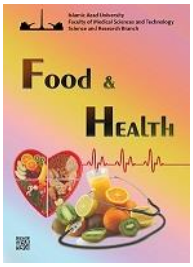

Food & Health Journal
Journal homepage: fh.srbiau.ac.ir

The effect of edible coating of chitosan and Baneh gum (*Pistacia atlantica*) containing propolis extract and ginger nanoemulsion (*Zingiber officinale*) on fresh salmon quality

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ARTICLE INFO

Original Article

Article history:
Received 10 February 2022
Revised 08 April 2022
Accepted 02 May 2022
Available online 20 June 2022

Keywords:
Chitosan
Beneh gum
Propolis extract
Nanoemulsion
Ginger essential oil

ABSTRACT

The use of new preservation methods based on natural substances of plant and animal origin in food is expanding. Edible coatings can improve the quality of fresh and frozen products such as fish by preventing microbial growth, and decreasing lipid oxidation and moisture loss. The aim of this study was to evaluate the effect of the edible coating of chitosan (0 and 2%), Baneh gum (0, 1 and 2 %), propolis extract (0, 1 and 2%), and nanoemulsion of ginger essential oil (0, 0.5 and 1 %) on the shelf life of fresh salmon fillets during 12 days refrigeration. The results showed that the coating had a significant effect on reducing the total count, psychrophilic bacteria, coliforms, and Pseudomonas count during storage. Also, coated samples showed lower pH and peroxide values than uncoated, but the coating had little effect on reducing thiobarbituric acid (TBARS) and total volatile nitrogen (TVBN) values. During the sensory evaluation, it was found that the chitosan coating with Baneh gum can maintain or improve the sensory properties and extend the shelf life of refrigerated fish.

1. Introduction

Fish is a rich source of essential fatty acids and protein for humans and plays an important role in the prevention and reduction of cardiovascular disease and certain cancers (1). Fish meat is a perishable food compared to chicken and red meat, and due to its high water content, large amounts of free amino acids, the presence of autolytic enzymes, and it is highly susceptible to chemical and microbial changes (2, 3). Salmon fish (*Salmo trutta aspius, Dorofeeva*), is one of the native species of the Caspian Sea of the *Salmonidae* family, which has exceptional economic value (4). The use of new preservation methods based on natural substances of plant and animal origin in food is expanding (5). Recently, studies on the use of biodegradable and environmentally friendly films and coatings are increasing (6). One of these compounds is chitosan, which is widely used as an edible coating. Studies show that chitosan (1–4)-2-amino-2-deoxy-β-D-glucan can be used to control microbial spoilage in meat and meat products (7-9). The combined use of chitosan with natural preservatives and antioxidants such as plant gums, extracts, and essential oils as alternatives to chemical compounds is highly recommended for food preservation (10). One way to utilize these compounds, rather than using them directly in food, is to use them in edible coatings, which reduces the possibility of inactivation in food and may be useful in food for a longer time (11). One of the limitations of essential oils is their low solubility in water. Therefore, the encapsulation process can stabilize the product and improve the biological activities of the essential oil and control the release of essential oils in the desired location. In addition, encapsulation stabilizes bioactive compounds during process and storage and prevents adverse reactions with the food matrix such as oxidation and hydrolysis process (12). Coatings play an essential role in the encapsulation process because they are responsible for protecting the essential oils and controlled release (13). Ginger

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(*Zingiber officinale*) as a common and widely used spice contains 1.5--3% essential oil which is rich in phenolic compounds and can be used as a natural preservative in food due to its antioxidant and antimicrobial properties (14-17). Baneh or wild pistachio (*Pistacia atlantica* subsp. *kurdica*), which is known as Persian turpentine, is a tree belonging to the *Anacardiaceae* family and is compatible with arid and semi-arid regions and occupy more than 1.200.000 hectares of forests in Iran (18). This area is under considerable cultivation and the unique properties of Baneh increase the need to use in the food products. The antimicrobial effects of Baneh have been reported in many studies (19-21). Propolis is a wax-like substance and by-product of beeswax, a dark brownish resinous substance with more than 300 compounds identified in its samples (22). One of the interesting effects of propolis is its antibacterial effects on human and animal bacteria (23, 24). Examination of propolis extract on 40 species of yeast fungi, including *Candida albicans*, *Candida eberta*, *Candida rosacea*, *Trichosperone*, and other types confirms its antimicrobial effects (25). The aim of this study was to evaluate the effect of the edible coating of chitosan, Baneh gum, propolis extract, and nanoemulsion of ginger essential oil on the shelf life of fresh salmon fillets during refrigeration.

2. Materials and methods

2.1. Sample preparation

Fresh salmon with an average weight of 900-1000 g was purchased from a fish farm (Amol province, Iran) and transported to the laboratory. Fresh ginger root was purchased at a local market (Tehran province, Iran). Chitosan powder with molecular weight 80 kDa and acetylation degree 7575 was purchased from Sigma (Sigma–Aldrich Chemie GmbH. Steinheim, Germany).

2.2. Extraction of ginger essential oil

Freshly ground ginger rhizomes (200g) were extracted for 4 hours at 70-80°C by water distillation with Clevenger type apparatus for essential oil extraction. The essential oil was dried over sodium sulfate and stored at 4 °C for future experiments (26).

2.3. GC/MS analysis

The ginger essential oil was identified and quantified using an Agilent 6890, USA GC instrument connected to a Hewlett-Packard 5973 MS and an HP5MS column (30 µm, 0.25 mm id, 0.25 mm film thickness). The temperature program was set at 150 °C, then increased by 6 °C/min to 280 °C, and kept at the final temperature for 5 min. One microliter of ginger essential oil was injected into a spitless injection at 250°C. Helium was used as the carrier gas at a flow rate of 1 ml/min. Compounds were identified by comparing retention times and mass spectrometry with available validated standards or by comparing retention indices (IR) and mass spectrometry with mass and reference libraries from NIST (National Institute of Standards and Technology).

2.4. Ginger essential oil nanoemulsions

First, the ginger essential oil was continuously mixed with Tween 80 (30% w/v) and distilled water using an ultrasonic homogenizer and heated at 3,000 rpm until the formation of the initial emulsion. The emulsion sample was then treated with an ultrasonic homogenizer at 100 W/cm^2 for 20 minutes for the preparation of nanoemulsions. Finally, the ginger essential oil nanoemulsions (GNE) were stored in a refrigerator (27).

2.5. Morphology of nanoemulsions

A light microscope (Labomed model LX400) was used to examine the morphology of ginger essential oil nanoparticles.

2.6. The particle size of nanoemulsions

The particle size of the nanoemulsions was measured using the DLS Cordouan technique (VASCO model). 10 ml of the essential oil nanoemulsion was fed to an ultrasonic device to form a stable suspension, and then injected into the device at 25°C.

2.7. Baneh gum preparation

The oleoresin gum of Baneh (*Pistacia atlantica* subspecies *kurdica;* Kordestan province, Iran) was extracted by piercing the trunk of the tree with a specialized tool and collected in a clay bowl attached to the trunk. Approximately 10 mg of Baneh gum (BG) was suspended in 1.0 ml of 0.2% (v/v) Tween 80 in distilled water (vehicle) just before use to obtain the solution.

2.8. Preparation of propolis extract

The propolis samples were obtained from individual beekeepers from several regions/locations of Tehran province in May 2020 and pooled. Hand-collected propolis samples were kept in the dark up to their processing and stored at 20°C. Then, 20 g of the dried propolis sample was immersed in a percolator in 100 ml of 80% ethanol, and after 48 hours, the resulting mixture was filtered through Whatman filter paper, freeze-dried, and stored at 4°C (28).

2.9. Edible coating preparation of chitosan/BG/propolis/GNE

The central composition of active edible coatings was designed with four-factor chitosan, Baneh gum (BG), propolis, and ginger essential oil nanoemulsions (GNE). To preparation of the solution, 2 gr of chitosan was added to 100 ml of acetic acid solution (1% v/v), and then 0.75 ml/g of glycerol as the plasticizer with 0.2% of Tween 80 as the emulsifier were added. The pH was then adjusted to 5.7–6 by adding 1 mol/L

NaOH, then the solution was steered at 30 °C for 30 min. The prepared solution was then filtrated through Whatman≠3 filter papers and autoclaved for 15 min at 121 °C (28). The propolis (0, 1, and 2%), BG (0, 1, and 2%) and GNE (0, 0.5 and 1 %) were added to solutions and homogenized for 10 min by magnetic stirrer.

2.10. Fish sample coating

The heads and tails of the fish were removed, the viscera were peeled and drained, then divided into 25 g pieces. The fish fillets were dipped in a chitosan coating solution containing a nano-emulsion of Baneh gum, propolis extract, and ginger essential oil (Table 1). After one minute, the fish fillets were removed and droplets of solution were separated with a sterile grid plate. After drying, the samples were transferred to sterile plastic bags and stored at 4°C for microbial, chemical, and sensory evaluation.

Table 1. The levels of chitosan, propolis, Baneh gum (BG), and ginger essential oil nanoemulsions GNE were used in this study.

Treatments	Chitosan	BG	Propolis	GNE
Control	-	-	-	-
T1	2%	-	-	-
T2	2%	1%	-	-
T3	2%	1%	1%	-
T4	2%	1%	1%	0.5 %
T5	2%	1%	1%	1%
T6	2%	2%	-	-
T7	2%	2%	2%	-
T8	2%	2%	2%	0.5 %
T9	2%	2%	2%	1%

2.11. Microbiological properties

Microbial counts were performed by methods using plate count agar (PCA) aerobic mesophilic and psychrotrophic bacteria, Pseudomonas agar for Pseudomonas spp., and violet red bile agar (VRBA) for coliforms. Fish samples (10 g) were aseptically taken in 90 mL of peptone water (0.1%), mixed in a sterile stomacher bag, and homogenized with stomacher (BagMixer 400, Interscience, France) at 200 rpm/min for 1 minute. The inoculated plates were incubated at 37 °C for 2 days for total viable counts, Pseudomonas spp., and coliforms. The incubation condition was 7°C for 10 days for psychrotrophic counts (29, 30). Microbial colonies were counted and expressed in log10 CFU/g.

2.12. Chemical properties

The pH of fish fillets was measured using a digital pH meter that had been calibrated with a buffer solution (pH: 4 and 7). Peroxide and thiobarbituric acid values were determined according to AOCS methods Cd8-53 and Cd-19-90, respectively (31). Total volatile basic nitrogen (TVBN), a measure of protein degradation, was applied according to the method of Goulas and Kontominas (32).

2.13. Sensory properties

A panel of seven semi-trained panelists was chosen from the University's staff based on their experience in sensory analysis. The uncoated/coated samples after cooking in a microwave at 185°C were evaluated based on sensory attributes (color, appearance, aroma, and texture) and the results were expressed on a 9-point hedonic scale. Number 1 represents the lowest score and number 9 represents the highest score (33). The sensory assessment of the samples was performed after 3 days of storage.

2.14. Statistical analysis

The experiments were carried out in a random pattern with three repetitions. The means were compared with the Duncan test at a probability level of 5% using the SPSS software version 21.

3. Results and discussion

The compounds identified in ginger essential oil by GC/MS are set out in Table 2. Among the identified compounds, α-Cedrene was the main component of essential oil. The other major constituents of ginger essential oil included Citral, Sylvestrene, α-Curcumene, Camphene. The major organic constituent reported by da Silva, da Cunha (34) and Noori et al. (16) was α-zingiberene, and these results contradict our results. The variation in the results may be due to genetic variation of the germplasm, plant age, climatic factors, and different source of origin (35).

Table 2. Chemical composition of volatile constituents of ginger essential oils by GC–MS.

Compounds	Retention time (min)	Area (%)
Camphene	12.1	10.95
L-β-pinene	13.18	0.57
β-Pinene	13.98	1.63
Thujene	14.40	0.33
Sylvestrene	15.64	12.72
Terpinolen	17.86	0.31
Linalol	18.43	0.77
Citronellal	20.45	0.29
Borneol	20.99	3.57
L-4-terpineneol	21.34	0.33
Terpineol	21.91	1.59
Cephrol	23.43	1.16
Citral b	23.89	8.44
Guaniol	24.44	1.49
Citral	25.03	13.25
Copaen	28.26	0.48
β-Elemen	28.81	0.80
α-Bergamotene	29.22	0.23
β-Cubebene	31.66	2.68
α-Curcumene	31.83	11.07
α-Cedrene	32.24	17.61
Farnesene	32.58	7.24
Elemol	33.85	0.94
Nerolidol	34.32	1.43
Total		**99.89**

3.1. The morphology and particle size of nanoemulsion

The morphology of the nanoemulsion of ginger essential oil is shown in Fig. 1. The advantages of the nanoemulsion

include the spherical morphology with narrow distribution and more nanoparticles (36). The particle size distribution of the nanoemulsion showed that the prepared dispersion had particles with a small size distribution (Fig. 2). The shape of each peak in the DLS diagram indicates the presence of particles with a broad or narrow size distribution. Therefore, a peak was observed in the size distribution of ginger essential oil nanoparticles, indicating that the suspension was enriched with particles with an average size of 15.56 nm. A similar result was obtained by Osanloo et al. (37) for Tarragon essential oil (14.5-15.6 nm). Lower particle size for blended cloves/cinnamon essential oil nanoemulsion was reported by Zhang et al. (38) that showed a steady-state with an average particle size of 8.69 nm. The particle size of ginger nanoemulsions produced through a solvent-displacement technique using Tween 20 and Arabic gum, ranged from 68 to 1,035 nm (39).

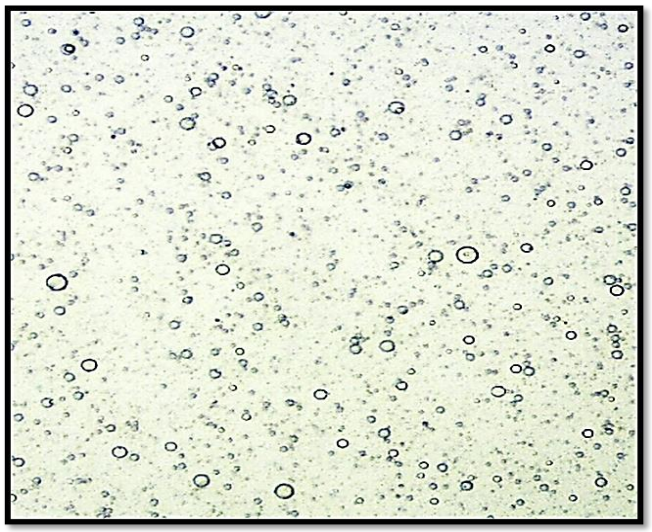

Fig. 1. Optical microscopy images of the nanoemulsions.

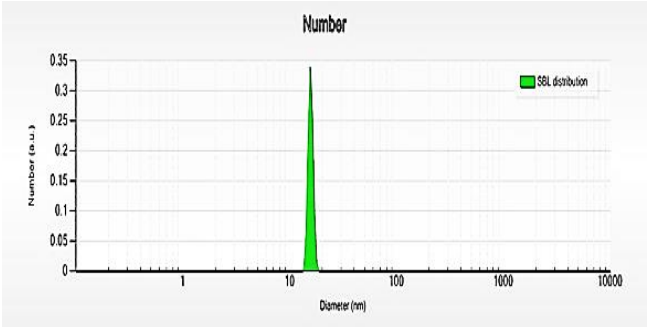

Fig.1. The particle size distribution of the nanoemulsions.

3.2. Microbiological properties

The results of microbial properties of coated and uncoated salmon fillets during the storage period are shown in Fig. 3. As can be seen, no significant difference was observed between different treatments ($p<0.05$) in 0 day. But after 3, 6, 9, and 12 days, the number of aerobic bacterial, psychrophilic, coliform, and pseudomonas count increased significantly ($p<0.05$). The coating had a significant effect on the reduction of bacteria count during storage. All treatments containing chitosan, Baneh gum, propolis extract, and ginger nanomaterial had better effects than other treatments in terms of aerobic bacterial and psychrophilic count. These results were in an agreement with Hesami et al. (40), who reported a reduction in the bacterial count with chitosan nanoparticles incorporated with *Pistacia atlantica* subsp. *kurdica* hulls' essential oil. They are also in agreement with those of Jonaidi Jafari et al. (28) reported a reduction of total number of microorganisms and *E. coli* by chitosan coating containing a 2% ethanol extract of propolis. T9, T5, and T8 that have a higher concentration of propolis extract, and ginger nanomaterial gave better results than the other treatments in reducing coliform and pseudomonas, respectively. Similar results were obtained by Noori et al. (16) in coatings containing a higher proportion of nanoemulsions in reducing the number of thermophilic bacteria. The chemical composition of ginger essential oils such as α-pinene, borneol, *eucalyptus*, and α-farnesene may be responsible for their strong antibacterial effect (14, 16, 41, 42). Research suggests that essential oils increase membrane permeability and cause microorganisms to swell and die (43). From the results obtained, it is clear that the compounds of essential oils and their synergistic role have an important effect on antibacterial activity (44). The mechanism of chitosan's antibacterial action is related to its structure and amine groups can enhance the antibacterial properties. Due to its high solubility, chitosan is more antibacterial since it penetrates the bacterial cell wall, binds to bacterial DNA, inhibits mRNA synthesis, and inhibits bacterial DNA replication and transcription (45). Chitosan also acts as a chelator and suppresses bacterial growth by adhering to structural metals. It acts as a water binder and thus prevents the activity of some enzymes (46). Similar findings have been reported in the literature about the antibacterial and antifungal effects of Baneh gum (*Pistacia atlantica*) (19, 20, 47) and propolis (48-51).

3.3. Chemical properties

3.3.1.pH

One of the variables during fish storage is pH, which can be considered as an indicator of fish freshness. The results of pH measurements in salmon fillets are shown in Fig. 4. As can be seen, no significant difference was observed between different treatments ($p<0.05$) in zero-day. But after 3, 6, 9, and 12 days, the pH increased significantly ($p<0.05$). The coating has a significant effect on lowering the pH during storage. T9 treatment had better effects than other treatments. Previous studies have indicated that nanoemulsion-based edible coatings with a composite mixture of essential oils have a better effect on lowering the pH (52, 53).

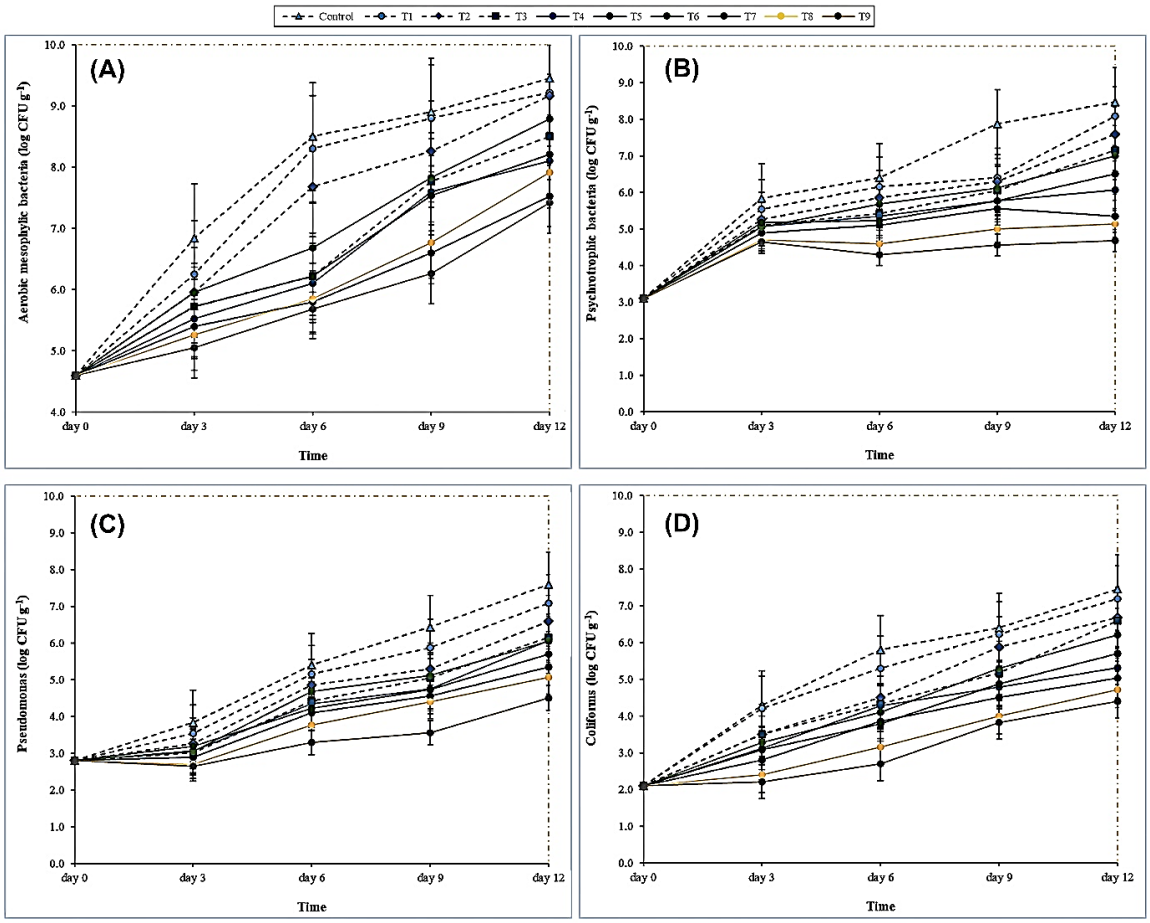

Fig. 3. Antimicrobial effects of active edible coating by chitosan and Baneh gum incorporated with propolis extract and ginger nanomaterial in salmon during 12 days storage at 4 °C. (A): Total aerobic mesophilic bacteria, (B): Total psychrotrophic bacteria, (C): Pseudomonas spp., and (D): Coliforms counts.

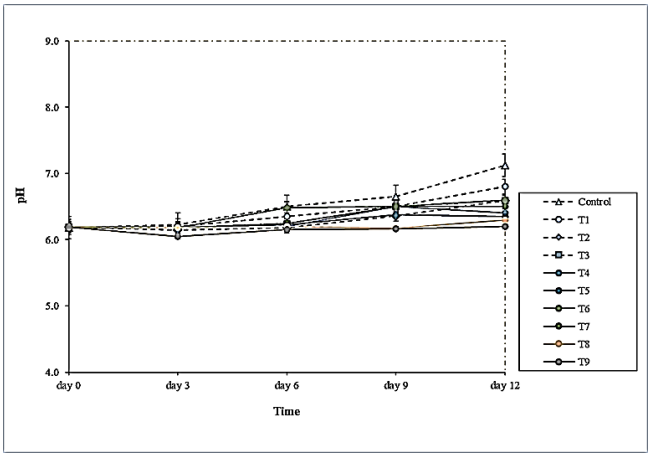

Fig. 4. pH in salmon fillet samples during 12 days.

3.3.2.Peroxide index

As a result of PV measurement, the coating had a significant effect on PV reduction during storage, and T9 treatment showed a better effect than other treatments (Fig. 5). The essential oil components involved in its antioxidant properties are α-pinene, borneol, eucalyptus, and α-Farnese. Phenolic compounds and monoterpenes also had a suppressive effect on this essential oil.

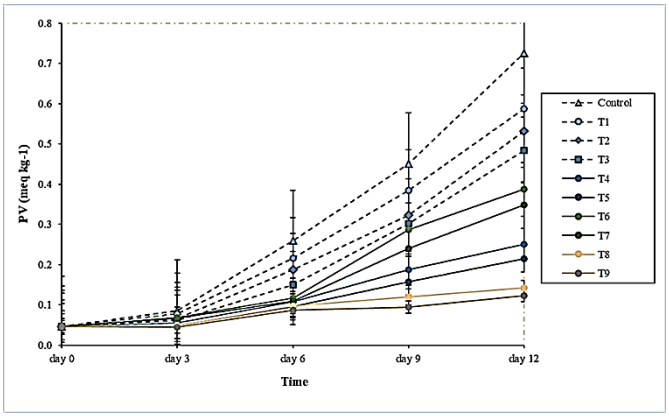

Fig. 5. PV value in salmon fillet samples during 12 days.

The antioxidant activity of these compounds has already been reported (42, 54, 55). The antioxidant activity of Baneh gum (*Pistacia atlantica*) has been reported in several studies

(19, 56, 57). Jonaidi Jafari et al. (28) reported that chitosan coating with 2% ethanolic extract of propolis has more effective in reducing PV and TBARS than other treatments. The role of propolis in oxidative stress in fish has been explained by Kakoolaki et al. (58). The results of this study demonstrated that propolis could improve some biochemical markers associated with oxidative stress in fish brains after cypermethrin exposure. Piedrahíta Márquez et al. (59) investigated the effect of chitosan-propolis edible coatings on the stability of refrigerated cachama (*Piaractus brachypomus*) vacuum-packed fish fillets and indicated that this combination has a barrier property against oxidative free radical. Spinelli et al. (60) also showed the antioxidant properties of microencapsulated propolis in fish burgers.

3.3.3.TBARS value

The results showed that coating had little effect on the reduction of TBARS after 3, 6 days (Fig. 6). At the end of storage, the TBARS value was significantly reduced compared to control samples. The results of this study are similar to those of Noori et al. (16), who reported that coating had little effect on reducing TBARS. The present study showed that all these compounds (essential oil of ginger, chitosan, Baneh gum, and propolis) played an important role in preventing oxidation and microbial growth in fish meat. Our results are in agreement with other researchers who have also reported a decrease in lipid peroxidation and microbial growth of fish using the essential oil of ginger and chitosan (61, 62).

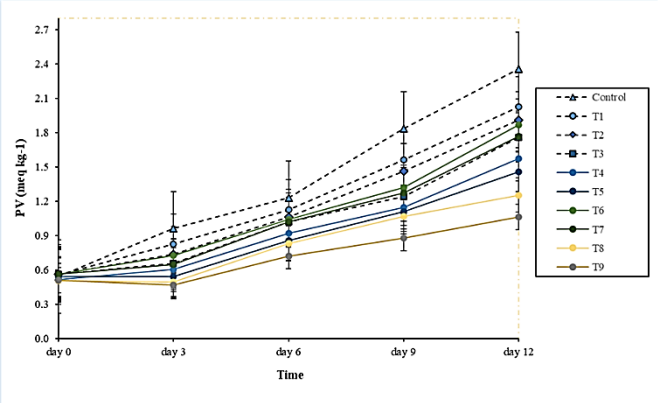

Fig. 6. TBARS value in salmon fillet samples during 12 days

3.3.4.TVBN

The results showed that coating had little effect on TVBN after 3, 6, and 9 days (Fig. 7).

3.4. Sensory evaluation

Sensory evaluation of salmon fillets after cooking at 180°C for 45 min on the third day of storage is shown in Fig. 8. The results of color and appearance evaluation show no significant difference between T4, T5, T8, T8 treatments ($p<0.05$). However, these scores were shown a significant difference compared to control samples ($p< 0.05$). The highest color and appearance scores for evaluators were assigned to treatments T1, control, T2. T6 and T7 respectively. The highest texture scores were attributed to treatment T9 and T8, followed by treatments T4 and T5.

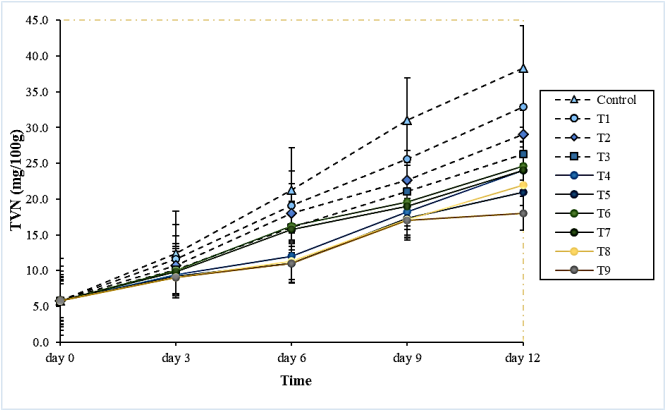

Fig. 7. TVN in salmon fillet samples during 12 days storage.

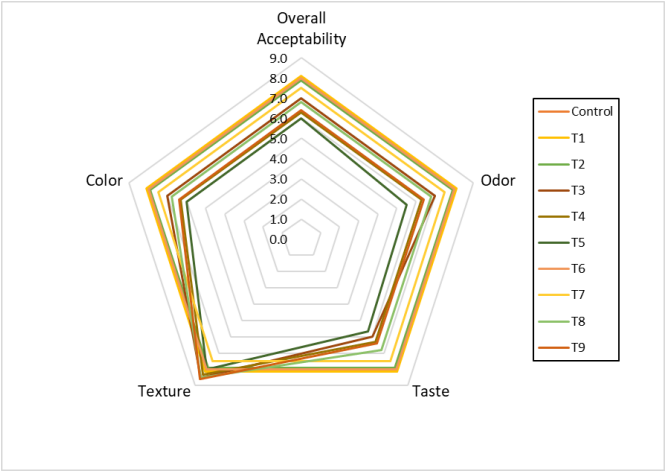

Fig. 8. Sensory evaluation of salmon fillet samples on the third day.

The highest score was reported to be associated with samples containing chitosan and Baneh gum. Therefore, the odor and appearance of the nanoemulsion-coated samples were also improved by controlling microbial spoilage. In a study by Jonaidi Jafari et al. (28) despite improved microbial and chemical properties, the control group received the highest rating, and the sample containing chitosan coating and 2% propolis extract received the lowest acceptance.

4. Conclusion

In this study, fresh fish fillets were coated with edible coatings of chitosan (0 and 2%), Baneh gum (0, 1 and 2 %), propolis extract (0, 1 and 2%), and nanoemulsion of ginger essential oil (0, 0.5 and 1 %) and the shelf life of fillets during 12 days refrigeration was evaluated. It transpired that the coating had a significant effect on reducing the number of

bacteria in storage. Moreover, coated samples had lower pH and PV than uncoated samples. During the sensory evaluation, it was found that the chitosan coating with Baneh gum can maintain or improve the sensory properties and extend the shelf life of refrigerated fish. According to the results, the coating can be used to maintain quality characteristics and extend the shelf life of fresh salmon fillets in the refrigerator.

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