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The Role of Gelatin-Based Film Coating Combined with Orange Peel Essential Oil on the Quality of Refrigerated Shrimp

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Research Article

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Abstract

In this study, application of gelatin-based film coating combined with essential oil obtained from orange (*Citrus sinensis* (L.) Osbeck) peel on the quality of deep-water pink shrimp (*Parapenaeus longirostris* Lucas 1846) was aimed. Gelatin film (GF) and gelatin film incorporated with orange peel essential oil (GF+EO) were used for film coating. Thickness and microstructure of the films, nutritional composition (protein, moisture, lipid, ash and free amino acid content), sensory and melanosis evaluation, chemical [Sulphur dioxide (SO_2), pH, total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), thiobarbituric acid (TBA), peroxide value (PV), free fatty acid (FFA)], physical (color) and microbiological [total viable counts (TVC), total psychotropic bacteria (PB), total coliform bacteria (TCB) and Enterobacteriacea (EB)] were carried out for 15 days. As a result, gelatin film coatings were effective on the quality and shelf life of shrimps than the control group. Gelatin films were more effective on melanosis than control and the essential oil protected the shrimp's quality more than the other groups. According to the sensorial and microbiological analysis results, control group samples (C) have 7 days' shelf life while gelatin group samples (GF) have 12 days, and gelatin film with orange peel essential oil coated group samples (GF+EO) have 15 days.

Keywords: Gelatin film coating, orange peel, essential oil, shrimp, shelf life.

Portakal Kabuğu Esansiyel Yağı ile Birleştirilmiş Jelatin Film Kaplamanın Buzdolabında Muhafaza Edilen Karidesin Kalitesi Üzerine Etkisi

Özet

Bu çalışmada, portakal (Citrus *sinensis* (L.) Osbeck) kabuğundan elde edilen esansiyel yağ ile birleştirilmiş jelatin bazlı film kaplamanın derin su pembesi karidesinin (*Parapenaeus longirostris* Lucas 1846) kalitesine uygulanması amaçlanmıştır. Film kaplaması için jelatin film (GF) ve portakal kabuğu esansiyel yağı (GF + EO) ile birleştirilmiş jelatin film kullanılmıştır. 15 gün boyunca filmlerin kalınlığı ve mikroyapısı, besin kompozisyonu (protein, nem, lipit, kül ve serbest amino asit içeriği), duyusal ve melanosis değerlendirmesi, kimyasal [Kükürt dioksit (SO2), pH, toplam uçucu bazik azot (TVB-N), trimetilamin azot (TMA-N), tiyobarbitürik asit (TBA), peroksit değeri (PV), serbest yağ asidi (FFA)), fiziksel (renkli) ve mikrobiyolojik [toplam canlı sayımı (TVC), toplam psikotropik bakteri (PB), toplam koliform bakteri (TCB) ve Enterobacteriacea (EB)) analizleri yapılmıştır. Sonuç olarak, jelatin film kaplamaları, karideslerin kalite ve raf ömrü üzerinde kontrol grubuna göre daha etkili olmuştur. Jelatin filmler melanosis üzerinde kontrolden daha etkili olup, esansiyel yağ, karidesin kalitesini diğer gruplardan daha fazla korumuştur. Duyusal ve mikrobiyolojik analiz sonuçlarına göre, kontrol grubu örneklerinin (C) 7 günlük raf ömrüne sahipken, jelatin grubu örneklerinin (GF) 12 gün, portakal kabuğu esansiyel yağı içeren jelatin film ile kaplı örneklerin (GF + EO) 15 gün raf ömrüne sahip olduğu tespit edilmiştir.

Anahtar kelimeler: Jelatin film kaplama, portakal kabuğu, esansiyel yağ, karides, raf ömrü

INTRODUCTION

Shrimps which results in the limited shelf life of the product are highly perishable due to the biochemical, microbiological or physical changes during storage (Aşık and Candoğan, 2014; Alparslan and Baygar, 2017). Lipid oxidation and rancid off-flavors may also occur even under refrigeration or freezing conditions of shrimps (Montville et al., 2012). Therefore, it is necessary to investigate methods to prevent or slow down the quality degradation of shrimp during storage time (Alotaibi and Tahergorabi, 2018).

Cold storage, freezing and chilling are traditional methods for shrimp (Arancibia et al., 2015). For the past decades, research on edible films or coatings in foods is driven by food engineers due to the high demand of consumers for longer shelf life and better quality of fresh foods as well as of environmentally friendly packaging (Siracusa et al., 2008). In addition to this storage technique, recently, there has been an increasing interest to develop materials with biodegradable film and containing an essential oil which help improve food safety and shelf life (Dutta et al., 2009). There is a growing interest in consumer demands for natural and safe foods recently. So, bioactive packaging systems including antimicrobial and/or antioxidant additives, edible coatings and films have become a novel option to maintain the freshness and quality of foods as an alternative to traditional methods (López-Caballero et al., 2007; Ojagh et al., 2010). Biopolymers such as polysaccharides and proteins have been used as edible food packaging materials (Artharn et al., 2009). Proteins are important biopolymers possessing good film forming ability. Among proteins, gelatin has been used as film-forming material (Gómez-Guillén et al., 2007). Gelatin has been attracted the attention for the developments of edible films due to its abundance and biodegradability (Jongjareonrak et al., 2006). To improve the water vapour barrier properties of the gelatin films, hydrophobic substances such as fatty acids and essential oils have been added (Bertan et al., 2005; Tongnuanchan et al., 2012; Nilsuwan et al., 2016). Gelatin films with strong water vapour barrier properties could be an alternative ecofriendly edible packaging material that could be used to preserve the shelf life of foods, especially those sensitive to the quality changes induced by moisture absorption.

In this study combined effect of edible gelatin film coatings incorporated with essential oil obtained from orange (*Citrus sinensis* (L.) Osbeck) peel on the shelf life of deep water pink shrimp (*Parapenaeus longirostris* Lucas 1846) was aimed.

MATERIAL and METHODS

Sampling

Deep water pink shrimps (*Parapenaeus longirostris* L. 1846; mean length 11.04±0.78 cm and mean weight 9.03±1.34 g) caught around Sığacık Gulf of Aegean Sea (İzmir) of Turkey were used as raw material. 30 kg shrimps were obtained from a vessel which harvesting shrimps using a trawl and were immediately iced and transferred to the laboratory for analysis. Proximate composition, sensory, physical, chemical and microbiological analyses were done initially and the remaining samples were prepared for coating as analysis groups.

Extraction and Analysis of Essential Oil

In this study, orange peel (*Citrus sinensis* (L.) Osbeck) were used. Orange peels obtained from fruit juice production in Köyceğiz were brought to our faculty laboratories. On average 50kg of orange peel were used in this study to obtaining the amount of essential oil. Orange peel essential oil was obtained after was milled and dried. The essential oil (EO) was obtained by hydro distillation, using a Clevenger apparatus (Edutek Instrumentation, Haryana, India) with 150g of dry plant material and 1500mL of water. The oil was obtained after 3 h of distillation at boiling temperature and stored at 4±1 °C in airtight glass vials covered with aluminum foil. The gas chromatography-mass spectrophotometer (GC-MS) analysis of the obtained essential oil was conducted at the ARGEFAR-ÇEG Laboratory of Aegean University (İzmir, Turkey), using an Agilent gas chromatograph model 6890 equipped with an Agilent mass selective detector (MSD) model 5973 (Agilent Technologies, Santa Clara, CA, USA). Identification of components in the essential oil was carried out with the Wiley 275 mass spectral library (NIST, Wiley, New York, NY, USA).

Preparation of Film-Forming Solution

Preparation of edible films was slightly modified from Gómez-Estaca et al. (2009). Food grade gelatin powder (8 g; Doga Drug and Raw Material Co. Ltd., Ankara, Turkey) was dissolved in 100 mL of distilled water (at room temperature) and the mixture was stirred until the gelatin completely dissolved (approx. 15 min). Glycerol and D-sorbitol (Merck) (0.15 g per g of gelatin) were then added to the gelatin coating solution, which was kept at 45 ºC for additional 15 min. Orange peel essential oil (OPEO) in a ratio of 0.5, 1 and 2% (by volume per mass of gelatin) was then added to the coating solution. To stabilize the emulsion at OPEO, Tween-20 was also added to the gelatin solution with a

ratio of 0.2% of the OPEO. Then the film-forming solution with and without OPEO was homogenized with an Ultraturrax T25 basic blender (21.500 rpm, position 5, for 1 min; IKA-Werke GMBH & Co. KG, Staufen, Germany). After cooling to room temperature, the film forming solutions (40 mL) were casted on 10 × 15-cm glass plates, and then dried for 3 days at ambient conditions (24 ± 1 °C). GF group was prepared in the same without adding OPEO. Each plate represented one piece of film.

Radical-Scavenging Activity of Gelatin Film Coatings

Gelatin film coating (0.1 g) was dissolved in distilled water (2 ml) at 45 ºC and, following the addition of ethanol (4 ml), were centrifuged (NUVE, NF 400R, Turkey) at 4000 × g for 10 min at 20 ºC (Cao, Xue, and Liu, 2009). The filtrate obtained was analyzed for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity.

DPPH radical-scavenging activity was determined according to the method of Yen and Hsieh (1995) with minor modifications. A 500 µl aliquot of ethanol extract was mixed vigorously with 5.5 mL of 0.036 mmol L^{-1} DPPH in ethanol and allowed to stand at room temperature in the dark for 30 min. The absorbance of the mixture at 517 nm was measured using a spectrophotometer. The control was measured in the same manner, except that ethanol was used instead of the sample. DPPH radical-scavenging activity was calculated according to the following equation:

Radical-scavenging activity (%) = [1 − (As − A0)/Ac] × 100

where as is the absorbance of the sample, Ac is the absorbance of the control and A0 is the absorbance of the mixture of 5.5 mL of ethanol and 500 µL of the sample.

Antimicrobial Activity of Gelatin Film Coatings Combined with Essential Oil

The antimicrobial activity of gelatin film coating combined with essential oil solutions was tested following a well diffusion assay over five microorganisms selected by potentially food microorganisms according to Vásconez et al. (2009). The standard strains were obtained from the Ankara Refik Saydam Hıfzısıhha Institutes Culture Collection: 2 (-) *Bacillus subtilis* (ATCC 25922) and *Stapylococcus aureus* (ATCC 43300), 2 (+) *Escherichia coli* (ATCC 35218) and *Pseodomonas aeruginosa* (ATCC 15442) and *Candida albicans* (ATCC 10231). The above mentioned bacteria were a culture in Nutrient Broth (NB) at appropriate temperatures. Inoculums were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland Standard Dilutions. 20 mL of Mueller Hinton Agar (Difco) sterilized in separated flasks and cooled to 45–50 °C. After injecting the microorganism cultures to sterile plates (1000 µl), media was distributed and mixed homogenously. 20 µL of solutions were injected to the wells of 6 mm in diameter. Three different concentrations of gelatin and orange peel essential oil combination were evaluated for antimicrobial activity; 0.5 %, 1 % and 2 %. After the proper incubation period for each microorganism, antimicrobial activity was evaluated by measuring the zone of inhibition against the tested microorganisms. Measurements were performed in triplicate.

Film Thickness and Microstructure of Gelatin Films

The film thickness of GF and GF+EO was measured using a digital micrometer screw gauge (model MDC-25M, Mitutoyo, Kanagawa, Japan), averaging ten different locations. Microstructure analysis of the gelatin films was carried out by using SEM technique in a JEOL JSM-7600F (Japan) field emission scanning electron microscope (Rivero et al., 2009). Gelatin films with and without orange peel essential oil were dried on foam plates under optimized conditions. Pieces were cut from films and mounted on copper stubs using double side adhesive tape. Samples were gold coated and observed, using an accelerating voltage of 15 kV.

Application of Film Coatings and Storage

Three different groups were created for analysis: non-coated control group (C), gelatin film coating without essential oil (GF) and gelatin coating with 2 % orange peel essential oil (GF+EO) For non-coated control group, 10 shrimps were put onto sterile foam dishes and vacuum packaged (Culinary, ATM Machinery 7483 BV, Haaksbergen, The Netherlands). For film coated groups (GF and GF + EO), one film was placed on styrofoam plate and after 10 shrimp were disposed, it was closed with the second film. Then, shrimps which were coated with film put inside into sterile foam dishes (poliamid, >160 cm^3/m^2/day O_2 and >8.5 g/m^2/day moisture permeability, Polinas trademark, Turkey) and vacuum packaged. 40 packages were prepared for each group and stored at (4±1) °C.

Nutritional Composition Analysis

The shrimp samples were analyzed in triplicate for nutritional composition: lipid content of fish by the Bligh and Dyer (1959) method, ash content by AOAC (1990) method, moisture content by AOAC (2006a) method and total crude protein analysis by AOAC (2006b) Kjeldahl method. In beginning and at the end of the storage period, total free amino acid analysis for shrimp was determined according to in house method (Kazlıçeşme Ar-Ge Test laboratory, İstanbul) using modified Agilent Eclipse AAA method by HPLC system (Agilent 1260 Infinity, high performance liquid chromatography) after pre-column derivatization with OPA and FMOC.

Sensory Analysis and Melanosis Evaluation

Sensory evaluation of the samples was conducted by six trained persons (24-36 years old) throughout the storage period. Panelists gave scores for sensory characteristics, such as appearance, color, odor, texture and general acceptability using a 5-point descriptive scale (Zeng et al., 2005). Melanosis was evaluated according to melanosis scale published by Otwell and Marshall (1986). Shrimps from each treatment were evaluated for degree of melanosis by an experienced seven-member panel. Panelists were asked to give the melanosis score (0–10), where 0 = absent; 2 = Slight, noticeable on some shrimp; 4 = Slight, noticeable on most shrimp; 6 = Moderate, noticeable on most shrimp; 8 = Heavy, noticeable on most shrimp; 10 = Heavy, totally unacceptable.

Color Measurement

The color of samples was measured by a lab color meter (Pen Color Art 1L model, Artoksi MSM, Istanbul, Turkey) and was in accordance with the recommendations of the International Commission on Illumination (CIE, 1976). The measured L*, a* and b* color parameters indicated lightness/brightness, redness/greenness and yellowness/blueness, respectively. The color meter was calibrated with a white standard and the color measurement was repeated 3 times on different parts of the surface.

Chemical Analysis of Shrimp Samples

A residual amount of SO_2 in fresh shrimp meat was determined according to AOAC (2000) method. The pH values were recorded by a digital pH meter (InoLab, WTW, Weilheim, Germany) after homogenization of each 10 g of sample in 100 mL of distilled water (Manthey, et al., 1988). Determination of total volatile base nitrogen (TVB-N) was carried out as described by Antonocoupoulos (1973). Homogenized shrimp samples were steam-distilled and the TVB-N value (in mg of nitrogen per 100 g of meat) was determined according to the consumption of 0.1 M HCl. TMA-N value within homogenized shrimp meat was determined according to Schormüller (1968) and the results were expressed as TMA-N value in mg of per 100g of shrimp meat. Thiobarbituric acid (TBA) reactive substances were determined according to Tarladgis et al. (1960) to evaluate the oxidation stability during chilled storage and the results were expressed as TBA value in mg of malonaldehyde per kg of shrimp meat. Free fatty acid (FFA) content and peroxide value (PV) in fresh shrimp meat was determined according to the AOCS (1998) method. FFA expressed in percentage of oleic acid, was determined by acidimetric titration of extracts according to Bligh and Dyer (1959), after the addition of ethanol and with phenolphthalein as an indicator. PV expressed in mEq of peroxide per kilogram of fat.

Microbiological Analysis of Shrimp Samples

The following groups of microflora were monitored: total viable count (TVC), psychotropic bacteria count (PBC) and total coliform bacteria (TCB). A sample of 10 g was removed aseptically from the fillet using a scalpel and forceps, transferred to a stomacher bag containing 90 mL of sterile peptone water (PW) solution (0.1 %), and homogenized at room temperature. For each sample, further serial decimal dilutions were prepared in PW solution (0.1 %). The appropriate dilutions were subsequently used for enumeration and differentiation of microorganisms. Total viable counts were determined using plate count agar (PCA, Code: 1.05463, Merck, Darmstadt, Germany) after incubation for 2 days at 37 °C, and psychotropic bacteria counts were determined after incubation at 7 °C for 10 days with the same medium (FDA/BAM, 2009). The sample was diluted in serial 10-fold

steps used as inoculum for the three-tube MPN procedure for total coliforms as specified in FDA/BAM (2002). Suspension: 1 ml from each diluted tube was transferred into lauryl sulfate tryptose broth and incubated at 35 °C for 24–48 h. Inoculum: one loopful from tubes with gas formation within 48 h at 35 °C was transferred to brilliant green lactose broth (BGLB), incubated at 35 °C for 24–48 h. The tubes with turbidity and gas formation in the Durham tube of BGLB indicated the presence of coliforms. Their numbers per 1 g of sample were calculated from the MPN table. *Enterobacteriaceae* were determined using double layer Violet Red Bile Agar (VRB Agar, Merck code 1.01406) after incubation for 2 days at 37 °C (ICMSF, 1982).

Statistical Analysis

Statistical analysis of data was performed using SPSS v. 21.0 program (IBM Corp., Armonk, NY, USA) and one-way analysis of variance (ANOVA), while for means where a statistical difference was performed, means comparisons were carried out with Duncan's multiple range tests. Data were presented as mean values±standard deviations and a probability value of $p>0.05$ was considered significant.

RESULTS AND DISCUSSION

Active Ingredients of Orange Peel Essential Oil

Active ingredient content of orange peel essential oil identified by GC-MS. Totally 16 components were detected and the major component was found to be D-Limonene with 82.24%. The citrus essential oils are a mixture of volatile compounds and mainly consisted of monoterpene hydrocarbon. The volatile components of citrus oil were mainly composed of limonene, the most abundant compound in citrus oil (Sawamura, 2011). Dijenane (2015) reported that the studied oils are made up mainly of limonene (77.37%) for orange essential oil (EO). A total of 18-22 compounds were identified in the *C. sinensis* peel essential oils. Limonene (80.9%) were the main constituents in the oils from fresh (Kamal et al., 2011). The findings were similar to other studies despite minor differences. Alparslan and Baygar (2017) were reported that climate, geographic conditions, variety of the plant, the drying period, the extraction method used, etc. are considered among these differences that may have a direct impact on the content of essential oils.

Antioxidant Activity of Gelatin Film Containing EO

Antioxidant activity results of gelatin film containing different concentration (0.5, 1 and 2%) of orange peel essential oil (EO) or not containing EO are given in Fig. 1. According to the free radical scavenging activity, the results of our study showed that gelatin film with 2 % orange peel EO was determined to have higher antioxidant activity than only GF and other concentrations and statistically was found significant ($P<0.05$). Terpenes that involves in the citrus oils have strong antifungal and antioxidant activities. Limonene, a monoterpene, has been reported to have significant antioxidative potential (Gürsoy et al., 2010; Tongnuanchan et al., 2012).

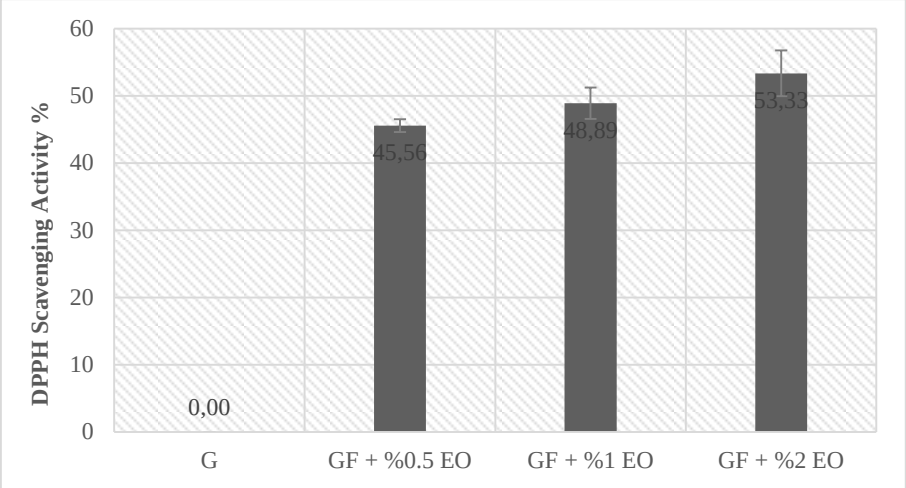

Figure 1. DPPH free radical scavenging activity of film forming solutions.
Letters show significant differences among the groups at P<0.05. Vertical bars represent means (n = 3) ± SE.
(GF: Gelatin Film, EO:Essential Oil)

Antimicrobial Activity of Gelatin Film Forming Solutions Containing EO

Antimicrobial activity of gelatin film forming solutions containing different concentrations (0.5, 1, and 2%) of EO or not containing EO were studied on against the five microorganisms (Table 1). According to the antimicrobial activity results of our study showed that gelatin film with 2% orange peel EO was determined to have higher inhibitory activity on all microorganisms than other concentration and statistically was found to be significant ($P < 0.05$). GF + 0.5% EO found to have no antimicrobial activity on *B. subtilis, P. aeruginosa* and *C. albicans.* And GF + 1% EO found to have no antimicrobial activity on only *B. subtilis*. Antimicrobial effect of the films changed depending on the concentration increase. Hammer, Carson, and Riley (1999) determined the minimum inhibitory concentration of orange (*Citrus aurantium*) essential oil as 1% against *C. albicans*, as >2% against *E. coli* and *P. aeruginosa* and as 2% against *S. aureus*. Similar results were also obtained within the present study. In a study of Gómez-Estaca et al. (2009) who investigated the antimicrobial activity of edible films based on fish-skin gelatin incorporated with chitosan and/or clove essential oil, gelatin films solely were found to have no antimicrobial activity. On the other hand, they resulted that gelatin films combined with clove essential oil have stronger inhibition activity against *E. coli*.

Table 1. Antimicrobial activity of gelatin film forming solutions containing EO (Inhibition zone: mm)

Microorganisms	GF	GF + 0.5 % EO	GF + 1 % EO	GF + 2 % EO
S. aureus	*	6.5 ± 0.7^{c}	8.5 ± 0.7^{b}	10.5 ± 0.7^{a}
B. subtilis	*	*	*	7.0 ± 0.0^{a}
E. coli	7.0 ± 1.4^{d}	9.0 ± 1.4^{c}	10.5 ± 0.7^{b}	13.0 ± 0.0^{a}
P. aeruginosa	*	*	6.0 ± 0.7^{b}	7.5 ± 1.4^{a}
C. albicans	*	*	6.0 ± 0.0^{b}	9.0 ± 1.4^{a}

* Not detected. Data were presented as mean values±standard deviations. Different small letters indicate significant difference among means in the same line (P<0.05). (GF: Gelatin Film, EO: Essential Oil)

Film Thickness and Microstructure of Edible Film

The thickness of gelatin films used in this study was measured as 14.5±0.94 and 18.0±2.11µm (N:10) for GF and GF + 2% EO, respectively. SEM images of the surface and sectional profile of gelatin films with and without orange peel EO provided remarkable data about the morphology (Figure 2). Films which did not contain essential oil had a more compact and homogenous structure when compared with the films including essential oil. Most of the studies reported that essential oil or plant extract incorporation had resulted in heterogenicity for film structures (Bao et al, 2009; Chana-

Thaworn et al., 2011; Tongnuanchan et al, 2012). Rattaya et al. (2009) measured the thickness of fish skin gelatin films incorporated with 6% seaweed extract and fish skin gelatin films without extract as 29.51 and 29.73 μm, respectively.

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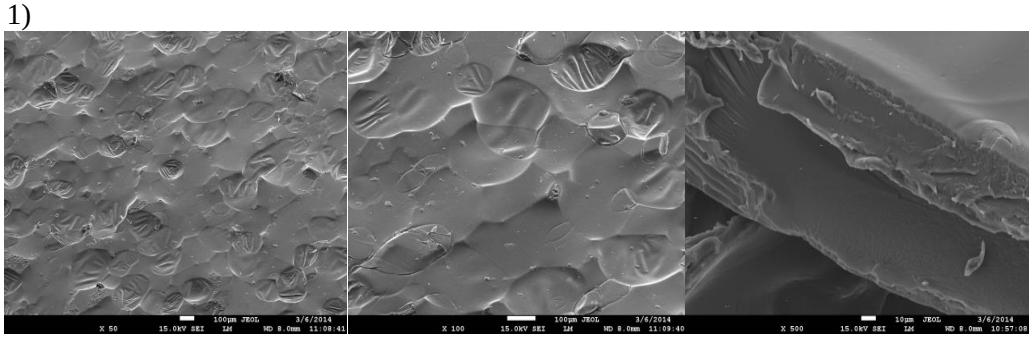

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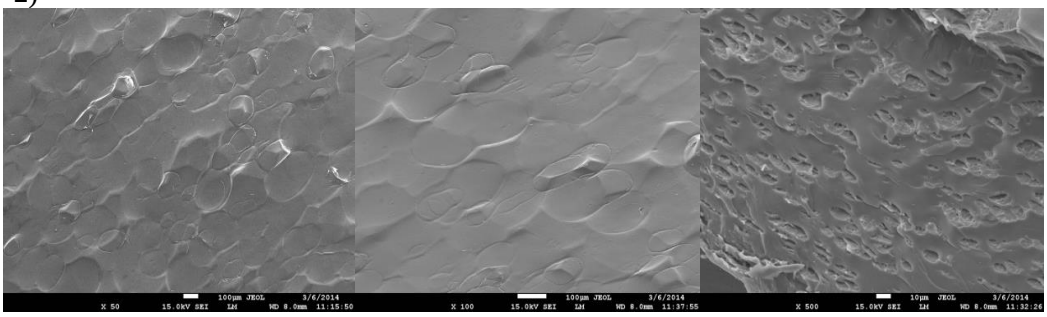

Figure 2. Microstructure images of the edible films (x50, x100, x500) [1) GF, 2) GF + 2% EO]

Nutritional Content of Shrimp

According to nutritional analysis results protein, lipid, moisture and ash values of fresh shrimps were detected as 18.51, 2.15, 74.22 and 2.45 % respectively. The free amino acid content of fresh shrimps, C, GF and GF + OPEO groups at the end of the storage period are shown in Table 2. In our study, ten essential and ten non-essential amino acids were identified in all groups according to free amino acid analysis results. the essential amino acids (EAA) arginine and non-essential amino acids (NEAA) glutamine, glycine, alanine, proline, and glutamic acid were found to be significantly high in fresh shrimp and all experimental groups ($P < 0.05$). The ratio of EAA / NEAA in free amino acid content was 0.58 in fresh shrimp. After 15 days of the storage period, this ratio was determined as 0.52, 0.60 and 0.64 in C, GF and GF + EO groups, respectively. There was a decrease in this ratio in the control group compared with fresh shrimp, whereas in the coated groups (GF and GF + EO) were an increase in EAA / NEAA ratio. The total amount of SAA decreased in the control group, whereas in the gelatin-coated groups, the total amount of SAA increased. The ratio of essential amino acids to non-essential amino acids is in *P. semisulcatus*. 0.60, and 0.59 in *M. monoceros* (Yanar and Çelik, 2006). Rosa and Nunes (2004) reported that the ratio of essential amino acids to non-essential amino acids was 0.93.

Table 2. The total free amino acid (ΣSAA) values (mg / 100g) of raw material, control and coated groups at the end of storage.

Total free amino acid (ΣFAA)	**Raw material**	**Groups**		
		C	**GF**	**GF+EO**
Histidine	55.05±6.29^{c}	52.50±2.12^{c}	65.00±1.41^{bc}	76.00±11.13^{b}
Arginine	775.35±33.87^{a}	103.00±16.97^{d}	593.50±28.99^{b}	506.00±1.41^{c}
Valine	42.95±13.22^{c}	86.00±1.41^{b}	67.00±1.41^{b}	74.00±9.90^{b}
Methionine	35.85±12.52^{c}	70.00±2.83^{bc}	64.50±2.12^{bc}	74.50±12.02^{b}
Tryptophan	30.05±4.03^{b}	33.00±2.83^{b}	15.50±0.71^{c}	10.50±0.71^{c}

Phenylalanine	17.35±0.49^{c}	58.00±21.21^{a}	34.00±1.41^{bc}	39.00±4.24^{abc}
Isoleucine	34.10±0.71^{c}	82.50±20.51^{ab}	51.00±35.36^{bc}	33.50±0.71^{c}
Leucine	59.55±0.35^{d}	151.00±0.00^{b}	100.50±0.71^{c}	141.00±2.83^{b}
Lysine	95.80±0.28^{e}	262.00±0.00^{c}	188.00±4.24^{d}	296.50±9.19^{b}
Threonine	4.95±1.77^{c}	140.50±6.36^{a}	15.00±0.00^{c}	25.50±36.06^{c}
ΣEAA	**1151.0±233.32^{d}**	**1038.5±66.88^{e}**	**1194.0±174.98^{d}**	**1276.5±156.73^{c}**
Cysteine	1.15±0.07^{c}	4.00±0.00^{a}	4.50±0.71^{a}	1.50±0.71^{bc}
Aspartic acid	0.00±0.00^{f}	25.50±0.071^{d}	19.50±0.71^{e}	34.50±2.12^{c}
Glutamic acid	68.20±2.97^{e}	287.50±0.071^{b}	139.50±2.12^{d}	157.00±0.00^{c}
Asparagine	34.75±0.064^{a}	1.00±0.00^{e}	16.50±0.71^{c}	18.00±1.41^{bc}
Serine	37.25±1.20^{a}	10.00±0.00^{c}	18.00±1.41^{bc}	24.00±1.41^{b}
Glutamine	240.85±29.49^{a}	119.50±3.54^{c}	166.50±3.54^{b}	182.00±5.66^{b}
Glycine	481.55±50.28^{a}	438.50±4.95^{ab}	490.00±29.70^{a}	474.00±8.49^{a}
Alanine	922.30±19.23^{c}	1015.00±15.56^{b}	1007.00±36.77^{b}	1007.50±16.26^{b}
Tyrosine	16.75±13.36^{b}	48.50±4.95^{ab}	42.00±33.94^{ab}	27.00±0.00^{ab}
Proline	188.05±18.03^{a}	49.00±1.41^{c}	104.50±2.12^{ab}	75.50±13.44^{c}
ΣNEAA	**1990.90±295.73^{c}**	**1998.50±320.69^{c}**	**2008.00±318.30^{b}**	**2001.00±317.22^{b}**
EAA/NEAA	**0.58±0.08**	**0.52±0.02**	**0.60±0.55**	**0.64±0.49**
ΣSAA	**3141.90±262.81^{c}**	**3037.00±230.78^{d}**	**3202.00±253.58^{b}**	**3277.50±246.34^{b}**

Data were presented as mean values±standard deviations. Different small letters indicate significant difference among means in the same line (P<0.05).

Sensorial Analysis and Melanosis Evaluation

The results of the sensorial analysis are important parameters to evaluate the seafood freshness. According to the storage conditions of shrimps, odor and flavor changes may occur in two or three days which negatively affect the sensory characteristics (Varlık et al., 2000). Sensory analysis results of the C, GF and GF+EO groups are shown in Table 3. The sensory assessment of shrimps was done using a category scale of 5 points and the initial score of the overall evaluation was found to be 4.93 points. After the 4th day of the storage, the control group was found to be inconsumable. GF and GF+EO groups were inconsumable on the 10th and 12nd days of the storage, respectively. It was evaluated by the panelists that EO-incorporation had a positive effect on the odor of the shrimps. Wan et al. (2007) coated the shrimps with chitosan solutions. They resulted that chitosan coated shrimps had better sensorial characteristics and the shelf life could be extended two or three days.

Table 3. Sensory assessment scores of shrimps coated with gelatin film coatings containing EO during storage

Sensorial analysis	**Groups**		
Storage time (Day)	**C**	**GF**	**Gf+EO**
0	4.93±0.10^{Aa}	4.93±0.10^{Aa}	4.93±0.10^{Aa}
1	3.05±0.57^{Bb}	3.97±0.42^{Ab}	4.03±0.42^{Ab}
4	2.44±0.34^{Bc}	3.54±0.47^{Ab}	3.90±0.18^{Ab}
7	1.18±0.18^{Cd}	3.02±0.22^{ABc}	3.40±0.38^{Ac}
10	0.72±0.25^{Be}	2.28±0.77^{Ad}	2.68±0.81^{Ad}
12	0.30±0.06^{Ef}	1.18±0.10^{Ce}	2.15±0.34^{Ae}
15	*	0.90±0.26^{BCe}	1.50±0.32^{Af}

*Not assessed. All values are the Mean±SD (n =6). Values with different letters in the same column (a, b…) and in the same row (A, B…) differ significantly (P<0.05).

Melanosis or black spot is a natural pathway that caused by enzymatic reactions. Melanosis occurs when the shrimps contact to the atmospheric oxygen just after they are harvested (Gökoğlu and Yerlikaya, 2008). Melanosis changes of the shrimps throughout the storage period are given in Figure 3. Melanosis of the shrimps was evaluated according to a 10-point melanosis scale and it was initially determined as 0.43 for the fresh samples. The increase was more rapid in the control group whereas

the scores of the coated groups were lower throughout the storage period. Gelatin coating and OPEO were found to be effective on melanosis and prevented the increase of melanosis throughout the storage. There are similar studies on the prevention of shrimp melanosis most of which resulted that herbal extracts may retard the melanosis formation (Gökoğlu and Yerlikaya, 2008; Nirmal and Benjakul, 2009). Yuan et al. (2016) resulted that chitosan coating combined with green tea extract inhibited the melanosis formation of Pacific white shrimp (*Litopenaeus vannamei*) significantly during storage in ice.

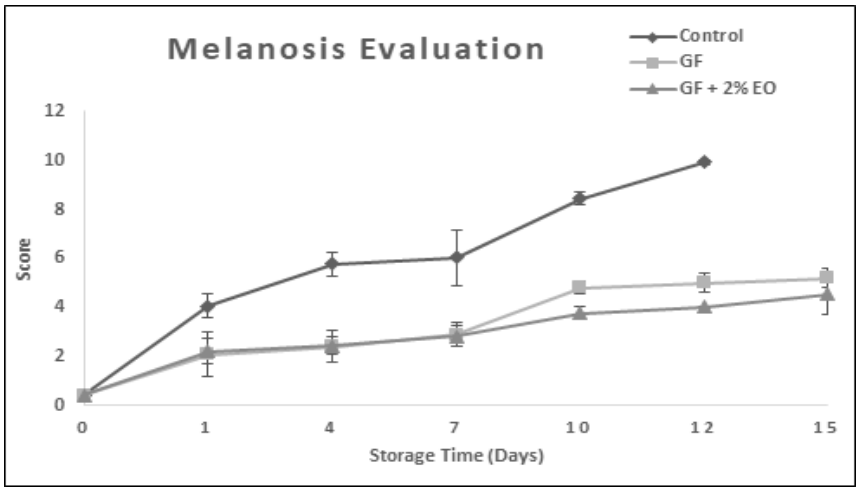

Figure 3. Melanosis changes in shrimp samples depending on the storage time (days)

Color

The L*, a* and b* values of the C, GF and GF+EO groups are shown in Fig. 4. At the beginning of storage, lightness (L*), redness (a*) and yellowness (b*) values of shrimps was determined as 53.56, 0.60 and 6.98, respectively. A rapid decrease was observed for the L* value of the control group. Gelatin coatings (for both GF and GF+EO groups) had a positive effect on the L* value. a* and b* values increased for gelatin coated groups while they decreased in the control group. EO incorpration positively resulted in higher redness values. It was observed that film coating and EO incorporation had a good effect on the L*, a* and b* values of the shrimps. In a study of Alotaibi and Tahergorabi (2018) who coated shrimps with sweet potato starch-based coating with or without thyme essential oil, the enhancement of L* value had been attributed to the light scattering that resulted from the emulsion created when oil was mixed with an edible coating. Gokoglu and Yerlikaya (2008) reported that the L*, a* and b* values of the shrimps are in good agreement with the sensory melanosis scores for their study of coating shrimps with grape seed extract. Aşık and Candoğan (2014) studied the effect of chitosan-based edible coatings (CC) incorporated with garlic (*Allium sativum*) oil at 0.5, 1.0 and 1.5% on *Parapenaeus longirostris* meat quality during refrigerated storage and resulted that CC application with or without GO generally resulted in higher lightness (L*), and lower redness (a*) and yellowness (b*) values.

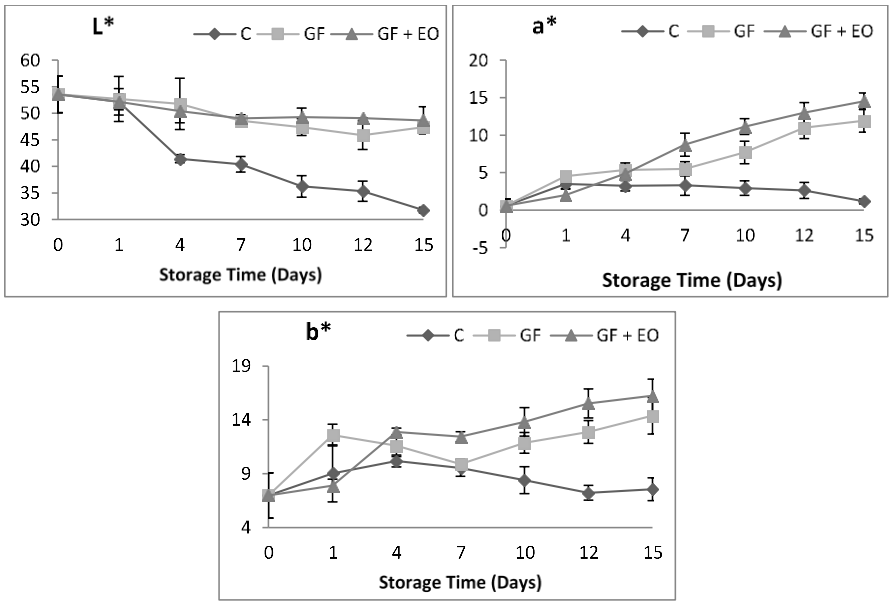

Figure 4. Changes in the measured instrumental color parameters of non-coating and coated shrimp samples during cold storage; a) lightness (L*), b) redness (a*) and c) yellowness (b*)

Results of Chemical Analysis

It is known that shrimps are dipped to sodium metabisulphite solutions as soon as they are harvested on board. So, to determine whether the test groups of the present study had been dipped into the sodium metabisulphite solution, SO_2 amount in fresh shrimp meat was determined. Residual sulfur dioxide (SO_2) of the fresh shrimps was detected as 0.25 mg/kg. This amount corresponds to 0.37 mg/kg as sodium metabisulfite residue. Measured residual SO_2 level of our study samples may be originated from the contaminated equipment's used during transportation and was below the limit values. In Europe, the amount of sulfites in the edible part of fresh *Penaideae* crustacean family is restricted to 0.15–0.3 g SO_2 kg^{-1} according to the size of the crustacean (EPCD, 1995).

The results of pH, TVB-N and TMA-N measurements of the analysis groups during refrigerated storage (4°C) for 15 days are shown in Fig. 5. It was reported that shrimp pH of 7.7 or less indicates prime quality, 7.70–7.95 shows poor but acceptable quality and 7.95 or greater represents unacceptable quality (Shamshad et al., 1990; Çolakoğlu et al., 2006). Initial pH value was measured as 6.44 for the present study. Group C, G and G+OL exceeded the acceptable upper limit of 7.95 on days 7, 12 and 15 of the storage, respectively. Aşık and Candoğan (2014) defined that chitosan coating resulted in the decrease of pH values of the shrimp samples, as compared with the control group, likely because of the low pH of the chitosan coating solution, resulting from acetic acid incorporation into the formulation.

It was suggested that 30 mg/100 g TVB-N value is an acceptable limit value for shrimps (Shamshad et al., 1990). The initial value of TVB-N content in shrimps was 17.32 mg/100 g of shrimp. There was a statistically signifcant increase for all test groups throughout the storage period ($P<0.05$). The control group (37.73 mg/100 g) exceeded the acceptable limit value on the 7^{th} day, while GF and GF+EO groups exceeded the acceptable limit value on days 10 and 12 of the storage, respectively. Aşık and Candoğan (2014) reported the initial TVB-N value (day 0) of shrimp meat as 20.72 mg/100g. They also indicated that TVB-N values for all groups showed significant increases over time and exceeded the upper limit for spoilage in the uncoated group at day 3, and in chitosan coated groups with or without garlic oil at day 5. Lopez-Caballero et al. (2018) studied the Melanosis-inhibiting formulations on quality of deepwater pink shrimpand resulted that TVB-N decreased in all groups after the treatments which was attributed to the release of basic compounds to the aqueous media, since the treatments were applied in solution.

The initial value of TMA-N content in shrimps was 1.15 mg/100g. Varlık et al. (2000) detected the initial TMA-N value of *P. longirostris* shrimps stored at 4°C as 1.75 mg/100g. Zeng et al. (2005) showed that the initial TMA value of the shrimp (*Pandalus borealis*) was 0.5 mgN/100 g at the

beginning of storage and observed a signidicant increase throughout the storage (P<0.05). Control, GF and GF+EO groups exceeded the acceptable limit value of TMA-N on days 7, 12 and 15 of the storage, respectively. There was a statistically significant difference between control and the others for TMA-N values (P<0.05). Varlık et al. (1993) suggested the consumable limit value of TMA-N as 1-8 mg/100 g for seafood as 8 mg/100 g TMA-N value indicates the inconsumable seafood.

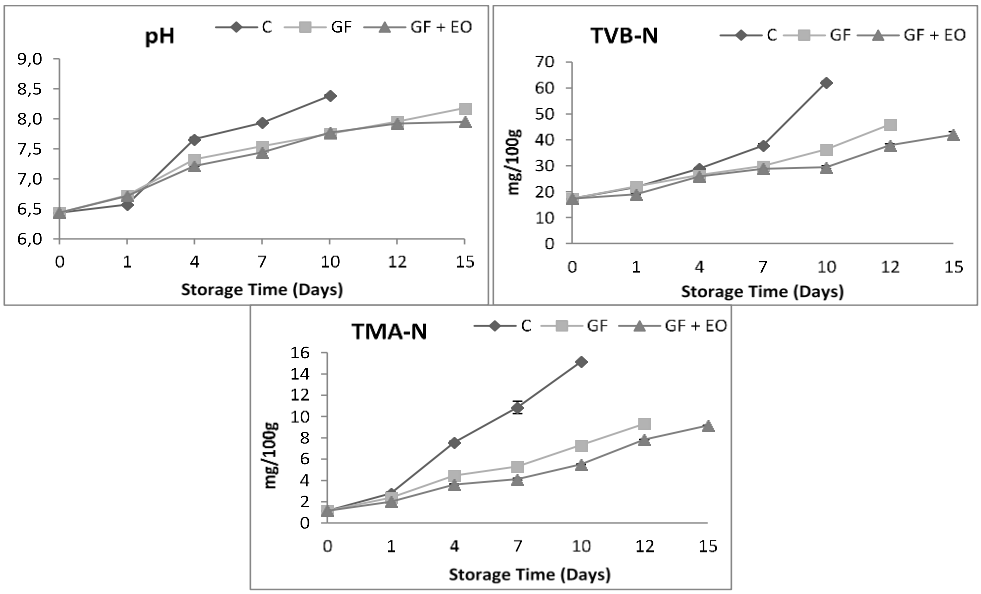

Figure 5. pH, TVB-N and TMA-N values of non-coated and coated shrimp samples during cold storage

The results of TBA, PV and FFA measurements of the analysis groups during refrigerated storage (4°C) for 15 days are shown in Fig. 6.

TBA has been used as a good indicator of the lipid oxidation (Ojagh et al., 2010). Oxidative rancidity is one of the most important reasons that cause seafood deterioration. Such deteriorations are detected by measuring malondialdehydes, end products formed via the decomposition of primary and secondary lipid peroxidation products. It has been proposed that a maximum TBA value is 5 mg malonaldehyde/kg, while the seafood may be consumed up to a level of 8 mg malonaldehyde/kg in TBA value (Schormüller, 1969). The initial value of TBA content in shrimps was 0.17 mg malonaldehyde/kg. There was a statistically significant increase in the control group when compared to other groups (P<0.05). According to Ojagh et al. (2010) TBA values of the chitosan plus cinnamon oil coated rainbow trout samples reached significantly (P<0.05) lower TBA value than the control and only chitosan coated groups, at the end of the 16 days of the storage.

The primary products of unsaturated fatty acid oxidation are peroxides. For this reason, peroxides that are formed initially are accepted as the quality indices. Ludorff and Meyer (1973) proposed that the peroxide values between 0-2 mmol O_2/kg are "very good", 2-5 is mmol O_2/kg "good", 5-8 mmol O_2/kg is "acceptable" and 8-10 mmol O_2/kg is "spoiled", for determining the freshness of fish. The initial value of PV in shrimps was 0.79 meq O_2/kg. PV values of all groups increased significantly throughout the storage (P<0.05). The increase of the PV value for the control group was higher than other groups and the limit PV level was exceeded after 10 days of the storage. EO-incorporation was more effective on peroxide value of the shrimps than the other groups. Okpala, Choo, and Dykes (2014) determined the shelf-life and quality changes in Pacific white shrimp and reported the initial PV value was 1.56±0.27 mEq of O_2/kg for fresh samples.

Free fatty acids (FFA) formed after lipid hydrolysis are highly contributed to rancidity (Chaouqy et al., 2008). The initial hydrolytic rancidity as evaluated by FFA value of the shrimps was 0.24 (expressed as a percent of oleic acid). Control group reached the acceptable FFA values on the 12th day of the storage with 8.92 %. During the 15 days' storage period, GF and GF+EO groups remained below the FFA limit values. Coating with chitosan with or without EO resulted in preventing the free fatty acid formation. In a study of Bhadra et al. (2012), it is recommended that a level of FFA in

seafood is 10-12 mg% as a limit of acceptability. They reported that the FFA values of the control and chitosan coated *Penaeus monodon* samples increased significantly ($p < 0.05$) with storage time.

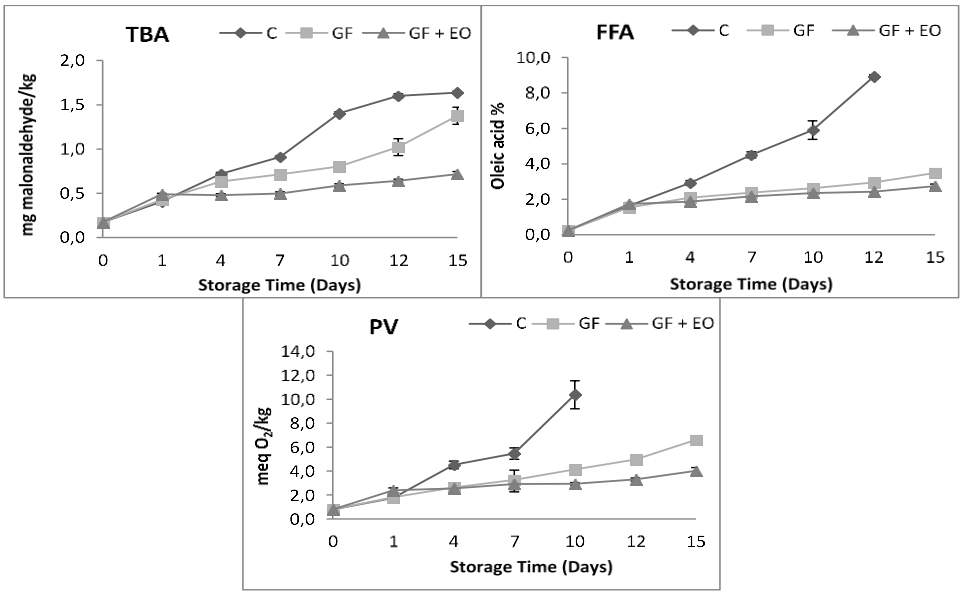

Figure 6. TBA, PV and FFA values of non-coated and coated shrimp samples during cold storage

Results of Microbiological Analysis

Total viable counts (TVC), Psychrotrophic bacteria (PB), Total Coliform Bacteria (TCB) and Enterobacteriaceae (EB) counts of shrimps during 15 days cold storage are shown in Fig. 7. For fresh seafood species, the microbiological limit for TVC and PB is reported to be 7 log/g or log/cm^2 (Çelikel and Kavas, 2008). The initial TVC of the shrimps was calculated as 3.7 log CFU/g. TVC value of the control group was found to be not significant until the 7th day of the storage but exceeded the limit value of 7 log CFU/g after 7 days of the storage period. Yuan et al. (2016) resulted that chitosan coating combined with green tea extract inhibited the total flora of Pacific white shrimp (*Litopenaeus vannamei*) significantly during 9 days' storage in ice. The initial PB of fresh shrimp was found to be 2.5 log10 CFU/g. There were significant increases for C, GF and GF+EO groups throughout the storage ($P<0.05$) and control group reached the limit value of 7 log CFU/g after 10 days of the storage period. The inhibition effect of OPEO against PB was observed during refrigerated storage of 15 days.

The acceptable limit value of TCB count of seafood is reported as 160–210 MNP/g (Jay, Loessner, and Golden, 2005). On the other hand, ICMSF (1986) and EU (2005) stated the limit for fresh and frozen fish as <100 MNP/g. For fresh shrimp, the initial TCB count was calculated as 1.5 MNP/g. A rapid increase was observed in the control group after 7 days of storage ($P<0.05$) and TCB value reached 9 MNP/g after 12 days. TCB count of all groups was below the limit value throughout the storage period. It was observed that OPEO incorporation the TCB growth of shrimps under refrigerated conditions. The initial amount of TCB may be due to poor post-harvest handling or poor personnel hygiene practices during transport.

The initial Enterobacteriaceae count of the fresh shrimps was detected as 3.2 log CFU/g. There was an increase of Enterobacteriaceae count for Group C after 10 days' storage which was found to be statistically significant ($P<0.05$). The effect of edible coatings on *E. coli* in the coliform bacteria group has been determined by many researchers. According to the results, the combination of coatings with essential oil and plant extracts has been effective on *E. coli* (Çelikel and Kavas, 2008; Gómez-Estaca et al., 2009; Dyahningtyas, 2010).

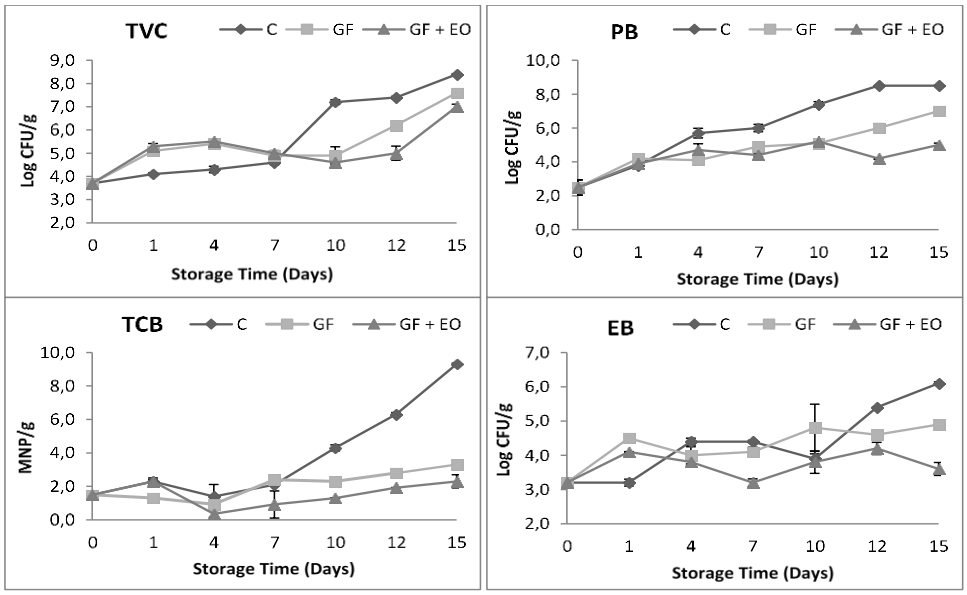

Figure 7. TVC, PB, TCB and EB counts of non-coated and coated shrimp samples during cold storage

CONCLUSIONS

GF+EO (gelatin and orange peel essential oil) maintained the sensory characteristics of shrimp within the acceptable limits during the storage period. The combination of gelatin film and OPEO was effective in extending the shelf-life of fresh shrimps to 15 days (Group GF+EO). Control group samples without gelatin film had a shelf-life of 7 days (Group C) and the only gelatin coated group had 12 days (Group G). The incorporation of OPEO to gelatin films extended the shelf-life of shrimps for nearly 8 days more when compared to the uncoated control group. OPEO improved the quality of shrimp during the 15 days' storage period in terms of chemical indices determined in shrimp meat. Lipid oxidation and microbial growth were successfully inhibited in refrigerated shrimp. Incorporation of OPEO to gelatin coating inhibited the growth of microorganisms involved in shrimp spoilage and showed effective antimicrobial characteristics throughout the storage. Thus, gelatin film forming solution combined with OPEO provides an effective coating that can be applied as a natural preservative for shrimp under refrigerated storage and maintains its quality.

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