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Combined effect of orange peel essential oil and gelatin coating on the quality and shelf life of shrimps

Wirkung ätherischer Öle aus Orangenschalen auf die Haltbarkeit von Garnelen

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Summary

Quality and shelf life of deep water pink shrimp (*Parapenaeus longirostris* Lucas 1846) coated by active edible coatings consisted of gelatin and orange (*Citrus sinensis* (L.) Osbeck) peel essential oil (OPEO) was determined. Orange peels were provided as a by-product from a fruit juice factory and its active chemical components were determined by GC analysis. Sensory and melanosis evaluation, microbiological [total viable counts (TVC), total psychrotrophic bacteria (PB), *Enterobacteriaceae* (EB), lipolytic bacteria (LB)], 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)] and physical (color and weight loss) analyses were carried out and shelf-life was determined on every other days throughout the storage period of 14 days.

Edible gelatin coating solutions enriched with orange peel essential oil were more effective on the quality and shelf life of shrimps when compared to control group. Sensory analysis results revealed that coating the shrimps with edible gelatin solutions were effective in inhibiting and retarding the melanosis formation for all groups. Incorporation of orange peel essential oil preserved the quality of shrimp chemically and microbiologically. It can be suggested that using orange peel essential oil as a natural compound for combining the preservative effects of edible coatings offers an alternative way to maintain the quality of the perishable foods.

Keywords: Deep water pink shrimp, edible coating, gelatin, orange peel, essential oil

Zusammenfassung

Während einer 14-tägigen Lagerzeit von Rosa Geißelgarnelen (*Parapenaeus longirostris* Lucas 1846), die mit aus Orangenschalen (*Citrus sinensis* (L.) Osbeck) gewonnenen ätherischen Ölen (in Gelatine-Form) behandelt wurden, wurde eine Verlängerung der Haltbarkeit nachgewiesen. Die aktive chemische Zusammensetzung der Orangenschalen wurde durch Gaschromatographie analysiert. Die mit ätherischen Ölen beschichteten Garnelen-Proben wurden periodisch sensorisch und auf das Auftreten von Melanose hin untersucht. Darüber hinaus wurden im Hinblick auf die Nährstoff-Zusammensetzung, Farbe, Gewichtsverlust, chemische (SO_2, pH-Wert, TVB-N, TMA-N, TBA, PV) und mikrobiologische (Gesamtkeimzahl (TVC), Gesamtzahl der psychrotrophen Bakterien (PB), *Enterobacteriaceae* (EB), lipolytische Bakterien (LB)) Parameter untersucht.

Die mit essbarer Gelatine beschichteten Garnelen wiesen gegenüber der Referenzgruppe eine verbesserte Qualität und Haltbarkeit auf. Die Ergebnisse der sensorischen Analyse zeigten, dass die Gelatinelösungen zu einer Hemmung und Verzögerung der Melanosebildung führten. Die Anwendung der Gelatine-Lösungen bewahrte zudem die chemische und mikrobiologische Qualität der Garnelen. Die verwendete Methode kann daher verwendet werden, um die Qualität dieser leicht verderblichen Lebensmittel zu erhalten.

Schlüsselwörter: Rosa Geißelgarnelen, essbare Beschichtung, Gelatine, Orangenschale, ätherisches Öl

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Introduction

Among seafood, shrimp is one of the most demanded and traded worldwide (Asik and Candogan, 2014; Tsironi et al., 2009). The most important causes of shrimp spoilage are accumulation of undesirable compounds as a result of microbiological growth and biochemical reactions, and melanosis (discoloration) originated by the polymerization of phenols into insoluble black pigments, i. e., melanins (Nirmal and Benjakul, 2011). Melanosis in shrimp, commonly referred to as 'black spot', is a defect caused by the activity of polyphenoloxidase (PPO) (Gokoglu and Yerlikaya, 2008). Black spots form on fresh shrimp and other shellfish within a few hours after harvest, without refrigeration. The reaction involves the oxidation of phenols to quinines by PPO (Taoukis et al., 1990). Because of being perishable seafood, shrimp has to be consumed as soon as possible or processed immediately with an appropriate processing method (Bilgin et al., 2006).

The main components of our everyday foods (e. g., proteins, carbohydrates and lipids) can fulfill requirements for preparation of edible films. As a general rule, fats are used to reduce water transmission; polysaccharides are used to control oxygen and other gas transmission, while protein films provide mechanical stability. These materials can be utilized individually or as mixed composite blends to form films provided that they do not adversely alter food flavor (Pavlath and Orts, 2009). Gelatin is a protein obtained by physical, chemical or biochemical denaturation and hydrolysis of collagen. Gelatin-based edible film coatings have already been proposed to extend the shelflife of various meat products (Alparslan et al., 2014).

In order to increase the efficiency of application of edible films on foods, natural plant extracts, especially essential oils possessing antimicrobial and antioxidant activities, are recommended (Asik and Candogan, 2014). The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS), as long as their maximum effects are attained with the minimum change in the organoleptic properties of the food (Viuda-Martos et al., 2008).

Citrus fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetics and folk medicine (Saidani et al., 2004). Citrus peels, widely processed as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils (Andrea et al., 2003). Citrus peel essential oils have also been searched for their natural antioxidant and antimicrobial properties (Viuda-Martos et al., 2008).

In this study, it is aimed to detect the effects of edible gelatin coating solutions incorporated with orange (*Citrus sinensis* (L.) Osbeck) peel essential oil on the quality and shelf life of deep water pink shrimp (*Parapenaeus longirostris* Lucas 1846).

Material and Methods

Sample

Deep water pink shrimps (*Parapenaeus longirostris* L. 1846; mean length 11.62 ± 0.74 cm and mean weight 7.89 ± 1.33 g) caught around Guzelcamlı Harbour (Soke, Aydın) of Turkey were used as raw material. 30 kg shrimps were obtained from a vessel 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) was used. Orange peels were collected from a fruit juice factory in Köyceğiz and were brought to quality-control laboratories of Fisheries Faculty at Muğla Sıtkı Koçman University. On average 50 kg of orange peel were used in this study to obtain of essential oil. Orange peel essential oil was obtained after drying and milling the peels. Essential oil (EO) was obtained by hydro distillation, using a Clevenger apparatus (Edutek Instrumentation, Haryana, India) with 150 g of dry plant material and 1500 mL of water. With 50 kg Orange peel, 500 mL orange peel essential oil was yielded and used throughout the analysis periods. 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 extracted 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. (2010). Commercial bovine gelatin (100 Bloom) (Nature Pharmaceutical Raw Materials Trade Co., Ltd. Sti., Istanbul, Turkey) was dissolved in 100 mL distilled water and the mixture was stirred until the gelatin completely dissolved (approx. 15 min). Glycerol (0.15 mL per g of gelatin) and D-sorbitol (0.15 g per g of gelatin) were then added to the gelatin coating solution, which was kept at 45 °C 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 gelatin solution. To stabilize the emulsion, Tween-20 was also added at 15 % (by volume), depending on the OPEO content. One of the gelatin groups was prepared without the addition of orange peel essential oil (0 % OPEO). Then the coating solutions including OPEO were homogenized with an Ultraturrax T25 basic blender (21 500 rpm, position 5, for 1 min; IKA-Werke GMBH & Co. KG, Staufen, Germany).

Microstructure of gelatin films added essential oil

Microstructure analysis of the films was carried out by using SEM technique in a JEOL JSM-7600F (Japan) field emission scanning electron microscope (Garcia et al., 2000). Gelatin films with and without orange peel essential oil were dried on foam plates. 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.

Radical-scavenging activity of gelatin coatings

Gelatin coatings (0.1 g) were 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 et al. 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 sample. DPPH radical-scavenging activity was calculated according to the following equation:

$$\text{Radical-scavenging activity (\%)} = [1 - (As - A0)/Ac] \times 100$$

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 sample.

Antimicrobial activity of gelatin coating solutions combined with OPEO

The antimicrobial activity of gelatin coating solutions combined with OPEO was tested using agar well diffusion assay over five food pathogen microorganisms (NCCLS, 2006). The standard strains were obtained from the Ankara Refik Saydam Hıfzısıhha Institutes Culture Collection; *Bacillus subtilis* (ATCC 25922), *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 15442) and *Candida albicans* (ATCC 10231). The above mentioned bacteria were cultured 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) were sterilized in separated flasks and cooled to 45–50 °C. After injecting the microorganism cultures into sterile plates (1000 µL), media were distributed and mixed homogenously. 20 µL of solutions were injected into 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.

Coating Treatment and Storage

Three different groups were created for quality analysis: non-coated control group (A), gelatin coating without essential oil (B) and gelatin coating with 2 % EO (C). 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 groups with gelatin coatings (B and C), 3 kg of shrimps were dipped into coating solutions for 30 seconds x 2 times and then were laid out on 1 m x 1 m square wire mesh plates for draining the excess amount of solution. Shrimps and the coating solutions were cold stored until the coating process and processing was done under ambient temperature (18 ± 1°C). After 2 minutes of drying on plates, 10 shrimps of each group were put onto sterile foam dishes and vacuum packaged. 40 packages were prepared for each group and stored at (4 ± 1) °C.

Sensory analysis and Melanosis Evaluation

Sensory evaluation of the samples was conducted by six trained persons (24–36 years old) every other day throughout the storage period. Similar to Zeng et al. (2005), panelists gave scores for sensory characteristics, such as appearance, color, odor, texture and general acceptability using a 5-point descriptive scale (5: Excellent, 4: Good, 3: Moderate, 2: Borderline-clearly not fresh, 1: Unfit spoiled).

Melanosis was evaluated according to melanosis scale published by Otwell and Marshall (1986). Shrimps from each treatment were evaluated for 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.

Proximate composition analysis

The shrimp/seafood samples were analyzed in triplicate for proximate composition: lipid content of fish by the Bligh and Dyer method (1959), ash content by AOAC (1990, 950.46) method, moisture content by AOAC (2006, 934.01) method and total crude protein analysis by AOAC (2006a, 984.13) Kjeldahl method.

Microbiological analysis of shrimp samples

The following groups of micro flora were monitored: total viable count (TVC), psychrotrophic bacteria count (PBC), *Enterobacteriaceae* (EB) and lypolytic bacteria count (LBC). 10 g of meat was removed aseptically from the peeled and deveined ten shrimps for each group 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 psychrotrophic bacteria counts were determined after incubation at 7 °C for 10 days with the same medium (FDA/BAM, 2009). *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). Lipolytic bacteria counts were determined using Tributyrin Agar (Code: 91015, Sigma-Aldrich, Buchs Switzerland) containing glycerol tributyrate after incubation for 3 days at 30 °C (Kurt et al., 1993).

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 of each group were recorded by a digital pH meter (InoLab, WTW, Weilheim, Germany) after homogenization of 10 g shrimp meat 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 fish samples were steam-distilled and the TVB-N value (in mg of nitrogen per 100 g of fish) 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

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100 g 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. Peroxide value (PV), expressed in millimole of peroxide per kilogram of fat, and was determined according to AOAC (1994).

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.

Statistical analysis

Experiments were performed in triplicate (N=3) for three independent samples and a completely randomized design (CRD) was used. Data were presented as mean values ± standard deviations and a probability value of $p>0.05$ was considered significant. Analysis of variance (ANOVA) was performed and the mean comparisons were done by Duncan's multiple range tests. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

Result and Discussion

Chemical Composition of Orange Peel Essential Oil

Active chemical components in orange peel identifed by GC-MS are shown in Table 1. In total, 19 components were detected and the major component was found to be limonene with 77.307 %. Njoroge et al. (2005) studied three varieties of *C. sinensis* and reported that monoterpene hydrocarbons are largely dominated in the volatile fractions of the orange peels. Ferhat et al. (2006) also reported that limonene, a monoterpene hydrocarbon, is the most abundant component present at 78.5 % with hydro-distillation. Similar to us, they determined limonene, α-pinene, sabinene and α-terpinene as the major components.

Antioxidant Activity of Gelatin Film Forming Solutions Containing OPEO

Antioxidant activities of edible gelatin film forming solutions containing or not containing OPEO are given in Figure 1. According to the free radical scavenging activity, solution with 2 % OPEO was found to have higher antioxidant activity than other groups ($p < 0.05$). Tongnuanchan et al. (2012) studied the antioxidant activities of films from fish skin gelatin incorporated with bergamot, kaffir lime, lemon and lime essential oil and figured out that film incorporated with bergamot essential oil showed the highest free radical scavenging activity ($p < 0.05$) followed by the film added with lemon essential oil. They also suggested that films incorporated with different essential oils containing 30 % glycerol mostly had the higher antioxidant activity analyzed by all assays than those with 20 % glycerol ($p < 0.05$).

TABLE 1: *Active ingredient content of orange peel essential (volatile) oil.*

	Active ingredient component	%	RT[a] (dk)
1	α-pinene	2.480	4.139
2	Sabinene	0.702	6.751
3	β-pinene	0.049	6.839
4	Octanol	0.969	7.298
5	β-Myrcene	1.788	7.398
6	α-Phellandiene	0.098	7.748
7	3-Carene	0.502	8.018
8	4-Carene	0.072	8.189
9	D-Limonene	77.307	8.760
10	Terpinene-4-ol	1.006	9.308
11	1,3,6-Octatriene,3,7-Dimethyl(Z)	0.080	9.378
12	α-Terpineol	2.748	9.646
13	1-Octanol	0.147	10.055
14	1,6-Octadien-3-ol 3,7-Dimethyl	2.055	11.157
15	6-Octenal, 3,7-Dimethyl (-R)	0.153	13.042
16	Decanal	0.268	15.328
17	Valencene	3.287	15.957
18	2,6-Octadienal, 3,7-Dimethyl	0.806	17.708
19	Caryophyllene	0.211	24.089

[a]: Retention Time

Antimicrobial Activity of Gelatin Film Forming Solutions with Essential Oil

Antimicrobial activity of edible gelatin film forming solutions containing or not containing orange peel essential oil are given in Table 2. Edible gelatin film forming solution with 2 % orange peel essential oil was found to have an antimicrobial effect on all microorganisms when comparing with the other groups ($p < 0.05$). Gelatin film forming solution without orange peel essential oil was found to have no antimicrobial activity. It was detected that 0.5 % and 1 % orange peel essential oil containing gelatin solutions did not have an antimicrobial effect on *B. subtilis*. 1 % essential oil containing gelatin solutions was the most active ($p < 0.05$) against *C. albicans*, so it was suggested that the increase of the volatile oil ratio did not change the antimicrobial activity on *C. albicans*. On the basis of the results obtained in the antioxidant and antimicrobial

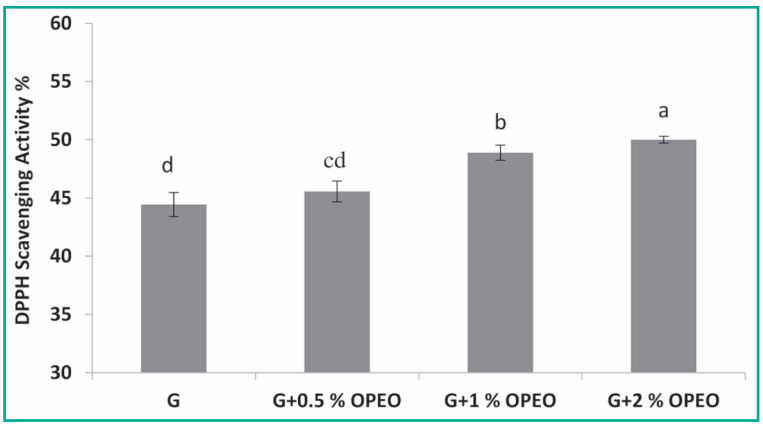

FIGURE 1: *DPPH free radical scavenging activity of film forming solutions. Letters show significant differences among the groups at $P < 0.05$. (G: Gelatin, OPEO: Orange Peel Essential Oil).*

TABLE 2: *Antimicrobial activity of gelatin film forming solutions containing different concentration of orange peel essential oil (Zone diameter: mm).*

Microorganisms	G	G+0.5 % OPEO	G+1 % OPEO	G+2 % OPEO
S. aureus	*	8.5 ± 0.7^{c}	11.5 ± 0.7^{b}	12.5 ± 0.7^{a}
B. subtilis	*	*	*	8.5 ± 0.7^{c}
E. coli	*	10.5 ± 0.7^{c}	11.0 ± 1.4^{b}	12.0 ± 1.4^{a}
P. aeruginosa	*	10.0 ± 0.0^{b}	10.5 ± 0.7^{b}	13.0 ± 1.4^{a}
C. albicans	*	10.0 ± 1.4^{c}	12.0 ± 1.4^{a}	11.5 ± 1.4^{b}

*: 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). (G: Gelatin, OPEO: Orange Peel Essential Oil)

analysis of different concentrations, 2 % OPEO concentration was selected to evaluate the combined effect of OPEO and gelatin coating on the shelf life of shrimps. Tao et al. (2009) determined the antimicrobial activities of OPEO against *Staphylococcus aureus, Penicillium chrysogenum, Bacillus subtilis, Escherichia coli* and *Saccharomyces cerevisiae,* with their inhibition zones ranging from 14.57 mm to 23.37 mm. Orange peel essential oil was also reported to be the most effective against *Aspergillus niger,* one of the moulds commonly associated with food spoilage (Viuda-Martos et al., 2008). Citrus oils are more effective on Gram-positive bacteria than on Gram-negative in vitro which is similar for other essential oil components (Burt, 2004).

Microstructure of the films

SEM images of gelatin films with and without orange peel essential oil provided useful data about the morphology (Fig. 2). The surface of the gelatin film (control) not containing OPEO was found to be smooth, compact and homogenous. There were not much grains and porous formations. Gelatin film with OPEO was flat but heterogeneous with holes and porous morphology. These holes might be related to the evaporation of the essential oil during the film forming process (Sánchez-González et al., 2011; Ahmad et al., 2012). Although some holes were observed on the surface of the gelatin films with OPEO, there might be active essential oil on the compact structure of the film which cause antioxidant and antimicrobial effect on the quality of the shrimps.

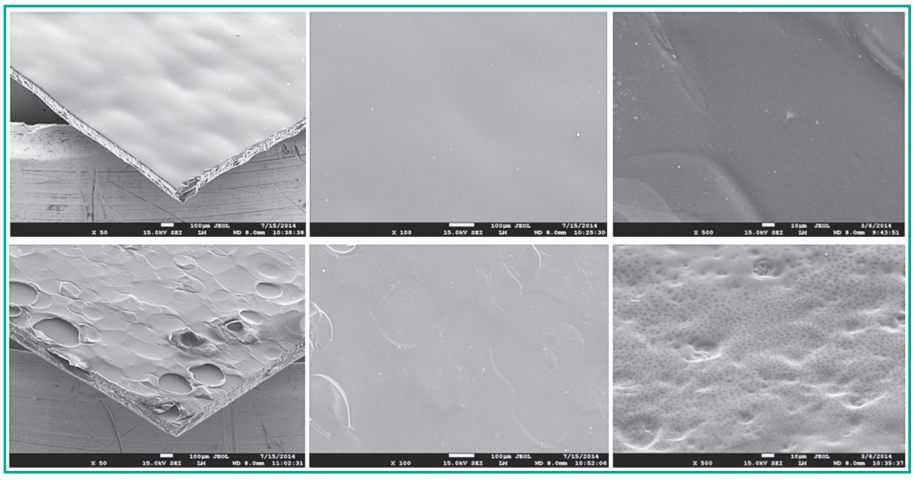

FIGURE 2: *Microstructure images of the edible films (x50, x100 and x500); Upper pictures: Gelatin film; Lower pictures: Gelatin film + 2% OPEO.*

Nutritional content of shrimp

Nutritional composition of the shrimps at the beginning (fresh shrimp) and at the end of the 14 day storage period, are shown in Table 3. Protein, fat, moisture and ash contents for fresh shrimp were determined as 18.57 ± 0.28 %, 1.52 ± 0.15 %, 74.21 ± 0.46 % and 1.64 ± 0.05 %, respectively. López-Caballero et al. (2007) reported similar values for the proximate analysis results of fresh shrimp as: crude protein 20.80 ± 0.62 %, total fat 0.41 ± 0.06 %, moisture 78.87 ± 0.15 % and ash 1.49 ± 0.04 %. At the end of 14 day storage period, protein content remained nearly same for Group A while it was increased for group B and C ($p < 0.05$). Alparslan et al. (2016) reported that the increase of the protein content of gelatin coated groups may be due to the protein structure of gelatin. At the end of the storage, fat content of group A, B and C were detected as 1.45 ± 0.03 %, 1.35 ± 0.08 % and 1.43 ± 0.05 %, respectively. Fat content did not show significant differences for all groups at the end of storage ($p > 0.05$). Ash content of group A, B and C were determined as 2.15 ± 0.05 %, 1.93 ± 0.05 % and 1.95 ± 0.06 %, respectively at the end of storage. An increase was observed in ash content of all of the groups ($p < 0.05$), and increase in group A was found statistically important according to other groups ($p < 0.05$).

TABLE 3: *Nutritional content of shrimps.*

Time (Day)	Groups	Protein %	Lipid %	Moisture %	Ass %
0	Raw material	18.57 ± 0.28^{b}	1.52 ± 0.15^{a}	74.21 ± 0.46^{a}	1.64 ± 0.05^{c}
14	A	18.29 ± 0.41^{b}	1.45 ± 0.03^{a}	73.12 ± 0.22^{b}	2.15 ± 0.05^{a}
	B	23.25 ± 0.03^{a}	1.35 ± 0.08^{a}	71.82 ± 1.26^{d}	1.93 ± 0.03^{b}
	C	23.46 ± 1.10^{a}	1.43 ± 0.05^{a}	72.70 ± 0.14^{c}	1.95 ± 0.06^{b}

Presented datas were as mean values ± standard deviations (n: 3). Different small letters indicate significant difference among groups in the same line (P < 0.05)

Sensorial Analysis and Melanosis

Quality assessment was carried out on a 5 point grading scheme for shrimps and general mean point was detected as 4.93 at the beginning of storage. Group A, B and C were under the consumable limit of 1 point on the 6th, 10th and 12th day of storage, respectively. Usage of gelatin and orange peel essential oil together had a positive effect on sensory characteristics of shrimps throughout the storage period. Orange essential oil gave a pleasant aroma and odor and received appreciation by panelists.

Melanosis changes that occurred throughout the storage are shown in Figure 3. For group A, the score was 10.00 on the 8th day of storage; however, for group B and C it was 6.72 and 6.28 at the same day, respectively. At the end of 14th day, these scores were 9.25 and 9.38 for group B and C, respectively. Since the first day of the storage, melanosis has increased continuously for Group A ($p < 0.05$). Melanosis score significantly increased from 0.10 to 8.90 and 8.75 for group B and C after the 12th day, respectively. Gelatin coating and orange peel essential oil had a combined prevention effect on melanosis of shrimps and also inhibited the increase of melanosis throughout the storage. Plant phenolic compounds such as ferulic acid (Nirmal and Benjakul, 2009) and catechin (Nirmal and Benjakul, 2009a) were found to be effective in retarding the melanosis. Fang

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et al. (2013) used pomegranate peel extract treatment and thereby significantly inhibited the formation of melanosis of Pacific white shrimp compared with the control.

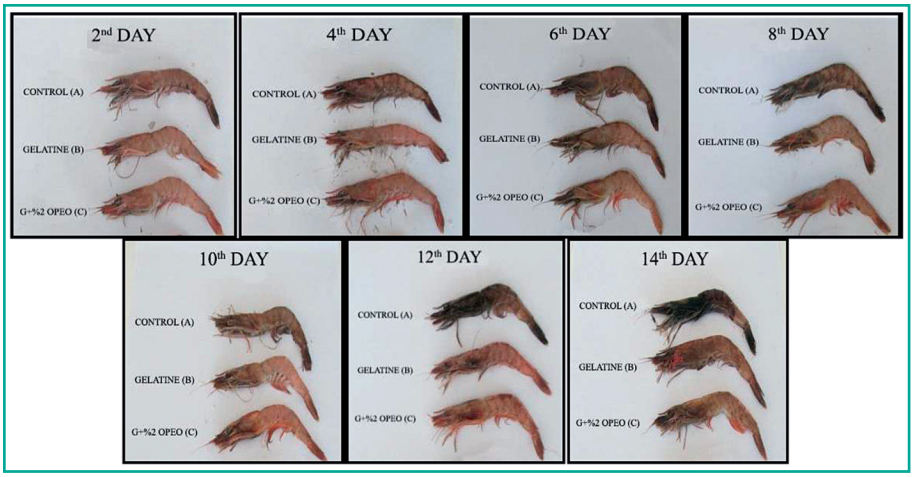

FIGURE 3: *Melanosis changes in shrimp throughout the refrigerated storage.*

Color

The L* (light/dark), a* (red/green) and b* (yellow/blue) values of group A, B and C are shown in Figure 4. Using the standard blank sample of the colour measurement, the L*, a* and b* value of fresh shrimps was determined as 52.78, 2.63 and 8.25 at day 0. For L* values during the storage, there was a significant decrease for group A ($p < 0.05$) when compared with group B and C. At the end of the 14th day, the L* value was determined as 29.10, 38.91 and 39.06 for group A, B and C, respectively. Gelatin coating enriched with orange peel essential oil had a positive effect on the L* value compared with the control group. This effect was also correlated with melanosis evaluation. At the end of the storage period, the a* and b* values of control group decreased while this values increased for B and C groups. As an important parameter of consumer's preference, this study resulted that the color was not changed by coating with gelatin+OPEO. Alparslan et al., (2016) also reported similar colour measurements for shrimps coated with gelatin-orange leaf essential oil.

Results of Chemical Analysis

SO_2 residue amount of fresh shrimp samples was detected as 0.25 mg SO_2 kg^{-1}. When calculated as sodium metabisulfite this value was detected as 0.37 mg SO_2 kg^{-1}. The use of sulfite compounds for food preservation is limited by authorities. In the European Union, the amount of sulfites in the edible part of fresh *Penaideae* crustacean family is restricted to 0.15–0.30 g SO_2 kg^{-1} (EU, 2008). SO_2 residue amount of shrimps analysed in this study was under this limit value. Hence, using natural extracts is a good alternative for eliminating the disadvantages of such chemicals.

pH, TVB-N and TMA-N analysis results of group A, B and C are shown in Figure 5. Initial pH value of fresh samples was determined as 6.47. Depending on storage time, a continuous increase was observed on pH value in all groups ($p < 0.05$). At the end of 14th day, pH values of group A, B and C were determined as 8.55 (8th day), 8.51 (12th day) and 8.62, respectively. pH values exceeded the consumable limit of 7.95 (Shamshad et al., 1990) on 6th day of storage for group A, on 8th day for group B and on 10th day for group C. This increase was also reported by Fang et al. (2013) who studied the effect of pomegranate peel extract on quality of Pacific white shrimp during iced storage. Throughout the study, Group A was found statistically significant in terms of pH value when compared with other groups ($p < 0.05$).

TVB-N value was detected as 17.14 mg/100 g for fresh shrimp on day 0. Depending on storage time, a statistically significant increase ($p < 0.05$) was observed for all groups. According to Finne (1982), the early increase in TVB-N content indicates that autolytic processes were involved in the production of volatile bases. TVB-N values for Group A reached 41.27 mg/100 g on 6th day, group B reached 32.57 mg/100 g on 8th day and group C reached 38.31 mg/100 g on 10th day. Despite there was a slightly moderate increase for OPEO treated group, all samples exceeded TVB-N consumable limit of 30 mg TVB-N/100 g (Cobb et al., 1976). The difference among all groups was statistically significant ($p < 0.05$). TMA-N value of fresh

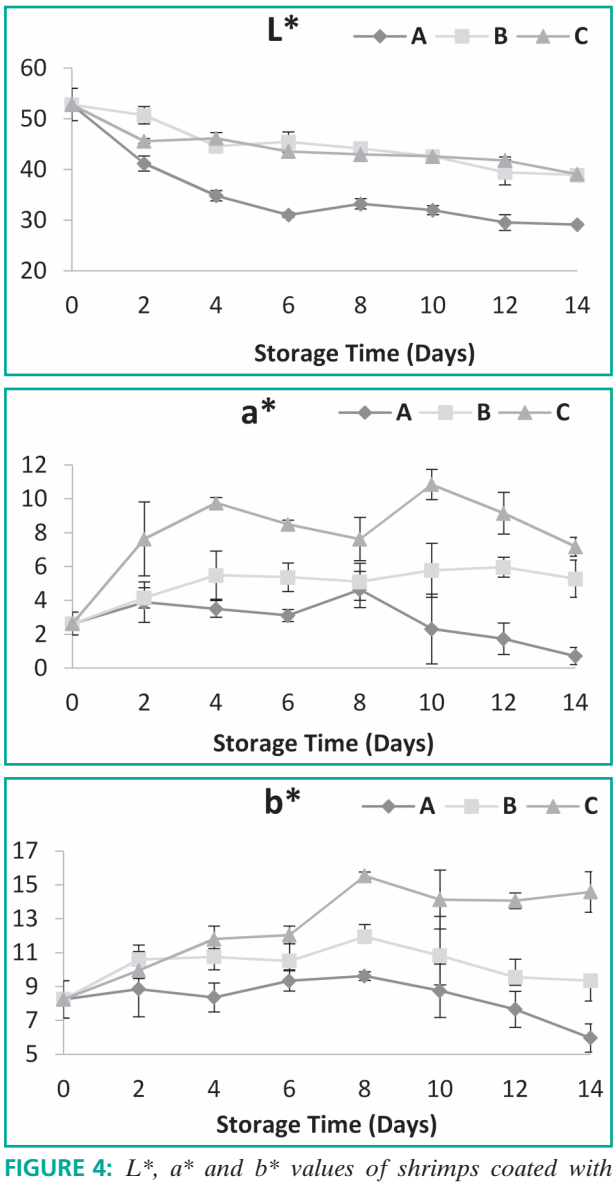

FIGURE 4: *L*, a* and b* values of shrimps coated with gelatin film solution.*

samples was detected as 0.97 mg/100 g. A statistically significant increase was observed for TMA-N values of all groups depending on the storage time ($p < 0.05$). Group A reached 10.35 mg/100 g on the 6th day, group B reached 8.88 mg/100 g on the 8th day and group C reached 10.11 mg/100 g on 12th day of storage. All groups exceeded 5 mg TMA-N/100 g level which is accepted as the consumable limit in shrimp (Cobb et al., 1976). Group A samples were found to have statistically significant TMA-N values ($p < 0.05$) when comparing with group B and C thoughout the storage.

TBA and PV analysis results of group A, B and C during 14 days of storage in refrigeration are shown in Figure 6. Initial TBA value of fresh samples were detected as 0.20 mg malonaldehyde/kg. Depending on storage time, TBA values of all groups increased significantly ($p < 0.05$). At the end of the storage period, TBA values of group A, B and C were detected as 2.37, 1.37 and 1.03 mg malonaldehyde/kg, respectively. The increase of TBA value was lower for the OPEO group. Nirmal and Benjakul (2011) and Fang et al. (2013) also reported similar decreases for TBA values when using plant extracts during storage, which is due to high antioxidant activity of their constituents.

Initial PV value of fresh samples was detected as 1.02 meq O_2/kg. During 14 days of storage, peroxide values of group A, B and C were determined as 11.72 (8th day), 9.70 and 5.44 meq O_2/kg respectively. Depending on the storage time, significant increase ($p < 0.05$) was observed statistically on peroxide values of group A and B. Control group (A) exceeded the consumable limits of peroxide values on the 6th day and group B on the 12th day. Boran et al. (2006) suggested 8 meq/kg as a limit of peroxide value for quality and acceptability of oils for human consumption. Group C which was coated with gelatin with essential oil did not reach this limit value throughout the storage. This might be due to the protective effect of gelatin as an oxygen barrier and also the antioxidative compounds found in OPEO.

Microbiological Analysis Results

TVC, PBC, EB and LB changes of group A, B and C during 14 days of storage in refrigeration are shown in Figure 7. At the beginning of the storage, total viable count of fresh shrimps was determined as 2.4 log CFU/g. This value is very close to Huidobro et al. (2002) who suggested that this is correlated to a correect handling process onboard. A rapid increase was observed for group A samples ($p > 0.05$) after the 2nd day. TVC was calculated as 7.3 Log (CFU/g) on 8th day of storage for group A, 7.4 Log (CFU/g) for group B on 12th day, 7.3 Log (CFU/g) for group C on 14th day. The microbiological limit recommended by the ICMSF (1986) for total viable count at 30 °C is 7 log/g for fresh water and marine species. It was observed that the consumable limit of 7 Log (CFU/g) was exceeded for all groups.

Initial PBC of fresh shrimps was determined as 3.6 Log (CFU/g). A significant increase ($p < 0.05$) occurred on PBC for the control group (Group A) and reached 7.4 Log (CFU/g) on day 8 which exceeded the limit of 7 Log (CFU/g) (ICMSF, 1986). PBC of group B and C increased throughout the storage ($p < 0.05$), and reached 7 Log (CFU/g) on the 14th day of storage, respectively.

It was determined that the initial *Enterobacteriaceae* count of fresh shrimps was 2.3 Log (CFU/g). Huidobro et al. (2002) reported 2.7 Log CFU/g EB count for fresh caught pink shrimp. At the end of the storage, EB counts

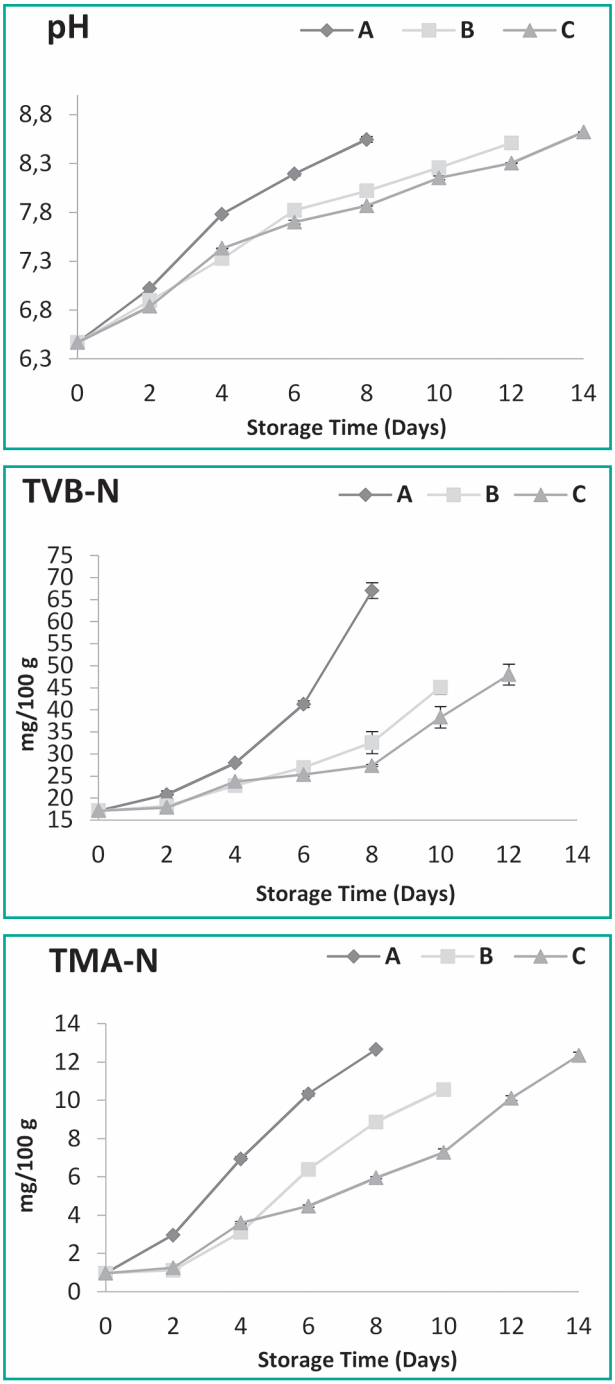

FIGURE 5: *pH, TVB-N and TMA-N analysis results of shrimps coating with gelatin film solution.*

of Group A, B and C were 6.0, 5.5 and 3.3 Log (CFU/g), respectively. In control group samples, an increase was observed at the 2nd day of storage and continued after 10th day, this increase was found to be significant ($p < 0.05$). Insignificant changes were observed on group B and C samples and it is concluded that orange peel essential oil is effective on this type of bacteria. Nirmal and Benjakul (2009a) reported that Pacific white shrimp treated with 0.1 % catechin had a lower EB count as compared to the control after 10 days of storage in ice. Mendes et al. (2002) figured out that chilling has a positive effect in the reduction of the growth of these bacteria in Pacific coast shrimp *(Pandalus jordani)* and deepwater pink shrimp. LB count of fresh shrimps was determined as 2.4 Log (CFU/g). At the

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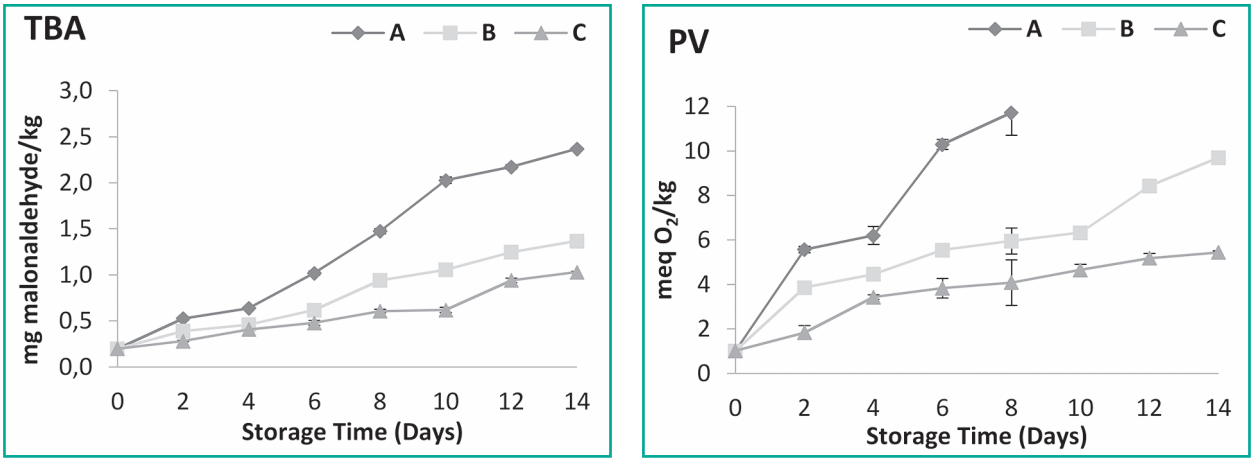

FIGURE 6: *TBA and PV values of shrimps coated with gelatin film solution.*

end of the storage period, LB counts of Group A, B and C were 8.5 (12th day), 8.5 and 8.2 Log (CFU/g), respectively. Statistically significant changes were observed in group A and B after 2nd day, in group C at the 6th day of storage ($p < 0.05$). Gelatin combined with OPEO found to not have much effect on lipolytic bacteria count. The increase of the pH and TVB-N results are in agreement with the microlobiological counts which are due to the autolytic processes that involved in the production of volatile bases.

Conclusions

Based on the results of sensorial analysis and melanosis evaluation, gelatin coating solution enriched with OPEO significantly advanced the quality of water pink shrimp during refrigerated storage. When compared to control, antimicrobial and antioxidant characteristics of the film were also emphasized by chemical and microbiological analyses. TBA and PV analysis results were all in agreement with the microbiological counts. Particularly, orange peel EO had a good effect on odor and aroma of shrimps and was appreciated more by panelists. The composite gelatin coating combined with OPEO was a transparent layer from a sensorial point of view and prevented black spot development of the shrimp. Use of edible gelatin solutions combined with natural protective compounds seems to be an alternative application which has advantages than using sodium metabisulphite, a chemical inhibiting agent for melanosis.

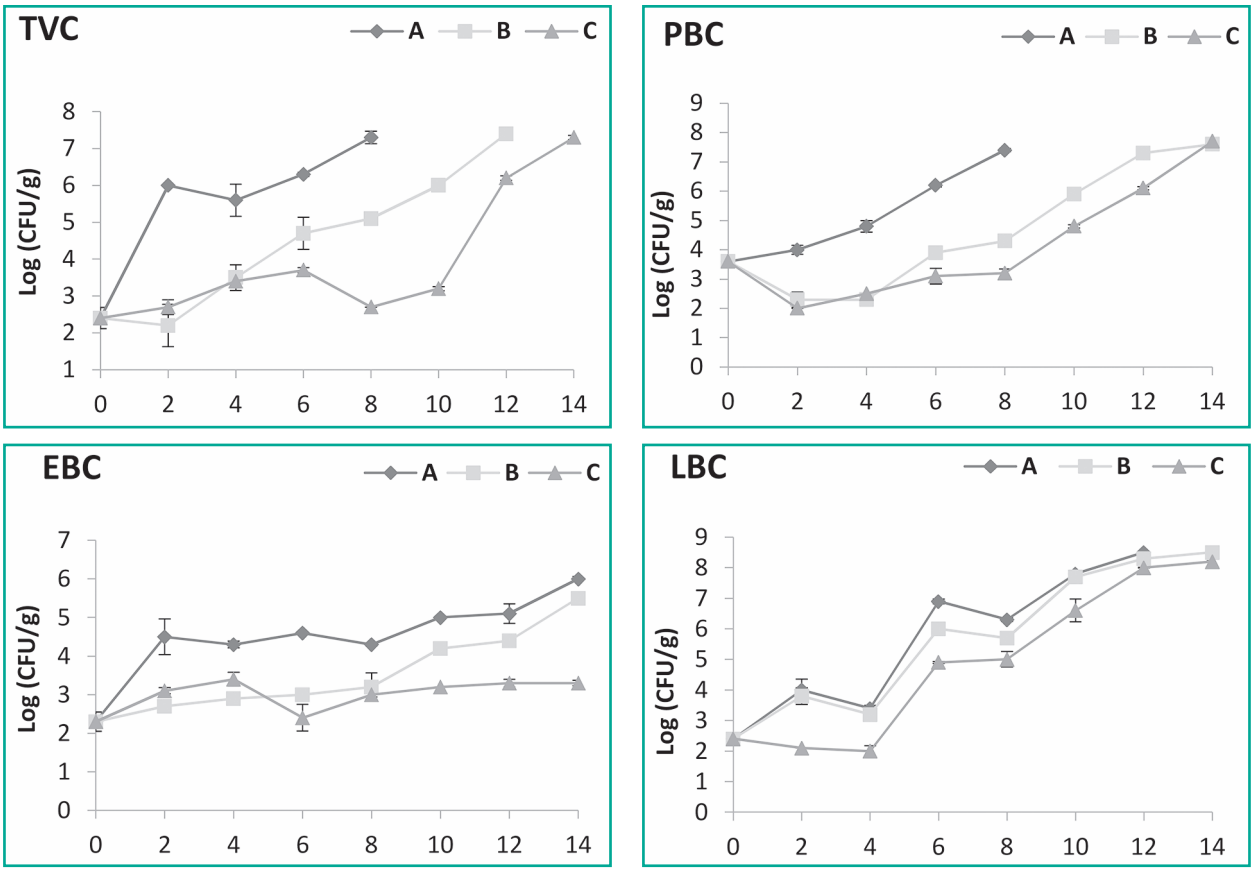

FIGURE 7: *TVC, PBC, EBC and LBC values of shrimps coated with gelatin film solution during storage time (days).*

According to the results of sensory and microbiological analysis; the group coated with OPEO-incorporated gelatin (Group C) had 12 days of shelf life whereas gelatin coated samples without essential oil (Group B) had 10 days and the control group (Group A) had 6 days. Gelatin coating combined with OPEO could therefore be useful for preserving shrimp quality during cold storage with a shelf-life extension in shrimps of about 6 days.

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Conflict of interest

The authors declare that no conflict of interest exits.

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