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The Effects of Nanochitosan Coating Integrated to *Zataria Multiflora* Boiss and *Polylophium Involucratum* Essential Oils on the Shelf-Life Extension of Silver Carp Fillets

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ABSTRACT

Introduction: Active antimicrobial food packaging prevents the growth of foodborne pathogens and spoilage microorganisms by incorporating antimicrobial agents into the film materials.

Methods: The effects of Nanochithosan (NC) coating containing various concentrations of *Polylophium involucratum* essential oil (PIEO) and *Zataria multiflora* Boiss. Essential oil (ZMEO) were investigated on microbial, chemical, and sensory characteristics of silver carp fillets within 12 days during refrigerated storage.

Results: The aerobic plate count (APC) exceeded 7 log CFU/g after day four and day six for the control and samples coated with pure NC, respectively. The samples coated with NC containing ZMEO 0.6% and PIEO 0.6% showed the lowest microbial count. In a control sample with NC containing ZMEO 0.6% and PIEO 0.6%, the total volatile base of nitrogen (TVB-N) reached 33.15 mg/100 g after eight days, but this value remained lower than 25 mg/100 g for the coated samples with NC containing ZMEO 0.6% and PIEO 0.6%. Generally, integrating the ZMEO and PIEO did not significantly and negatively affected the sensory characteristic of coated samples compared with those of control.

Conclusion: According to the results, NC coatings containing ZMEO and PIEO were capable of being used as novel active packaging for fish meat products without compromising their organoleptic characteristics.

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Introduction

The global aquaculture production for silver carp in 2016 was about 5301 tons and ranked second among freshwater fish species due to the low-cost production, availability, high feed efficiency ratio and easy cultivation (1-3). Fish meat is a valuable source of proteins and healthy lipids, which is classified as a highly spoilable food with a relatively short shelf-life under refrigerated conditions. The reduction of fish meat shelf-life could be attributed to its intrinsic factors like neutral pH, low connective tissue, high water holding capacity, free amino acids content and high concentration of polyunsaturated fatty acids (PUFAs) (4). The two major methods of preserving fish meat are freezing and canning, but these methods cannot be used for fresh fish meat.

Active packaging such as antimicrobial films and coatings based on chitosan and its derivatives has been used to prolong the shelf-life and improve food safety (3, 5-8). Chitosan, prepared by chitin deacetylation, is a nontoxic, biocompatible, and biodegradable polyaminosaccharide. The European Union considers chitosan safe at a daily consumption of 3 g (6, 9). Several published studies have reported that chitosan nanoparticles (sizes from 10 to 1000 nm) have higher antioxidant and antibacterial activity properties than chitosan due to their larger surface area and high affinity for bacteria (5, 8).

Avishan Shirazi, with the scientific name *Zataria multiflora* Boiss., belongs to the Lamiaceae family, which mainly grows in Southeast Asia and its leaves are utilized as a flavoring agent in foods and in treating diseases in folk medicine.

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Researchers have reported that Zataria multiflora essential oil (ZMEO) and its major components, carvacrol and thymol, are good antioxidants and antimicrobials.
Polylophium involucratum is a plant belonging to Umbelliferae, which is often wildly grown in northwestern Iran and extensively integrated into foods as a condiment in herbalism.
This study aimed to evaluate the effects of 2 g/100 ml NC solution containing different concentrations of ZMEO and PIEO on shelf-life, prolonging silver carp fillets within a 12-day refrigerated (+4°C) storage period.

Material and Methods

Essential Oil Extraction and Analysis

The dried leaves of *Zataria multiflora* Boiss. and seeds of *Polylophium involucratum* were ground and transferred to a Clevenger and steam distillated for three hours. The gathered EO was stored in a glass tube under the dark condition at 4°C until further use. The constituents of the obtained EO were analyzed by gas chromatography-mass spectrometry (GC-MS) as described previously by Khanjari et al. (2013) (10).

Sample Preparation

The fresh silver carp with an average weight of 2100 g were obtained from a fish farm in Tehran and delivered to the food hygiene laboratory in approximately 45 minutes. Then, the fish fillets were prepared in portions of about 60 g under a safety cabinet (JTLVC2X, Jaltajhiz, Iran).

Sample Treatment and Packaging

The coating solution was made by adding 2 g of chitosan nanoparticles (Nanonovin polymer Mazandaran, Iran) to 100 ml distilled water and stirring for 3 h on a magnetic stirrer at room temperature. Then, glycerol (0.75 ml per gram of nano-chitosan) was incorporated as a plasticizer, and the solution was stirred for another 10 minutes. The coating solution was then mixed with varying concentrations of ZMEO (0, 0.3, and 0.6%) and PIEO (0, 0.3, and 0.6%) mixed with Tween 80 (0.25% v/v). The final coating solution was homogenized at 12,000 rpm for 2 minutes. Afterward, fillet pieces were put in bowls containing 500 ml of coating solution with various EO concentrations for 1 min. In the next stage, the fillets were removed aseptically from the solution and placed on sterile metal mesh under a safety cabinet for 2 min to remove excess coating. Subsequently, the samples were taken into bags and stored at 4°C for 12 days (6, 7, 11).

Microbiological Analysis

Samples of each treatment (10g) were homogenized using stomacher bags containing 90 ml of diluent for 1 min each interval day. Then, suitable serial decimal dilutions of samples were made and cultured on plate count agar for both Aerobic plate count (APC), psychrotrophic bacteria counts (PSB), *Pseudomonas* agar for *Pseudomonas* spp. (PSE), on De Man Rogosa, and Sharpe agar for lactic acid bacteria (LAB) enumeration at seven intervals (0, 2, 4, 6, 8, 10 and 12 days), respectively (12).

Chemical Evolution

The samples were assessed for total volatile basic nitrogen (TVB-N) according to Wang et al. (2018).

Sensory Evaluation

The sensory properties were examined using a 6-member trained panel under the same light, location, and dish conditions. The chicken samples were cooked at the high power of a microwave oven (700 W). The color, taste and odor indexes were assessed using the acceptability scale, with 0 corresponding to the least liked sample and five corresponding to the most liked sample (7).

Statistical Analysis

First, the microbiological count of each treatment was calculated based on the logarithms of the number of colony-forming units (log CFU/g). Then, the logarithmic microbiological and chemical data were analyzed using a one-way ANOVA followed by Tukey's test (12).

Result and discussion

Microbiological analysis of samples

Figure 1 illustrates the changes in aerobic plate count within storage at 4oC. The APC of the silver carp fillet was 4.49 log CFU/g at the beginning day of the study. However, the APC of the control samples reached the maximum number of bacteria for acceptable quality in fresh fish recommended by the ICMSF (higher than 7 log CFU/g) on the fourth day (7.01 log CFU/g) (13). APC of NC-coated samples was significantly reduced (P0.05) by coating, possibly due to the antimicrobial properties of NC, ZMEO, and PIEO. The growth of PSB is an important factor in reducing fish fillet shelf-life under refrigeration.

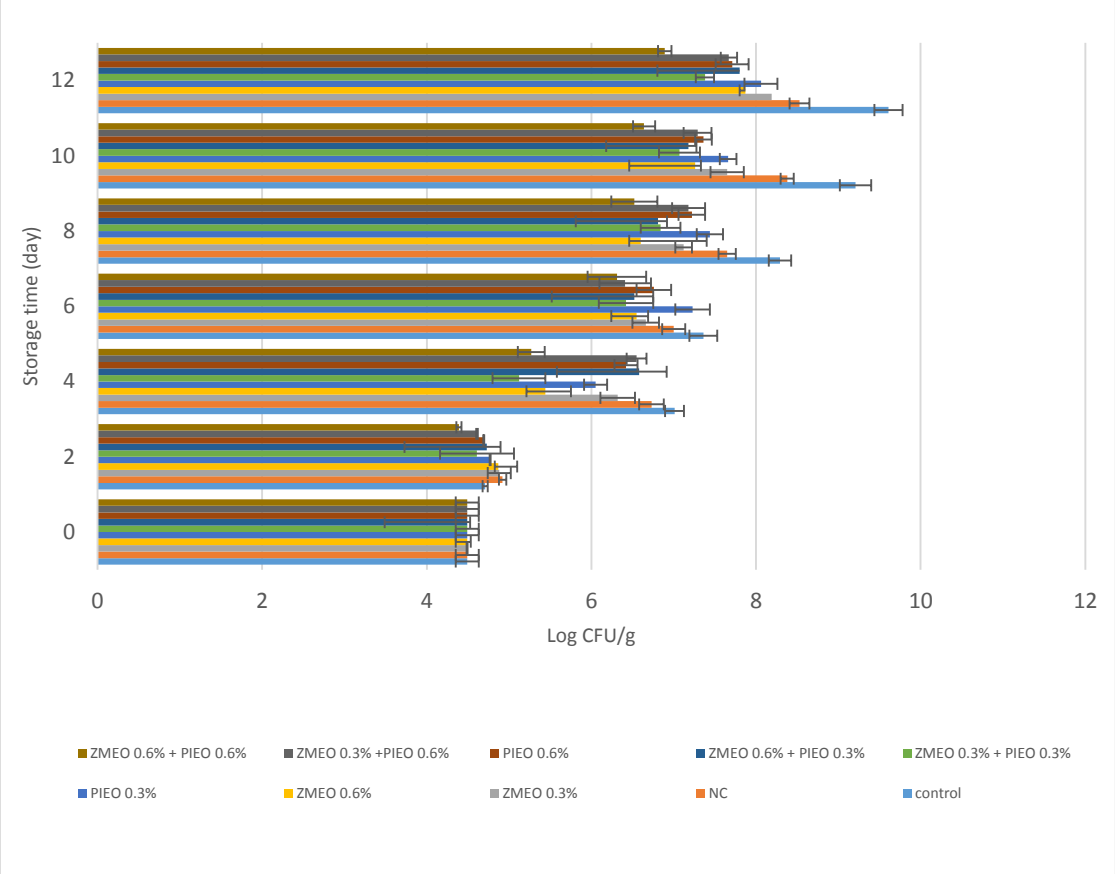

Figure 1. Changes in aerobic plate count (APC) of silver carp during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

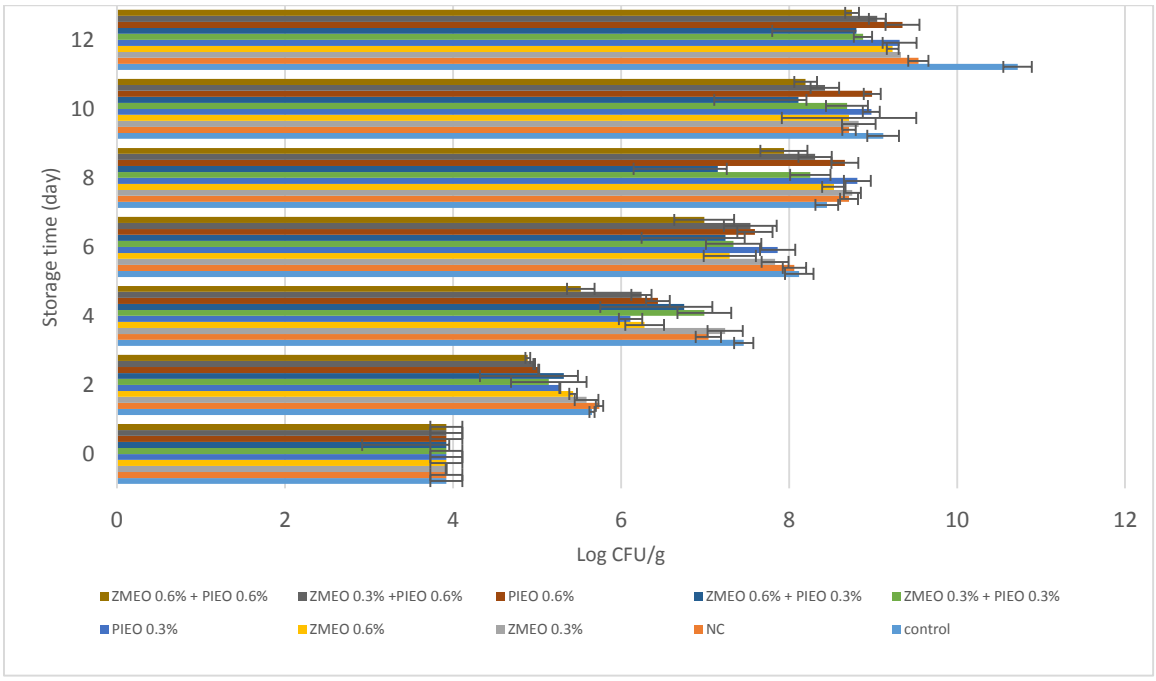

Figure 2. Changes in psychrotrophic bacteria (PSB) of silver carp during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

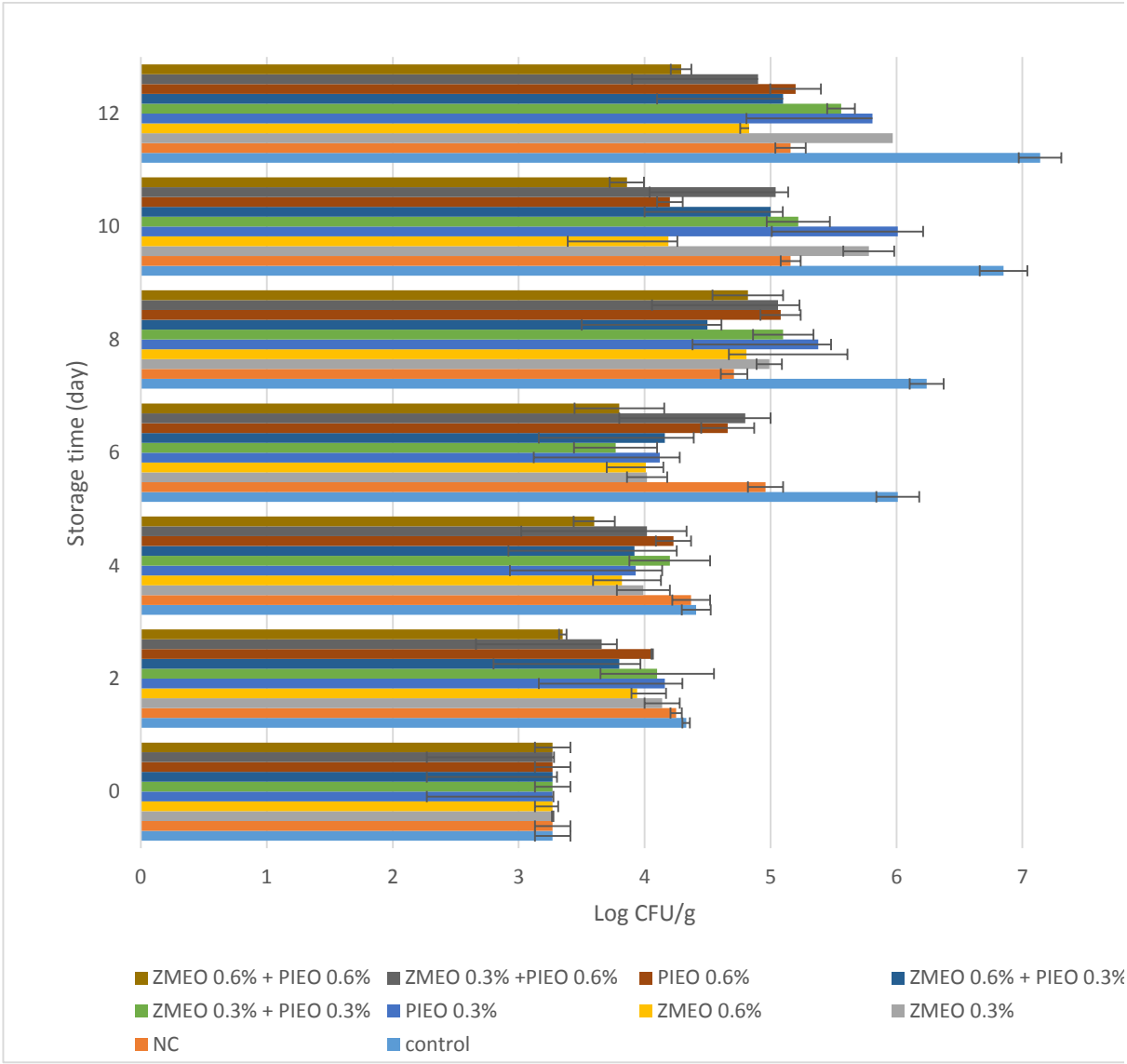

Figure 3. Changes in lactic acid bacteria (LAB) of silver carp during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

In this study, the number of PSB in fish fillets was 3.92 log CFU/g, which varied between 2 to 6 logs CFU/g in previous studies depending on the water temperature and conditions (14). The coating with pure NC and NC containing different concentrations of ZMEO and PIEO significantly decreased the PSB ($P < 0.05$) (Figure 2).

As shown in Figure 3, coating samples with NC containing ZMEO and PIEO alone or in combination resulted in a lower LAB count than control samples ($P<0.05$).

The PSE count of silver carp fillets was 2.73log CFU/g and a significant ($P<0.05$) decline in PSE was observed in samples coated with NC incorporated with both EOs compared to the control at the end of the study (Figure 4).

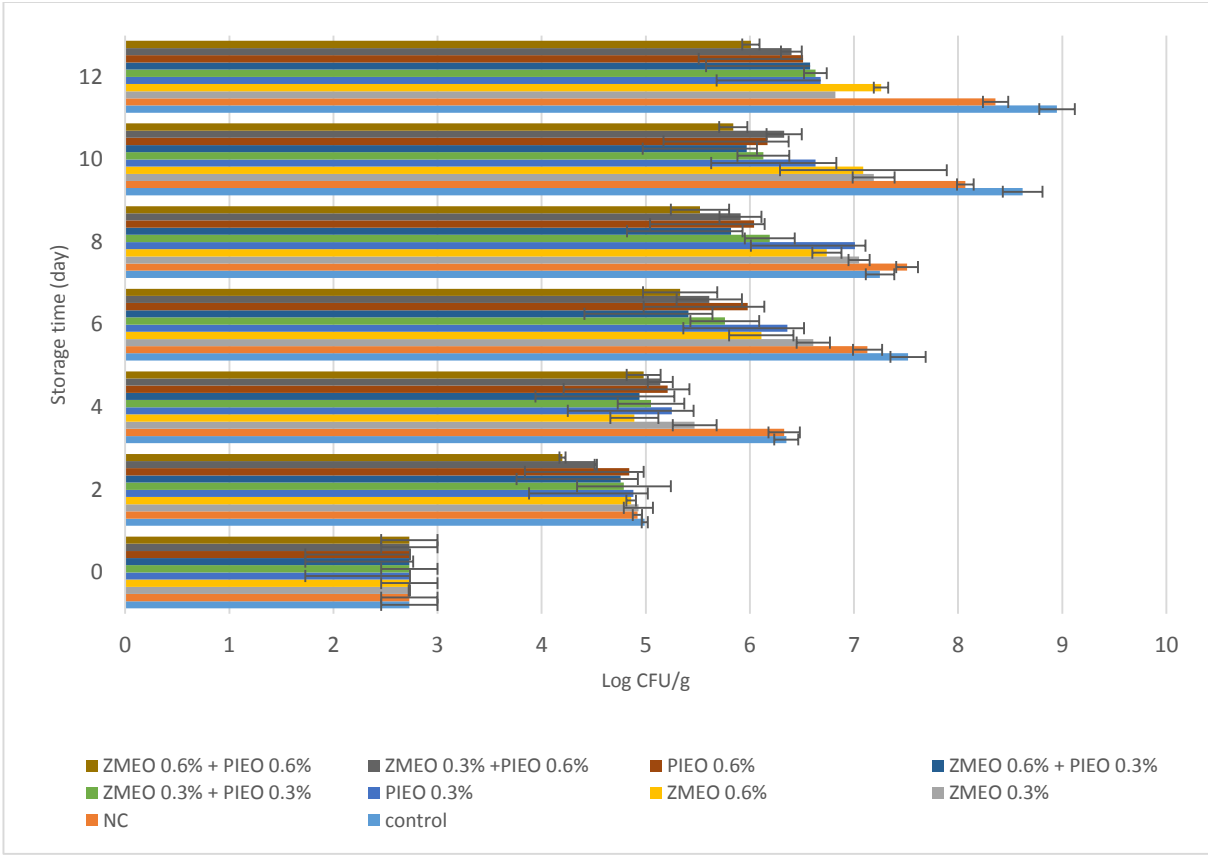

Figure 4. Changes in *pseudomonas* spp. (PSE) of silver carp during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

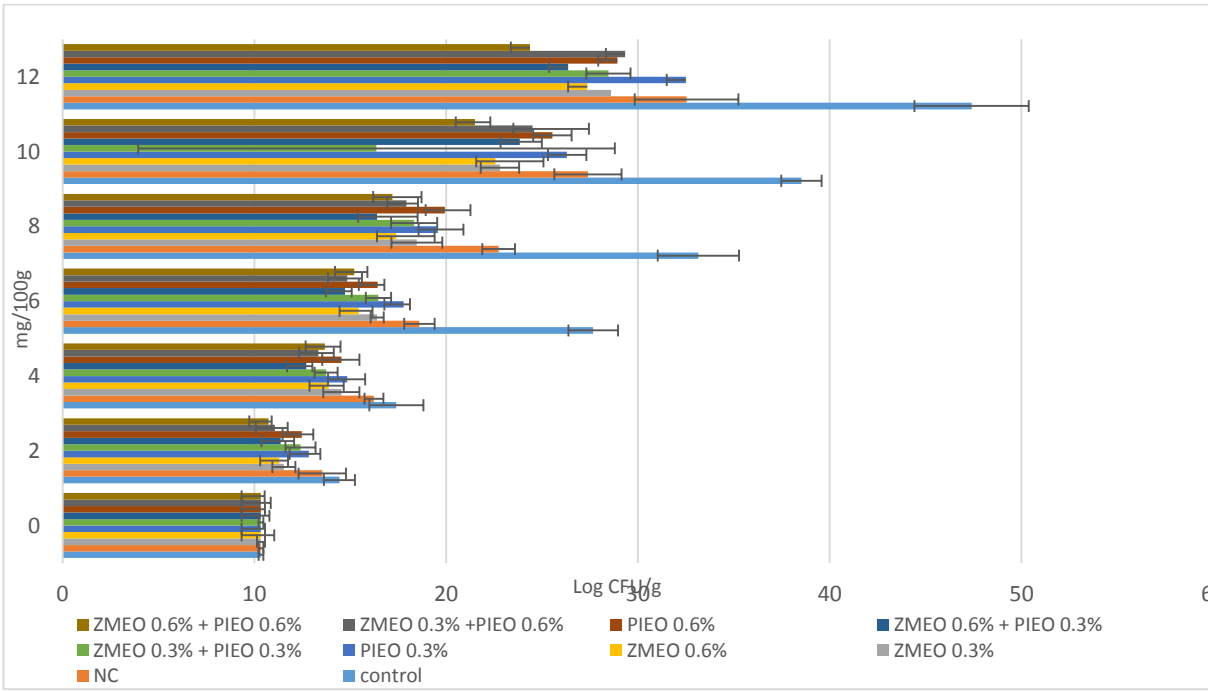

Figure 5. Changes in TVN of silver carp during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

Chemical Analysis Evaluation

According to Figure 5 a, both control and NC-coated samples increased TVB-N levels during the study. Nevertheless, the values were significantly lower in the NC-coated samples vs. control samples ($P < 0.05$). In this study, the amount of TVB-N in control samples was 10.34 mg/100g on the first day and reached and afterwards reached to 47.40 mg/100g after 12 days.

Sensory Evaluation

The studied sensory properties (color, odor and taste) of cooked fish fillets for all groups are presented in Figure 6 (a, b and c). The color, odor, and taste scores of the samples were vice versa to microbial and chemical values during storage time. The flavor of low concentrations of ZMEO and PIEO was very attractive to the sensory panel members, and Nanochitosan did not change sample sensory properties.

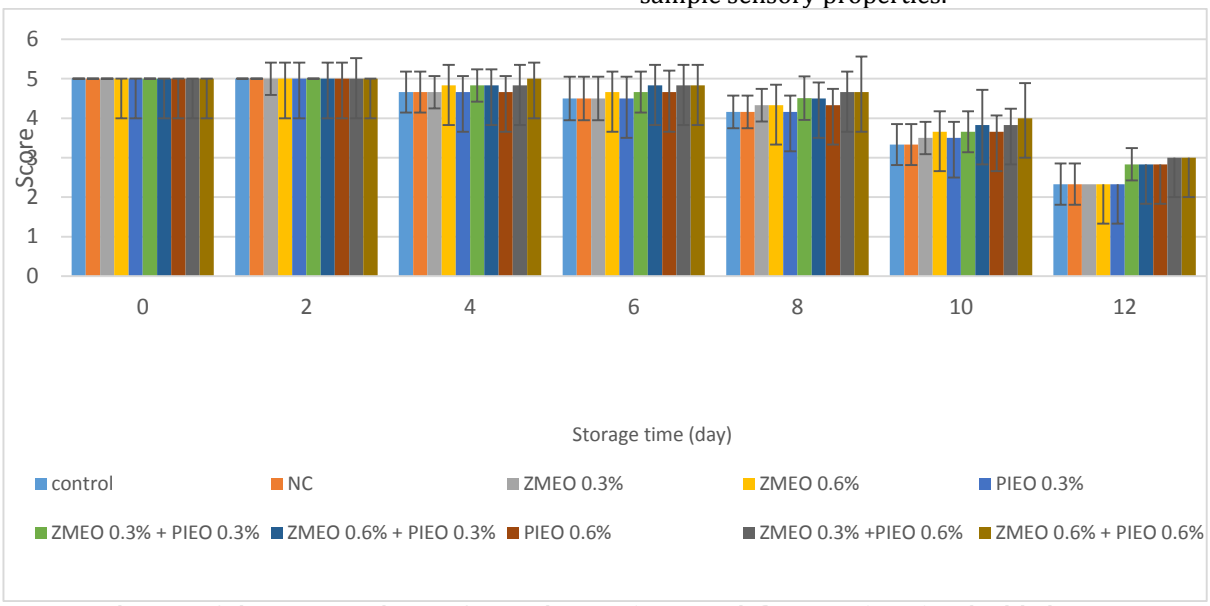

Figure 6. Color score of silver carp meat during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

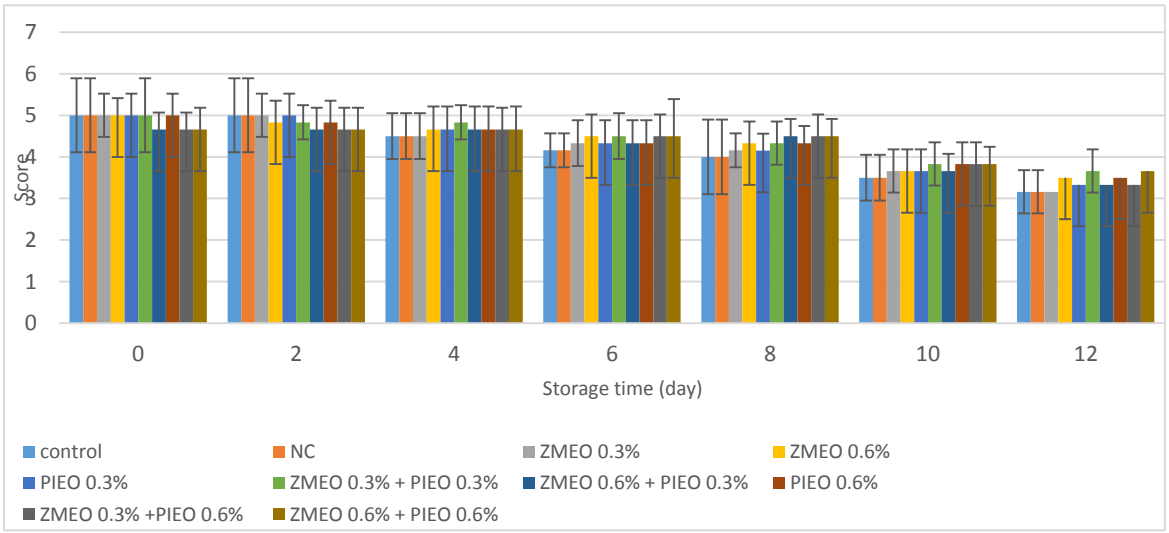

Figure 7. Odor score of silver carp meat during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

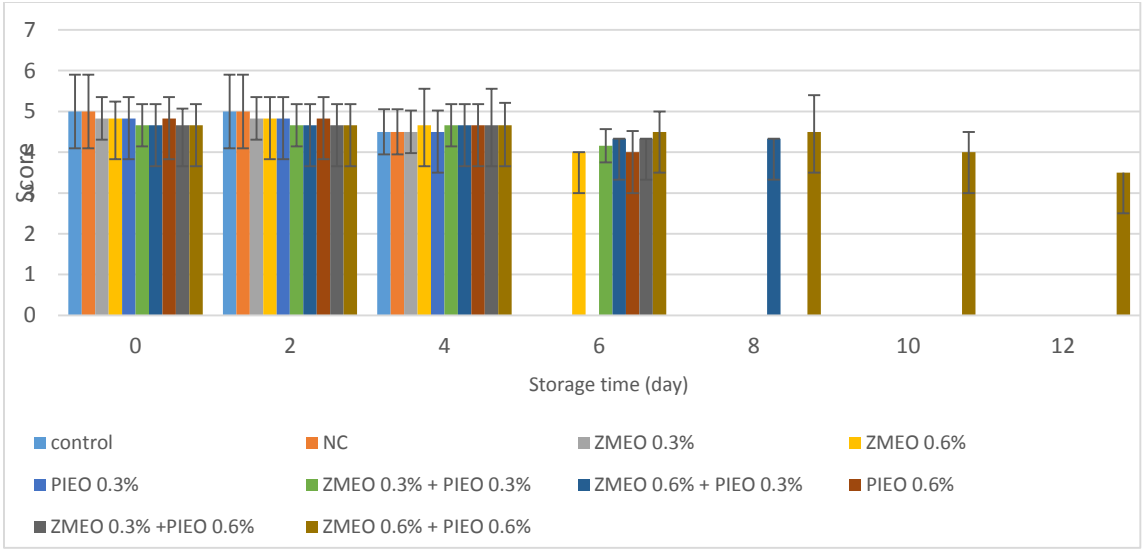

Figure 8. Taste score of silver carp meat during refrigerated storage (*Zataria multiflora* Boiss. (ZMEO), and *Polylophium involucratum* essential oil (PIEO) and nanochitosan (NC))

Table 1. Essential oil composition of *Polylophium involucratum* identified by GC-MS.

Row	Compounds	Kovats Retention Index	Percentage
1	Alpha-pinene	997	4.66
2	Sabinene	1037	.57
3	Beta-pinene	1044	.60
4	Myrcene	1052	1.83
5	Limonene	1106	33.08
6	Gamma-tripene	1139	.11
7	Linalool	1175	.23
8	Cis-limonene oxide	1219	.6
9	Trans-limonene oxide	1225	.83
10	Methylbenzoate	1393	49.15
11	Perilla Alcohol	1428	1.12
12	Limonene Glycol	1461	.21
13	Alpha-Copaene	1552	.12
14	Beta-Cubebene	1564	.21
15	Germacrene D	1682	.26
16	Spathulenol	1795	.16

Table 2. Essential oil composition of *Zataria Multiflora* Boiss. identified by GC-MS.

Row	Compounds	Retention Time (minutes)	Percentage
1	α– Pinene	6.291	2.08
2	β- Pinene	7.765	.26
3	Octanone	8.181	.74
4	β–Myrcene	8.356	.84
5	α–Terpinene	9.342	1.02
6	Cymene	9.727	5.52
7	1,8-Cineole	9.927	.24
8	γ–Terpinene	11.156	2.56
9	Linalool Oxide	11.74	.61
10	α–TERINOLENE	12.432	.72
11	Linalool	13.393	23.91
12	Borneole	15.884	.19
13	4-Terpineol	16.439	.93
14	α–Terpineol	17.312	.44
15	Carvacrol methyl ether	19.776	1.97
16	Thymol	22.447	4.13
17	Carvacrol	23.325	48.19
18	Carvacrol Acetate	25.723	1.27
19	Trans-Caryophyllene	27.372	.89
20	Caryophyllen oxide	32.913	.55

Discussion

The major compounds of ZMEO and PIEO used in this study were Carvacrol (48.19%) and Methyl benzoate (49.15%), respectively, with good antimicrobial activity (15). Hosseini et al. (2015) reported the same outcomes regarding the use of EO, and observed that the APC of rainbow trout fillets exceeded 6 log CFU/g in control samples and samples coated with oregano EO after 8 and 12 days, respectively (16). Fadiloglu and Coban (2018) found that the APC values of control samples were 6.77 log CFU/g at day 9, and the APC value of coated samples with chitosan alone and chitosan+sumac was lower than the 7 log CFU/g at days 9 and 12, which consistent with this study (17). In addition, these results are in line with those of Shahbazi and Shavisi (2019), who concluded that coating of silver carp fillets with sodium alginate incorporated with *Mentha spicata* EO significantly diminished the APC of treated samples in comparison with control ones (18). However, Andevari and Rezaei (2011) reported inconsistent findings so that coating the rainbow trout fillets with gelatin integrated with cinnamon EO did not significantly differed in the APC of coated samples from those of the control samples (19). The possible reasons for the observed differences could be attributed to the different compositions of ZMEO and PIEO and the general microflora of fresh fish. The dominant microflora of temperate water fish is gram-negative rod-shaped psychrotrophic bacteria (PSB) (20). The outcome of this research was consistent with Ojagh et al. (2010), who stated that the coating of chitosan enriched with *Cinnamomum zeylanicum* EO delayed the PSB growth in coated rainbow trout (7). Similar results were declared by Ramezani et al. (2016), who explained that the NC treatment significantly diminished the PSB bacteria population in silver carp fillets (8).

The most important spoilage bacteria in refrigerated proteinaceous products are lactic acid bacteria. This type of bacteria can metabolize amino acids and produce ammonia and biogenic amines, such as histamine, putrescine, and tyramine (21). Researchers have declared that coating prepared with various EOs can retard the LAB growth in refrigerated fish (22-24). LAB is among the most resistant Gram-positive bacteria against the antimicrobial activity of EOs (25). A possible explanation for this resistance may be their ability to adjust to osmotic stress conditions by producing ATP and responding more effectively to EO-induced K+ efflux (24, 26).

Pseudomonas (PSE) are among the commonest microorganisms associated with fish fillets' spoilage within cold storage. Species of this genus can produce protease enzymes and use nitrogen-containing compounds as energy sources, causing fish fillets to have an off-odor and off-flavor. Based on Jouki et al.'s (2014) study, NC coating incorporated with ZMEO and PIEO yielded similar results. Rainbow trout samples wrapped with quince seed mucilage edible films infused with 2% thyme extract were found to have a significant reduction in PSE populations (24). The results of this study are also consistent with those of Shahbazi and Shavisi (2019), who reported a significant reduction in silver carp PSE of 4.14-5.44 log CFU/g when treated samples were compared with untreated samples. By adding Mentha spicata EO (0.5 and 1%) to edible sodium alginate coatings after 14 days of storage, the control samples were significantly reduced in PSE (18).

TVB-N level of meat is related to the microbial growth and endogenous protease activity of meat. Thus, the shelf-life and freshness of meat could be evaluated by measuring the TVB N (27). Similar findings were declared by Raeisi et al. (2020), Andevari and Rezaei (2011), and Wang et al. (2018) regarding the utilization of NC coating integrated with ZMEO and PIEO. As a result of the antibacterial effect of Nanochitosan combined with ZMEO and PIEO, the TVB-N values are lower in the coated samples (2). Some compounds such as dimethyl disulfide, dimethyl sulfide and propylene sulfide are formed during the metabolism of Pseudomonads by the growth of the *Pseudomonas* spp. population. LAB metabolizes the amino acids to ammonia and biogenic amines after exhausting carbohydrates in fish meat. The taste and odor were unfavorable due to these compounds. Furthermore, producing chemical substances such as aldehydes and ketones from lipid oxidation could be the reason for the off-odor of fish fillets (21, 28).

Previously, color parameters were used to describe the freshness and acceptability of fish muscle. In general, the use of different concentrations of ZMEO and PIEO within the research period had no significant negative

effects on NC-coated samples' sensory characteristics compared to those of the control samples.

Conclusion

According to the results, NC coatings containing ZMEO and PIEO could be used as novel active packaging for fish meat products without adverse organoleptic effects. Silver carp fish fillets can be controlled by using alternative and non-toxic matrices that reduce spoilage bacteria populations.

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