on the Growth of Salmonella typhimurium in a Commercial Barley Soup. Food Research International. 2008; 41(10):1050-7.
9. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry. 2010; 120(1):193-8.
10. Williams Se. Official methods of analysis of the Association of official analytical chemists. 14th ed: Arlington (Va.): Association of official analytical chemists; 1984.
11. Arashisar Ş, Hisar O, Kaya M, Yanik T. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (Oncorhynchus mykiss) fillets. International Journal of Food Microbiology. 2004;97(2):209-14.
12. Ibrahim Sallam K. Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. Food Control. 2007; 18(5):566-75.
13.Jouki M, Yazdi FT, Mortazavi SA, Koocheki A, Khazaei N. Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf-life extension of refrigerated rainbow trout fillets. International Journal of Food Microbiology. 2014; 174(17):88-97.
14 .Egan H. Pearson's chemical analysis of food 9th edition. Longman Scientific and Technical. In: Kirk RS, Sawyer, R, editor. 1997: 609– 34.
15 .Kirk RS. Pearson's Composition and Analysis of Foods 9th edition. In: Sawyer R, editor. UK: Longman Scientific and Technical. 1991:640- 3.
16. Kilincceker O, Dogan İS, Kucukoner E. Effect of edible coatings on the quality of frozen fish fillets. LWT. 2009; 42(4): 868-73.
17. Khanjari A, Karabagias IK, Kontominas MG. The combined effect of N, O-carboxymethyl chitosan, and oregano essential oil to extend shelf life and control Listeria monocytogenes in raw chicken meat fillets. LWT. 2013; 53(1):94-9.
18. Mexis SF, Chouliara E, Kontominas MG. The combined effect of an oxygen absorber and oregano essential oil on shelf-life extension of rainbow trout fillets stored at 4 °C. Food Microbiology 2009; 26(6): 598-605.
19. Shafiee A, Javidnia K. Composition of Essential Oil of Zataria multiflora1997. Planta Med. 1997; 63(4): 371-2.
20. Asgharzadeh A, Shabanpour B, Aubourg SP, Hosseini H. Chemical changes in silver carp (Hypophthalmichthys molitrix) minced muscle during frozen storage: Effect of a previous washing process. Journal of Food Processing and Preservation. 2010; 61(1): 95-101.
21. Frangos L, Pyrgotou N, Giatrakou V, Ntzimani A, Savvaidis IN. Combined effects of salting, oregano oil, and vacuum-packaging on the shelf-life of refrigerated trout fillets. Food Microbiology. 2010; 27(1):115-21.
22. ICMSF, Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications 2nd edition. New York: University of Toronto Press; 1986.
23 .Bonilla J, Fortunati E, Vargas M, Chiralt A, Kenny JM. Effects of chitosan on the physicochemical and antimicrobial properties of PLA films. Journal of Food Engineering. 2013; 119(2): 236-43.