The shelf life extension of refrigerated *Nemipterus japonicas* fillets by chitosan coating incorporated with propolis extract

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Abstract

In this study, chitosan coatings incorporated with propolis extract (PE) were utilized to extend the shelf-life of refrigerated *Nemipterus japonicas* fillets. Microbiological (total mesophilic bacteria (TMB) and psychrotrophic (PTC) count), physicochemical (pH, TVB-N, TBARS, FFA) and sensory (color, odor and preference) characteristics of coated fillets were evaluated during 12 days. The results showed that incorporating PE into chitosan could significantly (p<0.05) improve the quality of fish fillets. The results showed that the chitosan+PE samples reduced the extent of lipid oxidation, as judged by TBARS and FFA, suggesting that there is a synergistic effect between chitosan coating and propolis. Moreover, the application of chitosan coatings with PE improved the TVB-N and pH values of the *Nemipterus japonicas* samples significantly compared to untreated sample, thus extending the shelf life of *Nemipterus japonicas* fillet approximately >10 days. All wrapped fillets in treatment samples incorporated with PE had lower bacterial counts compared to control (p<0.05). However, the bacterial counts of chitosan coating combined with PE were lower than those treated by chitosan coating or PE alone during the storage. The fillets treated with chitosan and PE had the best sensory properties. The increase (>10 days) in the shelf-life was attributed to the antioxidant and antimicrobial characteristics of chitosan and PE. These results confirmed that chitosan coating enriched with PE can be an improving method to reducing deterioration of refrigerated *Nemipterus japonicas* fillets.

Keywords: *Nemipterus japonicas*, Chitosan, Propolis extract, Coating, Shelf life.

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Introduction

Japanese thread fin bream (*Nemipterus japonicas*) is one of the demersal species belonging to the family of Nemipteridae, mainly caught in the Red Sea, East coast of African, all parts of the Persian Gulf, North Coast of the Oman Sea, Mediterranean Sea, Indian Ocean, Pacific Ocean (Russell, 1990; Golani and Sonin, 2006; Nematollahi *et al*., 2018). Japanese thread fin bream has become one of the seafood resource which enthusiastically welcomed by consumers in the south of Iran due to its light flesh color, low fat content and high protein content (Nurnadia *et al*., 2011). Japanese thread fin bream is commonly sold for immediate consumption at local stores as whole (ungutted) fish, while chilling storage has been widely used to extend its shelf life. Fish is very susceptible to lipid oxidation during refrigerated storage and it produces hydroperoxides that are readily decomposed into to secondary products. Secondary products (such as aldehydes, ketones and carboxylic acids) can lead to the formation of off-odor and off-flavor, texture and color changes in seafood, even when produce in low content. Moreover, loss of quality and spoilage of seafood products occur as a result of bacteriological activity and produce metabolites that result in off-odors and off-flavors (Gram and Huss, 1996). Therefore, the use of suitable preservation methods such as active packaging and natural antioxidants can protect the quality of fish products against oxidation reaction. Combination of chilling along with active packaging and natural food additives has been applied to augment the shelf life. In last decade, various biopolymer coatings alone/ or in combination with plant additives have been tried by researchers to improve the shelf life of fish products. Among edible coatings, chitosan is hydrophilic one with strong antimicrobial and antioxidant activities against food born pathogen and free radicals (Nowzari *et al*., 2013), and have been used successfully in food industry because of edibility, biodegradability, aesthetic appearance and barrier properties, being nontoxic and non-polluting, as well as carrier of foods additives. Propolis is a natural additive that is collected from leaves, flowers buds, stems, and cracks in the bark of some tree species, to which bees add saliva, wax, and pollen to elaborate the final product (Ghisalberti, 1979). Propolis extract is prepared by three methods including ethanol, glycerol, and/or water (Pobiega *et al*., 2019)., that ethanol extract is useful for extracting of propolis because of low content of wax (Mello *et al*., 2010; Mello and Hubinger, 2012). Propolis extract (PE) contains the major source of polyphenols such as flavonoids, phenolic acids and their esters and has various biological effects, including antimicrobial and antioxidant properties (Al-Naggar *et al*., 2016; Seibert *et al*., 2019). The use of PE in food preservations is often limited because its volatile aroma compounds can be influenced the unique flavor and odor (Pobiega *et al*., 2019). Therefore, it is

very important to determine the concentration of PE that can be applied without changes of sensory characteristics of the food products. A minimal addition of PE for acceptance sensory of food product was determined ≤0.5% by several researchers (Duman and Özpolat, 2015; Pobiega *et al*., 2019). Spinelli *et al*. (2015) showed that the PE could be used as an effective antioxidant on fish burgers (Spinelli *et al*., 2015). Moreover, Payandan *et al*. (2016) reported the antibacterial activity of PE on minced common carp against Gram-positive and Gram-negative bacteria such as lactic acid bacteria (LAB) and *Staphylococcus aureus* (Payandan *et al*., 2017). Previous studies have shown the in vitro antibacterial activity of PE against several Gram-positive and Gram-negative bacteria (Boyanova *et al*., 2006; da Silva *et al*., 2006; Kalogeropoulos *et al*., 2009; Al-Naggar *et al*., 2016; Silva *et al*., 2018). In food industry, beneficial effects of chitosan in combination with PE has been studied by Jonaidi Jafari *et al*. (2017) and Rollini *et al*. (2017). Moreover, Piedrahíta Márquez *et al*. (2019) reported synergistic antimicrobial and antioxidant effects by combining PE and chitosan against spoilage bacteria and lipid oxidation of cachama (*Piaractus brachypomus*) (Piedrahíta Márquez *et al*., 2019). A mixture of fish chitosan in combination of PE would seem to be suitable to protect seafood products. A little published data exist on the associated usage of chitosan coatings in combination with PE for fishery products. Therefore, the aim of the present assay is to investigate a comparative basis antimicrobial and antioxidant effect of chitosan coating enriched with PE on quality of Japanese thread fin bream (*Nemipterus japonicas*) fillets in refrigerated condition (4±1˚C).

Material and methods

Preparation of propolis extract and sample preparation

The propolis was cut into small pieces, and 1 gr of ground propolis was extracted with 10 ml of 70% ethanol by shaking at 150 RPM at 25°C for 48 h. The ethanolic extract of propolis was filtered (Whatman N0. 4 filter paper) and concentrated under vacuum with a rotary evaporator (IKA, RV 3 V, Germany) at 50 °C and the extracts were freeze dried. The samples were stored in the dark at 4°C before use (Yaghoubi and Satari, 2007).

Chitosan solution was prepared with 1% (w/v) chitosan (Sigma Chemical Co., medium molecular weight, viscosity 200-800 cP) in 1% v/v acetic acid. To achieve complete dispersion of chitosan, the solution was stirred at room temperature to dissolve completely. Glycerol was added at 0.75 mL/g concentration as a plasticizer and stirred for 10 min (Ojagh *et al*., 2010). The freeze dried ethanolic extract of propolis was added to the coating at 0.1% concentrations and homogenized for 10 min by magnetic stirrer. Fillet samples were randomly assigned into four treatment lots consisting of: one control lot (un-coated), two lots coated

in chitosan, three lots coated in PE, four lots treated with chitosan coating in combination with PE. To create coating, fillets were soaked in coating solution containing 0.1% propolis extract for 30 s, then the fish fillets were removed (Ojagh *et al*., 2010). The coated samples were allowed to drain for 5 min under a biological safety cabinet. All samples were placed in polyethylene bags, stored at 4±1˚C for 12 days. Microbiological, physicochemical, color and sensorial analysis were performed at 3-day intervals.

Microbiological analysis

Samples were collected aseptically. The minced fillet (25g) was placed in a Stomacher bag (Stomacher® 400 Circulator, West Sussex, UK) containing 225 mL of 0.85% saline water. After mixing for 2 min in a Stomacher blender, further serial dilutions were done using the same diluent. Thereafter, 0.1 mL of the appropriate dilution was used for microbiological analysis using the spread plate method. The media and conditions used were plate count agar (PCA, Merck, Darmstadt, Germany) incubated for total psychrotrophic count (PTC) at 4°C for 10 days and for total mesophilic count (TMC) at 30°C for 24-48 h. The microbial count was expressed as $\log_{10}$ CFU/g (Sallam, 2007).

Physicochemical analysis

Determination of pH

The measurement of pH was carried out on 5 g of sample homogenised in 45 mL distilled water. The pH value of the sample was determined using a digital pH meter (Suvanich *et al*., 2000).

Determination of total volatile base nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) value was estimated by the micro-diffusion method [27]. The micro diffusion method was determined by distillation after the addition of MgO to homogenised fish sample. The distillate was collected in a flask containing aqueous solution of boric acid and methyl red as an indicator. Afterward, the boric acid solution was titrated with sulphuric acid (H_2SO_4) solution. The TVB-N value (mg N/ 100 g of fish) was determined according to the consumption of sulphoric acid. The constant 14 was used to calculate the TVB-N number using Eq. 1.

TVB-N value = 14×V (1)

V=mL of sulphuric acid (H_2SO_4) solution for titration

Determination of 2-thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid (TBA) measurment was determined following Siripatrawan and Noipha (2012) with some modification. 10 g of homogenised sample were added with 97.5 ml of distillated water and 2.5 ml of 4 M HCl. The mixture was heated with steam distillation. 5 ml of distillate was added

to 5 ml of thiobarbituric reactive reagent containing 0.02 M TBA in 90% glacial acetic acid and incubated in boiling water for 35 min. After cooling, the absorbance of the pink solution was measured at 532 nm using spectophotometer. The constant 7.8 was used to calculate the TBA number using Eq. 2. The TBA value is expressed as mg malonaldehyde equivalents/ kg sample.

TBARS value = 7.8 Abs_{532} (2)

Determination of free fatty acid (FFA)

The free fatty acid value was determined in the lipid extract by the procedures of Woyewoda *et al*. (1986) according to the Eq. 3. Results were expressed in % of oleic acid.

Color measurement

The color of the fish samples was measured by a Hunter lab color meter (Shanghai Precision & Scientific instrument Co., Ltd., Shanghai, China) and was reported by the CIE system. The colorimeter was calibrated with a white standard. In this system, L^* represents the color lightness on a 0-100 point scale from black to white; a^* is the position between red (+) and green (-); and b^* is the position between yellow (+) and blue (-).

Sensory evaluation

Sensory analysis of raw *Nemipterus japonicas* fillets was carried out using the quality index method (QIM) as shown in Table 1 by 13 panelists. Scores were given for each quality attributes according to descriptions, ranging from 0 to 5. On the first and last days of storage (day 0 and day 112), *Nemipterus japonicas* received a freshly: QI (quality index) =0 and completely deteriorate: QI=5. A preference score of 3 was viewed as the threshold for acceptable quality.

Table 1: Sensory evaluation criterions of *Nemipterus japonicas* fillets by chitosan coating incorporated with propolis extract.

Quality attributes	Characteristics	Score
Color	Bright/ Glossy/Brightness intense	5
	Slightly bright/discoloration on the side/ Brightness loss/Partially glossy	4
	Slightly bright/ Dull/pale	3
	Loss of glossiness/ a little yellow surface	2
	Loss of glossiness/opaque/ yellow surface/ opaque	1
Odor	Fresh/Specific species	5
	Destruction of fish specific smell/neutral	4
	Low rancidity	3
	Medium rancidity	2
	High rancidity/ Stinking	1
Overall acceptance	Bright/Vivid and defined /Glossy/Brightness intense/ fresh/totally acceptable	5
	Slightly bright/discoloration on the side/ Brightness loss/Partially glossy/neutral/acceptable	4
	Loss of glossiness/Dull/pale/ opaque/ reluctant acceptance	3
	Not fresh/ Rancid/Slightly ammonium/unacceptable	2
	Not fresh/ Putrid /otally unacceptable	1

Statistical analyses

All experiments were performed in triplicate and a completely randomised design was used. Mean values ± standard deviation was reported for each case. Significant differences between means were determined by one-way analysis of variance (ANOVA) using SPSS18.0 for Windows. Duncan's test was used to compare the means. A significance level of $p<0.05$ was used. Pearson correlation analysis was used to determine the relationship between bacterial counts and physicochemical values. P values less than 0.05 were considered statistically significant. Friedman test were performed for analyses of the non-parametric data (sensory analysis).

Results and discussion

Changes in bacteriological changes

Total mesophilic bacteria (TMB) and psychrotrophic (PTC) counts of *Nemipterus japonicas* fillets of different treatments stored at 4°C for 12 days is shown in Figure 1.

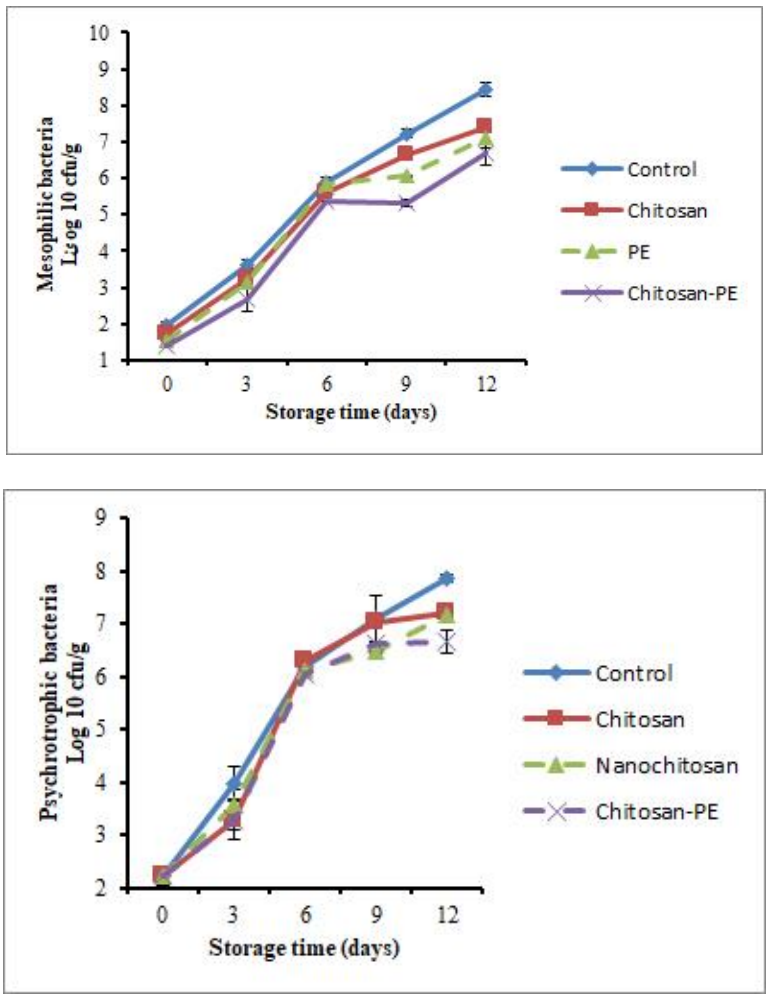

Figure 1: Combined effect of chitosan and propolis extract on microbiology properties (Mesophilic and psychrotrophic bacteria) of *Nemipterus japonicas* during storage at refrigerator. Mean values and standard errors from the three replicates are presented.

The initial TMB (log_{10} CFU/g) in the all samples of *Nemipterus japonicas* fillet varied from 1.42 to 1.95 log_{10} CFU/g, indicating a good quality of fisheries products (Sikorski *et al.*, 1990). The initial PTC of fresh fillets was 2.24 log CFU/g. The lower bacterial counts of coated treatments compared to control samples can be due to the antibacterial activity of chitosan and propolis, resulting in lower microbiological growth (Pereda *et al*., 2011; Pobiega *et al*., 2019). All of the treatments led to a dramatic reduction in TMB and PTC in *Nemipterus japonicas* fillet compared to the control samples. By the day 9 of storage, TMB in *Nemipterus japonicas* fillet were reached to about 7 log_{10} CFU/g for control samples, which is higher than the maximal recommended limit in raw fish (Sallam, 2007), showing a microbiological shelf life about 7 days for the control samples. However, samples treated with chitosan or PE achieved this count at the end of the storage time. TMB values for chitosan+PE coatings did not exceed the maximal permissible limit at the end of the storage period. Kakaei and Shahbazi (2016), Mohebi and Shahbazi (2017), and Berizi *et al*. (2018) showed that chitosan containing plant extract could extended the shelf life of seafood during storage (Kakaei and Shahbazi, 2016; Mohebi *et al*., 2017; Berizi *et al*., 2018). The positively charged on the NH_3^+ group of glucosamine monomer in chitosan molecules interact with negatively charged macromolecules on the microbial cell surface, leading to the leakage of intracellular constituents of the microorganisms (Mohebi and Shahbazi, 2017). Furthermore, Disruption of the lipopolysaccharide layer of the outer membrane of gram-negative bacteria by chitosan and its function as a barrier against oxygentransfer are shown by Pereda *et al*. (2011) and Jeon *et al*. (2002). Moreover, Propolis extract has antimicrobial activity against a wide spectrum of microorganisms (Khodabakhshi *et al.*, 2019). Results are in accordance with other studies reporting a reduction in TMC and PTC with the addition of plant extracts in seafood (Van Haute *et al.*, 2016; Rezaei and Shahbazi, 2017). Hassanin and El-Daly (2013) and Payandan *et al.* (2016) reported to be effective as antimicrobial agents on inhibition of the growth of spoilage bacteria of fishery products (Hassanin and El-Daly, 2013; Payandan *et al.*, 2016). Thus, the incorporation of PE in chitosan coating can remarkably delay the growth of bacteria. Jonaidi Jafari *et al*. (2017) showed that chitosan edible coating containing Propolis extract could extended the shelf life of chicken fillet during storage at refrigerator (Jonaidi Jafari *et al*., 2017). The antimicrobial activity of propolis extract has been ascribed to the presence of bioactive compounds such as flavonoids (e.g., tectochrysin, pinobanksin, pinocembrin, chrysin, galangin, apigenin, kaempferol), which show antibacterial effect (Pobiega *et al*., 2019). Moreover, the *in vitro* and *in vivo* antimicrobial activity of propolis against bacteria has been reported by

Seibert *et al*. (2019) and Khodabakhshi *et al*. (2019). Duman and Özpolat (2015) stated how bacterial growth in PE coated *Barbus grypus* reached less than that uncoated samples (Duman and Özpolat, 2015). The result of the present study indicated that the propolis extract used in fresh fillet coated with chitosan leads to a reduction in microbial contamination during long storage time.

Changes in physicochemical changes

Changes in pH value

Figure 2 shows the changes in pH value of *Nemipterus japonicas* fillets during refrigerated storage with different treatments. The initial pH value of *Nemipterus japonicas* fillets was between 6.16 and 6.33. According to He and Xiao (2016), the initial pH value of fish varies between 6.0 and 7.0. Such variations in the chemical composition of fish is related to the species, diet, season, and the level of activity or stress during the catch as well as the type of muscle (He and Xiao, 2016). During storage, the pH values gradually increased for control sample and samples treated by chitosan/ or PE and chitosan+PE. Storage time had a significant (p<0.05) effect on the pH values until the end of the storage period (day 12). However, no significant is observed for coated samples with chitosan+PE (p>0.05) during storage. The increase of pH might be attributed to accumulation of alkaline compounds (such as biogenic amines and ammonia) generated from both endogenous enzymes and enzymatic actions of psychrotrophic bacteria (Nirmal and Benjakul, 2011; Songa *et al*., 2011). The pH values of samples coated with treated by chitosan/ or PE and chitosan+PE were consistently lower than that of the control sample during storage. It was found that controls samples had higher pH than coated treatments (p<0.05). Overall in terms of the whole storage period, significant difference was observed in the pH values between control (7.62) and coated samples with chitosan+PE (p>0.05). Piedrahíta Márquez *et al*. (2019) reported that chitosan alone or in combination with propolis could decrease pH values of cachama (*Piaractus brachypomus*). Moreover, Jonaidi Jafari *et al*. (2017) showed that chitosan+propolis coating is effective in reducing pH values on chicken breast meat (Jonaidi Jafari *et al*., 2017). Berizi *et al*. (2018) showed that chitosan in combination with natural additives could notably decrease pH value in food product (Berizi *et al*., 2018) The pH value is positively correlated with TVB-N as increase in volatile amine lead to increase of pH which is showed in this research. Moreover, There was a positive correlation between TMC and pH in treated samples (R^2=0.483-0.947) and controls (R^2=0.905).

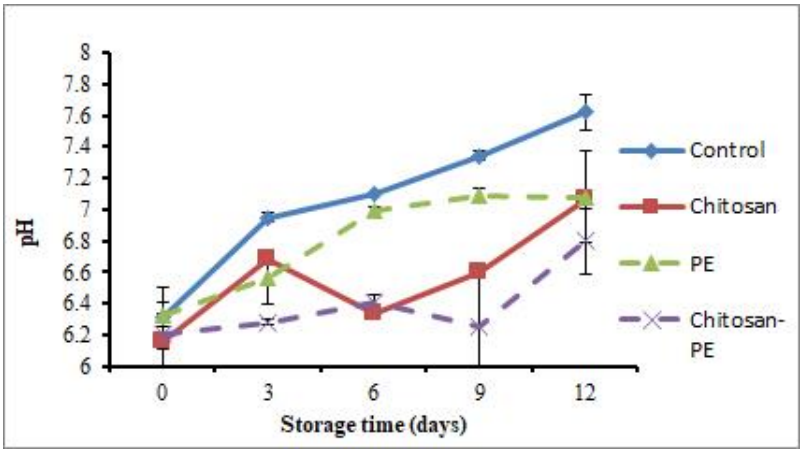

Figure 2: Combined effect of chitosan and propolis extract on pH content of *Nemipterus japonicas* during storage at refrigerator.
Mean values and standard errors from the three replicates are presented.

Changes in TVB-N value

Changes of TVB-N content of all samples during the storage are shown in Figure 3. The initial TVB-N varied from 5.93 mg N /100 g in samples treated with chitosan to 6.86 mg N /100 g in control samples. As shown in Figure 3, TVB-N content of fresh *Nemipterus japonicas* fillets progressively increased along with the time of storage for both control and coated samples. At day 9 of storage, the TVB-N value of control was higher than the others and the lowest value was 18.27 mg N /100 g in the samples coated with chitosan+PE at the end of the storage. The increase of TVB-N is probably due to the activity of endogenous enzymes, spoilage bacteria and the subsequently increase of microbial degradation products including ammonia, primary, secondary, and tertiary amines (Kakaei and Shahbazi, 2016). There was a positive correlation between TMC and TVB-N in treated samples (R^2=0.774-0.897) and controls (R^2=0.911). Based on our findings, the samples coated with chitosan+PE delayed the increase speed of TVB-N compared to the other treatments (p<0.05) due to the presence of bioactive phenolic compounds in propolis extract, which could remarkably inhibit capacity of bacteria for oxidative deamination of non-protein nitrogen compounds. It can be concluded, chitosan coating in combination with PE is more effective than coating alone in controlling TVB-N of fillets, suggesting that chitosan coating demonstrated synergism in retarding of TVB-N value when used in combination with PE. Similarly, Payandan *et al*. (2017), Hassanin and El-Daly (2003), and Bodini *et al*. (2013) reported that presence of propolis extract could be effectively inhibit the increase of microbial count (Payandan *et al*., 2017; Hassanin and El-Daly, 2003; Bodini *et al*., 2013).

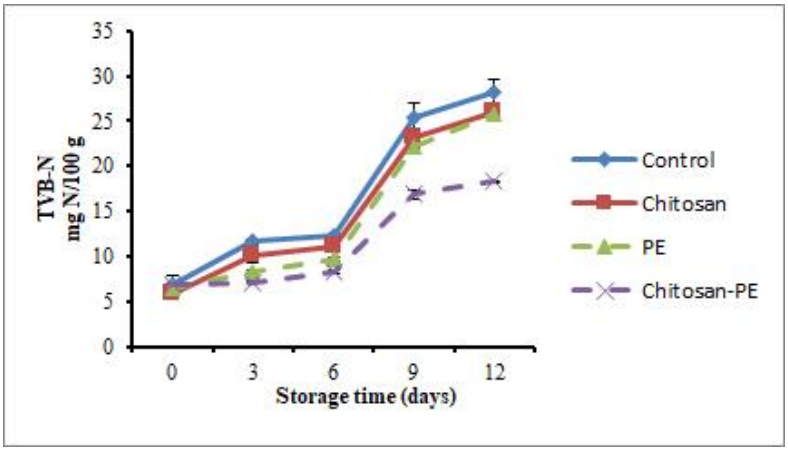

Figure 3: Combined effect of chitosan and propolis extract on TVB-N content of *Nemipterus japonicas* during storage at refrigerator.
Mean values and standard errors from the three replicates are presented.

Jonaidi Jafari *et al*. (2017) also found lower TVB-N contents in treatments of chicken muscle with chitosan coating containing ethanolic extract of propolis compared to control sample (Jonaidi Jafari *et al*., 2017. Piedrahíta Márquez *et al*. (2019) reported that chitosan coatings combination with propolis extract were highly efficient in the prevention of microbiological development in cachama (*Piaractus brachypomus*), resulting in lower TVB-N compared with other treatments (Piedrahíta Márquez *et al*., 2019). According to the highest acceptable level of TVB-N (25 mg N/100 g fish muscle) (Kilincceker *et al*., 2009), the control, fish coated with chitosan/ or PE were spoiled on days 9 and 12, respectively. However, TVB-N value of samples coated with chitosan+PE was less 25 mg N/100 g fish sample after 12 days.

Changes in TBARS value

Changes in TBARS value of *Nemipterus japonicas* are shown in Figure 4. The initial TBA values were ranged from 0.01 mg malonaldehyde/kg sample to 0.03 mg malonaldehyde/kg sample. During storage, TBARS value of the control samples were significantly higher than other treatments (p<0.05), which are in accordance with the results reported by Jonaidi Jafari *et al*. (2017) and Piedrahíta Márquez *et al*. (2019). The increase in TBARS of control samples during storage may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids (Al-Qurashi and Awad, 2018). Generally, no significant difference was presented in the TBARS values of chitosan, PE and chitosan+PE samples throughout the storage time.

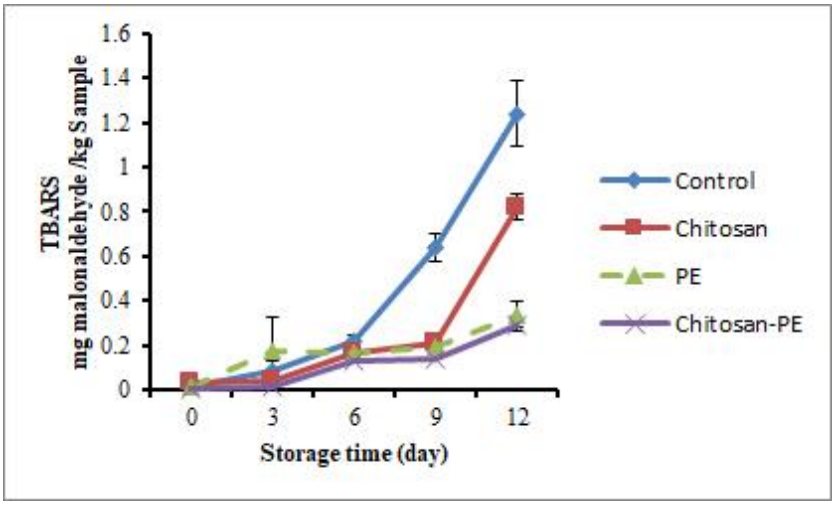

Figure 4: Combined effect of chitosan and propolis extract on TBARS content of *Nemipterus japonicas* during storage at refrigerator.
Mean values and standard errors from the three replicates are presented.

This showed that chitosan coatings alone or with PE as natural antioxidant can be used to retard lipid oxidation in *Nemipterus japonicas* fillets during storage. According to Jeon *et al.* (2002), chitosan coatings on herring and cod fillets had lower contents of TBARS than those of the uncoated samples during storage time (Jeon *et al.*, 2002). This protection against oxidation of lipid in fish samples may be associated to the synergism between the coating properties of an oxygen barrier and the antioxidant activity of the chitosan (Ojagh *et al.*, 2010; Antoniewski *et al.*, 2007). The addition of PE to chitosan-coated samples can increase the antioxidant activity of the chitosan. However, in this study, there was no significant difference in the TBARS content of fillet between chitosan and chitosan-PE coated samples. The antioxidant mechanism of propolis could be through radical scavenging capacities with antioxidant agent (polyphenolics compounds such as flavonoids and cinnamic acid devrivatives), and /or protection of antioxidant food ingredients through forming a biodegradable coating (oxygen barrier properties) treated samples during storage (Pobiega *et al*., 2019; Al-Qurashi and Awad, 2018). A lower TBARS content for rainbow trout fillets treated by propoils extract was reported by Adnani *et al*. (2017). Spinelli *et al*. (2015) added 5% spray-dried propolis to a suitable fish-burger formula and showed lower lipid oxidation than the control because of higher phenol content and higher sequestrating activity on 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Spinelli *et al*., 2015). In another study, the lipid oxidation of the burger meat was reduced by using microencapsulation propoils extract (Reis *et al*., 2017). According to our study, similar effects were observed. TBARS values of 1 to 2 mg malonaldehyde/kg muscle are an

acceptable sensory limit (Shakila *et al*., 2005). In the current study, TBARS values for all samples were less than 2 mg malonaldehyde/ kg muscle at the end of the storage. This indicates that the PE with or without chitosan coatings are able to reduce the lipid oxidation, resulting in lower rancidity compared with other samples.

Changes in FFA value

Low initial FFA values (0.30 to 0.36 % of oleic acid) suggested that the fillets were good quality (Fig. 5), which is in agreement with the relatively low PTC of 2.24 log CFU/g. FFA value gradually increased in all samples over the time, which could be due to the enzymatic hydrolysis of fish fats during storage at refrigerator (Serdaroğlu and Felekoğlu, 2005). According to our results, FFA value in the samples treated by propolis coatings, chitosan coatings with or without PE was significantly lower than control group (p<0.05). This reduction may be due to antioxidant and antibacterial effect of propolis extract (phenolic and flavonoid compounds of propolis) and chitosan, which could reduce of free radicals and secondary compounds of lipid oxidation such as TBARS, as well as psychrotrophic bacteria population. There was a positive correlation between PTC and FFA in treated samples (R =0.761-0.828) and control ones (R=876). Antioxidative feature of propolis was demonstrated in the study of Seibert *et al.* (2019) which is in agreement with our research (Seibert *et al.*, 2019). According to Pobiega *et al*. (2019), there is significant relationship between the phenolic compounds and free-radical scavenging activity of PE (Pobiega *et al*., 2019). Some researchers indicated that chitosan coating has positive effects on reducing FFA (Ojagh *et al*., 2010; Nowzari *et al*., 2013).

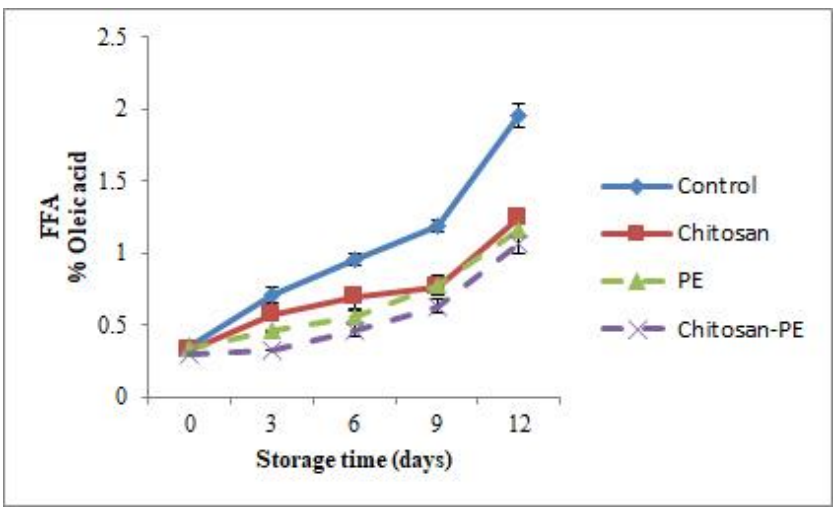

Figure 5: Combined effect of chitosan and propolis extract on FFA content of *Nemipterus japonicas* during storage at refrigerator Mean values and standard errors from the three replicates are presented.

Changes in color

Color values of *Nemipterus japonicas* fillets coated with and without PE during refrigerated storage for 12 days are shown in Figure 6. *Nemipterus japonicas* fillets of control and chitosan+PE samples showed a decrease in L* values with increasing storage time, which is associated with oxidation of red oxymyoglobin to metmyoglobin, yielding muscle with an unattractive brown color. However, Lightness of the chitosan and / PE treatments did not change significantly over time. The samples coated with chitosan+PE had lower changes in L* values compared to the control, chitosan and PE (p<0.05). The increase in lightness of chitosan+PE compared to other treatments may be explained by the protein denaturation and hence decreasing in bond water (Kamani *et al*., 2017). In all samples, a significant increase in a* values was observed throughout the storage time, indicating the increase in redness of samples. The highest change was observed in control samples, while coated fillets showed a slight increase in a* values, compared to control samples. Thus the application of chitosan coatings either without or with PE, on fish fillet surface could increase the stability of the red meat color to some extent. The increase in b* value was possibly associated with the increase in TBARS value. Samples coated with chitosan/PE and chitosan+PE showed the lower changes in color values, probably due to the antioxidant and antimicrobial properties of chitosan and PE. Thus, chitosan coatings and PE could retard the color changes of fillets to some extent during the storage. At day 0 of storage, b* values were not significantly different between treatments. Yellowness of the control, chitosan and PE did not change significantly over time. Nevertheless, the chitosan+PE application techniques influenced a slight decrease in yellowness during storage compared to the initial b* values. These results showed that chitosan coatings containing PE were capable of retarding discoloration of fish fillets during storage.

Sensory analysis

The effect of chitosan coating incorporated with PE on the general aspect of *Nemipterus japonicas* fillet is shown in Figure 7. Sensory attributes of fish were divided into 3 elements including color, odor and overall acceptance, whose scores were given for each quality attributes according to descriptions, ranging from 1 to 5, the higher preference level, the higher element score. At day 0, all samples had the score 5 for attributes tested, but the scores decreased significantly with extended time (p<0.05), with slower decrease for the chitosan coating fillets incorporated with PE. The fish with scores of below 3 were unacceptable with a sign of putrid odor, no shiny color and overall unacceptability.

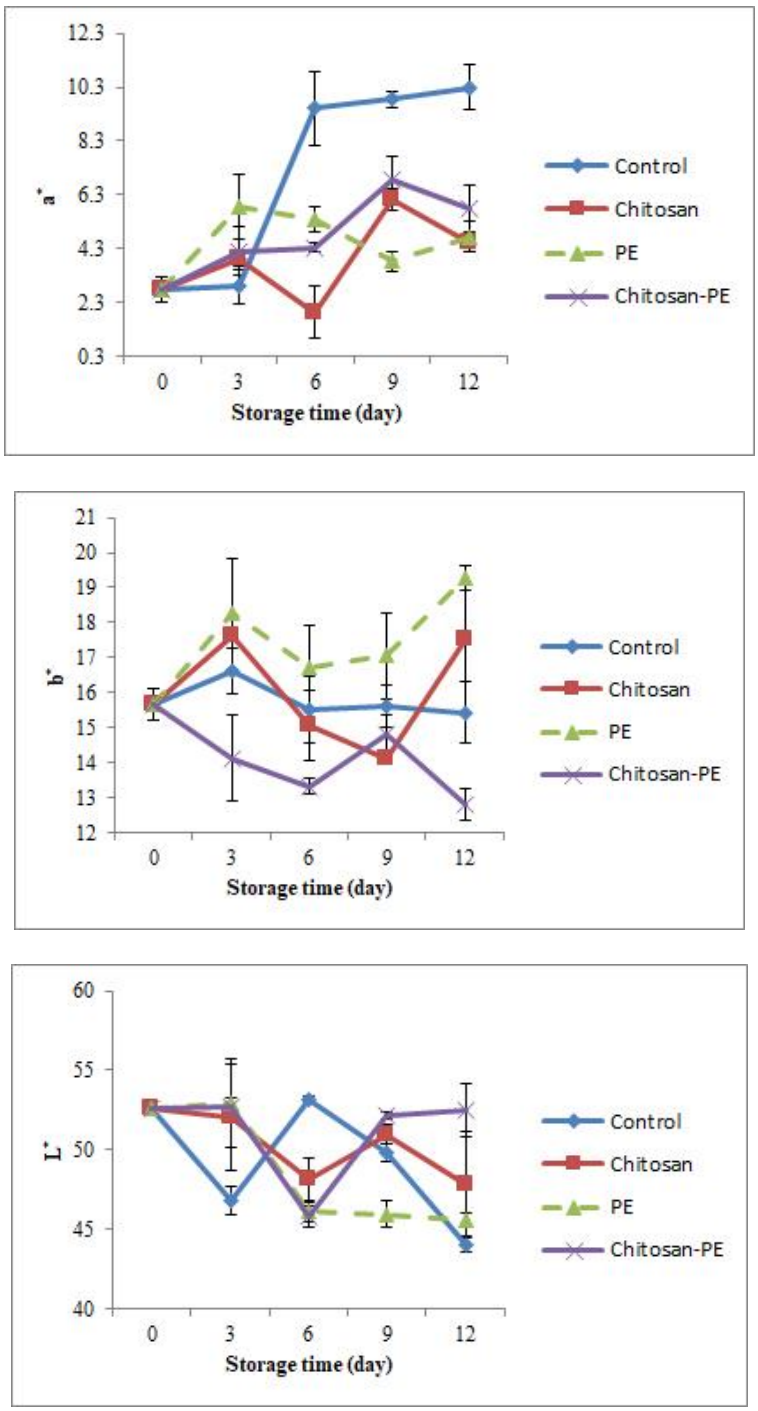

Figure 6: Combined effect of chitosan and propolis extract on color changes of *Nemipterus japonicas* during storage at refrigerator.

The increasing off-odor of uncoated samples could be smelt from 6th days backwards and the fish fillet was unaccepted after 9 days of storage (score=1). However, samples coated with chitosan+PE obtained higher scores than PE/or chitosan-based coated fillets during storage, suggesting that PE and chitosan can improve organoleptic quality of refrigerated fillets.

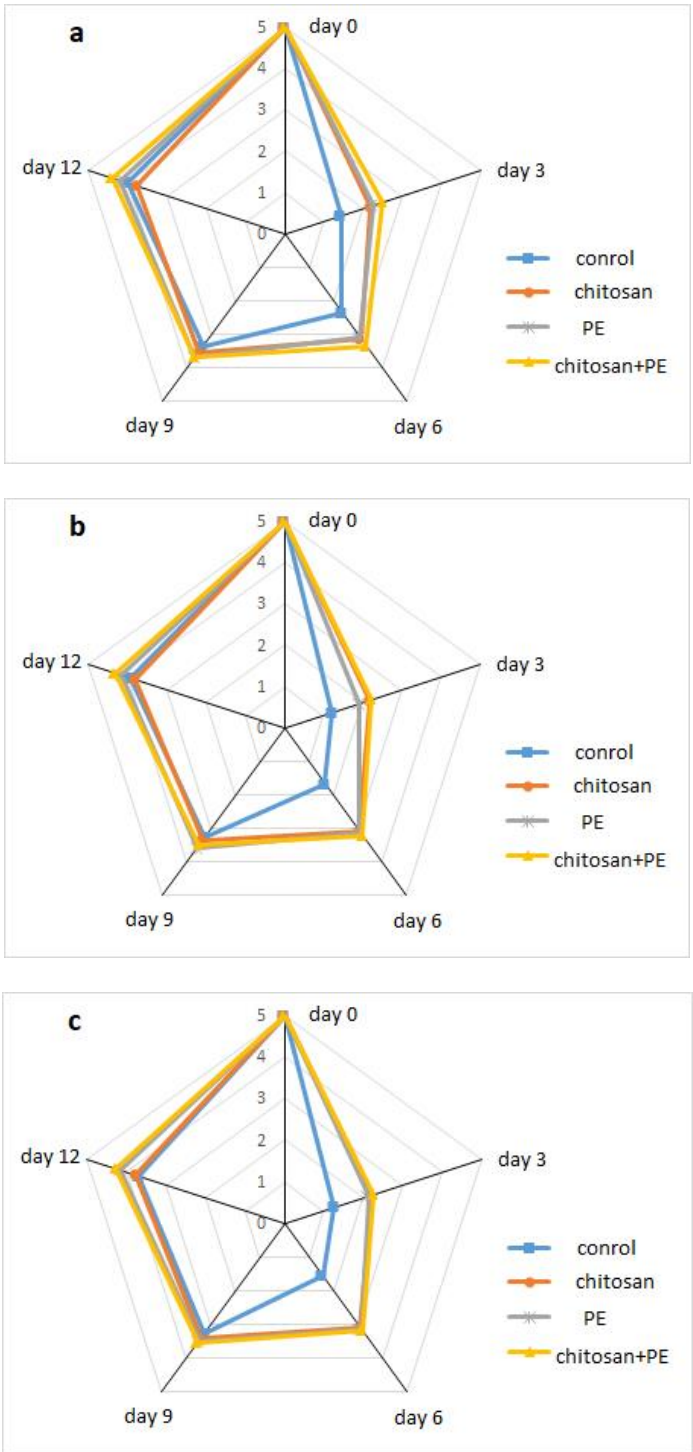

Figure 7: Changes in a) color; b) odor; c) overall preference of *Nemipterus japonicas* during storage at refrigerator.

The deterioration of the organoleptic quality was correlated with the microbial spoilage and physicochemical analysis, caused the change of surface of color of samples (Hui *et al*., 2016). The antioxidant and antimicrobial effects of chitosan and PE has been shown to extend the shelf life of fish approximately 3 days for chitosan, PE and chitosan+PE coated samples. This

result was in agreement with that of TVB-N values and bacterial counts, which suggested that coated treatments was effective to delay sensory changes of fish fillet.

Conclusion

The results presented in this study indicated that chitosan coating incorporated with PE of *Nemipterus japonicas* fillet effectively retarded the growth of total mesophilic bacteria and psychrotrophic bacteria. This study showed that chitosan in combination with PE treatment could effectively inhibit lipid oxidation and protein decomposition, and could improve color, and sensory attributes within acceptable limits during the storage, and could extend the shelf life of fish fillets for >10 days compared with the control. Therefore, natural antioxidant and antibacterial (chitosan and PE) may be a promising method of maintaining the storage quality of *Nemipterus japonicas* and extending its shelf life.

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