Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

# Study the biodegradable bilayer film based on hydrolyzed protein and its effect on the shelf life of *Penaeus semisulcatus*

**Amir Ghalandari[1], Laleh Roomiani[2*]**

1- MSc. Department of Food Science and Technology, Ahv, C., Islamic Azad University, Ahvaz, Iran
2-Associate of Professor, Department of Fisheries, Ahv, C., Islamic Azad University, Ahvaz, Iran

**ARTICLE INFO**

**Article History:**

Received:2024/10/23

Accepted:2024/6/9

**Keywords:**

Film,
Shelf life,
Microbial and Chemical indicators,
Shrimp

**DOI: 10.22034/FSCT.22.166.75.**

*Corresponding Author E-

laleh.roomiani@iau.ac.ir

l.roomiani@yahoo.com

**ABSTRACT**

In this study, we aimed to produce environmentally compatible double-layer films with fish processing waste and orange leaf extract as additives. The double-layered films were based on carboxy-methyl-cellulose hydrolyzed protein obtained from fish waste (first layer) and using orange extract at levels of 10, 20 and 40 (weight/volume) (second layer). Evaluation of the effect of films on the shelf life of green tiger shrimp during 15 days of storage at 4 degrees Celsius was analyzed through microbial, chemical and color parameters. The obtained results showed that the shrimp samples closed with double-layer films decreased the number of total aerobic bacteria, mold and yeast and cold-loving bacteria by 3, 1 and 3 log compared to the control treatment. The double-layer film containing 40% orange extract was able to reduce K-value, H-value, TVB-N and TBARS in shrimp compared to other treatments. Histamine, cadaverine, spermidine and spermine increased with increasing storage time in the treatments. The amount of $a^*$ in shrimp samples did not have a significant difference between the treatments ($P>0.05$), but the values of $b^*$ and $L^*$ of shrimp showed a statistically significant difference between the study treatments ($P < 0.05$). The results of the correlation coefficients were confirmatory and consistent with the results of the principal components analysis. The results of this study showed that double-layer films can be a substitute for plastic materials.

## 1-Introduction

According to the World Food and Agriculture Organization (2024), the raw material residues obtained from the fishing industry constitute up to 70% of its original weight. The majority of the remaining raw materials are currently converted into flour or oil or used to produce animal feed, but these processes and products have low profitability. Therefore, it is important to develop solutions to utilize fish waste by processing them to produce new materials for human and animal consumption [1]. According to the Food and Agriculture Organization of the United Nations (2022), about 1.3 billion tons of the total food resources produced for human consumption are wasted annually worldwide. The composition of food waste varies depending on the type and source of food. Food waste is preferably used for animal feed, but it can lead to food-borne diseases. Therefore, products produced from food waste, with proper valuation, can have practical and commercial applications [2]. The processing industry (heading, skinning, finning, gutting, scaling, filleting) of commercial fish generates waste. Canning of aquatic animal's accounts for 70% of the solid waste, including dark meat, head, bones, and skin. On the other hand, shrimp processing generates approximately 50% of the waste [3]. Studies have shown that aquatic animal waste is rich in proteins, polyunsaturated fatty acids, minerals, vitamins, and carotenoids that have functional properties [4, 5, 6]. However, fishery waste can carry pathogenic bacteria that, if not properly managed, pose a threat to human health and the environment [7]. On the other hand, there are current concerns about environmental pollution due to plastic packaging. This has led to the development of biodegradable films and packaging. Films can be made from carbohydrates, proteins, and lipids that are biodegradable, nontoxic, and derived from renewable resources. Biodegradable films may be used as coatings, wrapped, or sprayed onto foods, acting as a barrier to the transmission of salts, vapors, and gases, improving food quality [8].

Various food proteins such as corn, milk, soy, wheat and whey have been used to prepare biodegradable films. Also, due to the economic use of by-products and wastes of aquatic processing, there is interest in using myofibrillar proteins [2]. On the other hand, plant extracts can be used in edible films due to their safety, preservation and stability of food at low concentrations [9]. Plant extracts are rich in antioxidants, having high amounts of phenolic acids, flavonoids, anthocyanins, tannins and other polyphenol subgroups [3,10].

Several works have been carried out on films based on fish myofibrillar proteins. Lima et al. (2024) investigated the properties of films based on myofibrillar proteins obtained from hydrolyzed protein of tuna waste [11]. Another study investigated the production of edible films based on pectin, gelatin and hydroxypropyl methylcellulose and evaluated their application as biodegradable packaging materials for shelf life. *Golden* breathes films produced were able to increase the shelf life of fish fillets by 7-8 days compared to the control film [12]. Leite et al. (2024) used Nile tilapia waste to produce myofibrillar protein film. Their results showed that 1% hydrolyzed protein in film production could provide the best result [13]. Protein obtained from shrimp waste was used to produce biodegradable film. The results showed that higher protein concentrations could affect the film properties [14]. By-products produced from aquatic waste with high protein content (approximately 17%) [15, 16] are a valuable opportunity for film production that can contribute to the main functions of food packaging related to product protection and its integrity, protection and external control. Therefore, the aim of this study was to extract hydrolyzed myofibrillar protein from the waste of Hover fish (Katsuwonus pelamis) and then producing a protein-based film, orange

leaf extract (*Citrus aurantium)* and its use in increasing the shelf life of green tiger shrimp (*Penaeus semisulcatus*) was.

## 2- Materials and methods

### 1-2- Collecting waste and performing hydrolysis

Bluefin tuna waste, including head and skin, were purchased from canned fish factories in the industrial town of Sistan and Baluchestan province - Chabahar city, then packaged in sterile bags and sent to the laboratory in boxes containing ice and stored at 4°C. Then, after washing several times, they were frozen at -20°C until laboratory procedures were performed.In order to perform the hydrolysis, papain enzyme purchased from Novozyme, Denmark, was used and stored at 4°C until the time of the experiment. 50 g of the waste sample was poured into a 250 ml Erlenmeyer flask, then 100 ml of distilled water was added to it in a 2:1 ratio, homogenized with a digital mixer for 2 minutes, and then placed in a water bath at 85°C for 20 minutes to inactivate the internal enzymes. For the hydrolysis, the frozen samples were stored in the refrigerator for 24 hours until thawing. Then, they were diluted with 0.2 M phosphate buffer solution (8.5 M) in a ratio of 1:2 pH was diluted. They were completely homogenized for 2 minutes using a homogenizer. Then the homogeneous solution was diluted using 1N sodium hydroxide Dilute the solution to 5/8 ph. The optimal enzyme is papain. Papain enzyme was added to the samples at a ratio of 1% and transferred to a shaking incubator at a temperature of 55 ° C with a constant speed of 200 rpm. After the above periods of time, in order to stop the enzymatic reaction, the samples were placed in a water bath at a temperature of 95 ° C for 15 minutes. After cooling to normal room temperature, the samples were incubated at a temperature of 10 ° C for 30 minutes at a speed ofgThey were centrifuged at 7000 × g. The supernatant was collected for further analysis and placed in a freeze-dryer at -60 to -70 °C to become a powder [17].

### 2-2- Film production

To prepare the film, a solution of 1% carboxymethylcellulose (wt/vol) was mixed with 1% (wt%) glycerol (plasticizer) (along with Tween 80 in a suitable solvent which was a mixture of 34 ml ethanol and 66 ml distilled water) and then stirred for 12 hours at room temperature. 5 g of hydrolyzed fish waste powder was diluted with 100 ml distilled water and stirred for 30 minutes at 50°C. Then it was added to the solution containing carboxymethylcellulose and glycerol. Then concentrations of 10, 20 and 40% (v/v) of orange leaf extract (CA) (*Citrus aurantium*) (Provided by the Safiabad-Dezful Agricultural and Natural Resources Research and Education Center) (Previous studies by the corresponding author showed that the leaves of the orange plant have antimicrobial and antioxidant properties and were selected in this study [18 and 19]) were added separately to the final solution and stirred for 20 minutes. The control group was without orange extract. Then, 0.2% Tween 80 (emulsifier) was added to the samples and mixed using a homogenizer for 25 minutes. In the final stage, the resulting solutions were poured into 15 cm diameter Petri dishes to form a gel state and then allowed to dry completely and take the shape of a plate [20].

### 3.2- Fourier transform infrared spectroscopy

Spectrum ATR-FTIR[1] All films obtained using the model spectrometerIFS-48 Made in Germany, manufacturer User, to show the functional groups. 1.5 mg of film powder was mixed with potassium bromide powder in a ratio of 1:100 and made into a 1 mm tablet. Then it was placed in a spectrometer. The percentage of light transmitted in the frequency range$cm^{-1}$4000 to 450 was calculated and then the resulting graph was drawn.Device resolution$cm^{-1}$It was 0.9.

1-Attenuated total reflectance Fourier transform infrared spectroscopy

### 4.2-Preparation of shrimp samples

Green tiger shrimp(*Penaeus semisulcatus*), as one of the most important native and commercial species of Iran and with a wide distribution in the Persian Gulf and the Sea of Yemen [21],Shrimp were purchased from shrimp fishing centers in Bushehr and sent to the laboratory in ice-filled boxes and stored at -20°C until the study was conducted. At the beginning of the experiment, the shrimp were defrosted at 4°C and washed several times, then the head and carapace were removed and their intestines were removed. Then the shrimp were immersed in the prepared film solution for 35 minutes and 4 treatments were obtained. Treatment 1: Shrimp without orange extract, Treatment 2: Shrimp in a film containing 10% orange extract, Treatment 3: Shrimp samples in a film containing 20% orange extract, Treatment 4: Shrimp samples in a film solution containing 40% orange extract. After rinsing the shrimp for 3 minutes, they were placed at 4°C for 15 days and microbial, chemical and color quality tests were performed on days 0, 4, 7, 9 and 15.

### 5-2-Microbial tests

To count total aerobic bacteria (TVC[2]) fromISO 4833-1:2013For this purpose, 5 grams of shrimp samples from each treatment were placed in a sterile Stomaker bag with 45 ml of distilled water (InterScience, France) and homogenized for 180 seconds. Then the sample was diluted to$^{5-}$Diluted to 10 ml. 1 ml of each dilution was placed in a plate containing Count Agar medium. After a few minutes, all plates were inverted and placed in an incubator for 48 hours at 30°C.To count psychrophilic bacteria according toISO 17410:2019, Culture was performed superficially on the culture medium of the Count Agar plate and after keeping the plates at 10°C for 7 days, the number of colonies on the plate was counted. Shrimp samples were taken at specified intervals for mold and yeast tests. To prepare the initial suspension, dilution$^{1-}$10 (10 grams of shrimp sample with 90 milliliters) of Ringer's solution were homogenized in a Stomaker for 2 minutes. Then, decimal dilutions were prepared from the resulting suspension. In culture mediumSDAand incubation temperature 25°C according toISO 21527-1:2008For 120 hoursDone.

### 2-6-Chemical experiments

#### 1-6-2-MeasurementK-valueandH-value

2 grams of shrimp samples from all four treatments were homogenized with 10 ml of trichloroacetic acid (6%) for two minutes. 4 ml of 1 M potassium hydroxide was added to the solution and vortexed.g× 3438 was centrifuged for 12 minutes at 4°C. The supernatant was filteredμm0.45 smooth and then to enter the vialsHPLC (Shimadzu، LC-10ATseries (Made in Japan) with columnCOSMOSIL 5C18-OAQStationary phase and phosphate buffer inpH=6.7 as mobile phase and methanol in a ratio of 95:5 for 20 minutes at 254 nm [22]. The amountKandHIt was calculated using equations 2 and 3:

$$\text{K-value (\%)} = \frac{INO+Hx}{ATP+ADP+AMP+IMP+INO+Hx} \times 100$$

$$\text{H-value (\%)} = \frac{Hx}{IMP+INO+Hx} \times 100$$

Hx: Hypoxanthine,NOW: Inosine,IMP: Inosine monophosphate,AMP: adenosine 5-monophosphate, ADP: adenosine 5-diphosphate, ATP: Adenosine triphosphate.

### 2-6-2- Assay of Biogenic Amines

2 ml of the supernatant prepared above was mixed with 1 ml of 2 M sodium hydroxide. Then 1 ml of 2% benzoyl chloride was added and the solution was vortexed and kept at room temperature for 20 minutes to facilitate the extraction of biogenic amines. In the next step, 2 ml of saturated sodium chloride solution was added and the benzylamines were extracted with diethyl ether. The solution was evaporated under liquid nitrogen at 30°C and the residue was mixed in 500 µl of acetonitrile and water in a ratio of 90:10 and was taken to the device.HPLCIt

2-Total Viable Count

was prepared for the detection of biogenic amines cadaverine, histamine, spermidine and spermine. Using the device (Shimazdzu LC-10A, Japan)HPLCWith columnC18(5 µm, 250 × 4.6 mm) and fluorescent marker were injected. Acetonitrile (Sigma, USA) and distilled water (75:25 v/v) were used. The flow rate of the mobile phase was 1 mm/min. Biogenic amines were determined based on the matching of the peak retention times of the unknown samples with the standard samples (Sigma-Aldrich, St. Louis, MO, USA) and according to the area under the curve, the peaks were determined using the corresponding standard curve [23].

**3-6-2-MeasurementpH**

AmountpHWith a digital devicepHGauge (MI150, Milwaukee Martini Italy) was determined, for which 10 grams of shrimp were homogenized in 100 ml of distilled water and then measured [24].

**4-6-2- MeasurementTBARSandTVB-N**

Thiobarbituric acid was measured using a colorimetric method. 200 mg of shrimp samples from all four treatments were transferred to a 25 ml volumetric flask and made up to volume with 1-butanol. 10 ml of the resulting mixture was placed in dried capped tubes and 5 ml of thiobarbituric acid reagent was added to them. The tubes were placed in a hot water bath at 96°C for 2 hours and then cooled to room temperature. The results were measured using a spectrophotometer (UV-VIS-NIR, modelUV-3600Made in Japan (Absorption value at 530 nm)$A_s$(vs. absorption of control sample)$A_b$) was read. The amountTBAIt was calculated in milligrams of malondialdehyde per kilogram of shrimp tissue based on equation 1 [25]:

$$\text{TBA (mg MDA/ kg)} = \frac{(As-Ab)\times 50}{200}$$

For measurementTVB-N5 grams of shrimp muscle from each treatment was mixed with 90 ml of distilled water and homogenized for 4 minutes at 25,000 rpm. Then, at 4°C,×g3438 was centrifuged for 10 minutes. The supernatant or supernatant liquid was filtered with Whatman paper and 50 ml of it was transferred to the distillation tube of an automatic Kjeldahl apparatus and 6.5 ml of 20% w/v sodium hydroxide was added to it. The distilled material was transferred to a flask containing 40 ml of boric acid solution and the volatile bases present were titrated with 0.01 M hydrochloric acid until reachingpH=5The results were expressed as milligrams of nitrogen/100 grams of shrimp muscle and calculated according to the following formula [26]:

$$\text{TVB-N (mg N/ 100 g)} = \frac{(Vs-Vb)\times 0.14\times 2\times 100}{m}$$

The volume of hydrochloric acid consumed for the sample and control is respectively$In_s$and$In_b$It was shown.mThe weight of the sample is in grams.

**5-6-2- Color analysis**

Using a Hunterlab 4510 colorimeter, made in the USA, color indices include:$a^*$، $b^*$and $L^*$Which respectively indicates$a^*$ < 0= Vegetable,$a^*$> 0= redness,$b^*$< 0= blue,$b^*$> 0= jaundice,$L^*$ = 0Blackness and$L^*$ = 100 The whiteness was measured. The color difference (ΔE) was obtained using the following equation [27]:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

*$L_0^*$، $a_0^*$ and $b_0^*$ Color parameters are zero on the day.*

**6-6-2-Statistical analysis**

To analyze information from softwareSPSSVersion 17 was used. All analyses of this completely randomized design were performed with 3 replications. Data normality was tested using the Kolmogorov-Smirnov test. One-way analysis of variance (One-way ANOVA) and at a probability level of 5%, was used to analyze the data. Means were compared using the Tukey test. The data results were expressed as mean and standard deviation. Excel software was used to draw graphs.

## 3-Results and Discussion

**1-3-FT-IR**

Spectrum resultsFT-IRThe films of the four treatments studied are shown in Figure 1. No significant change was observed in the

spectra of the films with increasing concentration of orange extract. This result was also observed in the study of Jamrooz et al. (2019) who studied the film of forsalaran and several plant extracts and its effect on fish fillets [28]. The broadening of the 3600-3000 band in the two-layer films containing orange extract can be related to the formation of hydrogen bonds between the polyphenols of the extract and the polysaccharide [29]. The absorption peak $cm^{-1}$3500-3200 can be related to the functional groupO-HandN-HAvailable in glycerol and water. Functional groupC-HIt can be in the range of$cm^{-1}$2850-2880 can be seen. The peak is related to$cm^{-1}$1700-1740 to the working group$cm^{-1}$ C=OThe strong peak at 1689 is related to the presence of aldehyde groups.C=Cwhich was seen in the fourth treatment. Peaks$cm^{-1}$1440-1450 related to the linkC-Hmethyl groups. Peaks$cm^{-1}$1200-1250 related to stretchingC-Oare related to the properties of cellulose and hemicellulose derived from lignocellulosic materials [30].

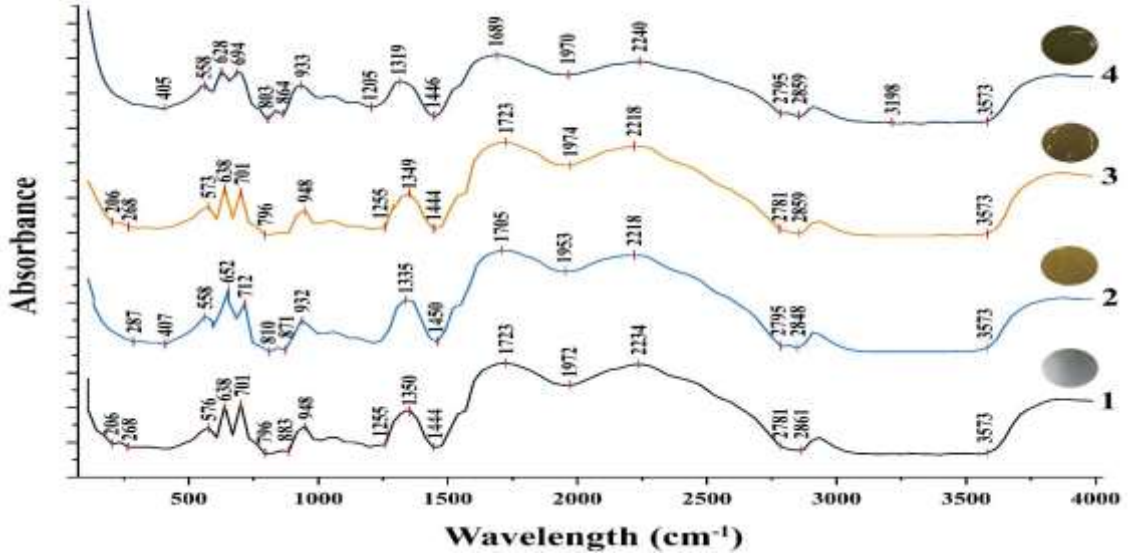


Figure 1- FTIR spectroscopy for double-layered film groups. Film 1: control (without CA); Film 2: 10% CA; Film 3: 20% CA; Film 4: 40% CA

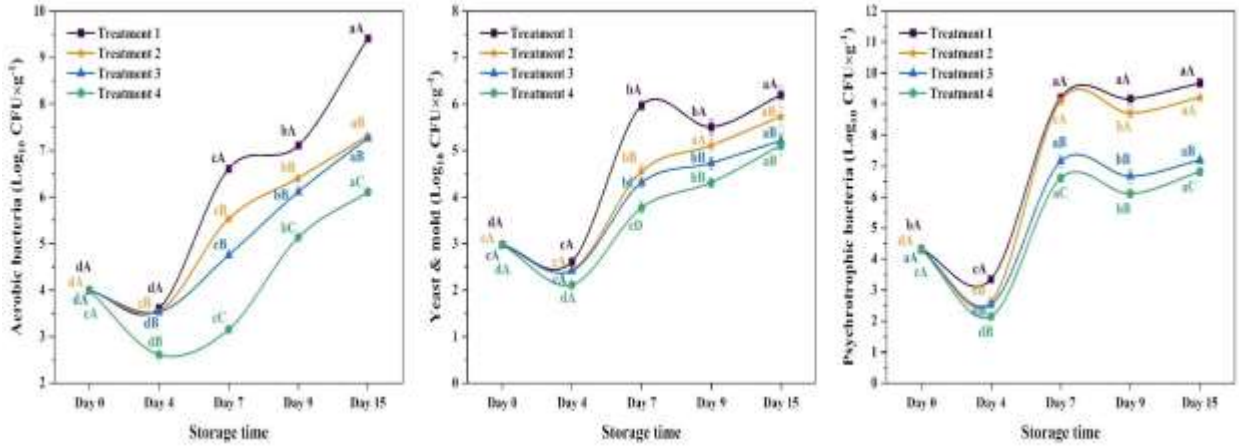


Figure 2- Change in wrapped shrimp quality microbial count during storage. Film 1: control (without CA); Film 2: 10% CA; Film 3: 20% CA; Film 4: 40% CA

## 2-3-Microbial changes

The results of microbial changes in shrimp samples in the film treatments prepared are shown in Figure 2. On all sampling days, the amount ofTVCTreatment 1 or control showed the highest value, which had a statistically significant difference with other treatments ($P<0.05$).P<Initial amount of bacterialog CFU/g During the storage period, the effect of the film on bacterial growth was determined, so that from day zero to the fifteenth in the

control group or treatment 1, the rate ofTVCIn shrimp samples, there was a 6-log increase and a 3-log increase in the fourth treatment. Before the fourth day, a significant decrease was shown in the treatments compared to day zero, but from the fourth day to the last day of storage, the amount of bacteria increased, the lowest amount being in treatment 4, which had a statistically significant difference with other treatments ($p<0.05$).P<).

Throughout the storage period, the amount of mold and yeast increased from the fourth day onwards in all shrimp samples packaged in film. On the seventh day of storage, the most statistically significant difference between the studied treatments was shown in the amount of mold and yeast ($P < 0.05$).P<On the fifteenth day, the amount of mold and yeast in treatment 4 waslog CFU/ g 11.5 and in the control treatment tolog CFU/ g6/19. In the two-layer films containing orange extract, on the fifteenth day, no statistically significant difference was observed in the amount of mold and yeast in the shrimp samples (0.05).P>Film packaging in the fourth treatment was able to reduce the amount of mold and yeast in shrimp samples by 3 logs compared to the control group (4 logs). The growth pattern of psychrophilic bacteria from the growth behaviorTVCInitial levels of psychrophilic bacteria in shrimp samples packaged with two-layer film without extract and containing orange extractlog CFU/ gwas 4.31. In the control group, the amount of psychrophilic bacteria increased from 4.31 on day zero tolog CFU/ g It reached 9.67 on the fifteenth day. On the fourth day, in all shrimp samples that were wrapped with the film containing the extract, a significant decrease was observed in the amount of psychrophilic bacteria, which was statistically significantly different from the control group (0.05).P<). The limit set for the number of bacteriaCFU/g $10^7$-$10^6$But spoilage can be detected mainly due to odor in most foods with more thanlog CFU/g6 was detected [31]. Therefore, the shelf life of the control samples was 7 days. Shrimp samples wrapped in film containing 20% orange extract contained the following microorganisms:CFU/g $10^7$They were stored until the fifteenth day, which increased the shelf life of shrimp by 8 days compared to treatment 1. The amount of microbes in shrimp samples from the fourth treatment, on the fifteenth day of storage, wasCFU/g $10^6$They went beyond, but even after 15 days, below the surfaceCFU/g $10^7$remained. The inhibitory effect of microorganism growth in shrimps up to the fourth day of the study may be due to the presence of hydrolyzed protein in the films, as such films increase the oxygen diffusion barrier and thus prevent the proliferation of microorganisms by forming a protein biofilm around the shrimp samples [32]. In various studies, it was shown that hydrolyzed protein from various aquatic wastes contains di-peptide alanine-tyrosine, which has antioxidant effects and has a low molecular weight [33]. Studies have shown that caryophyllene and limonene were the most abundant compounds in the leaves of the orange plant [18, 19]. The inhibitory effect of orange leaf extract on shrimp microorganisms may be due to the action of hydrolyzable tannins, anthocyanins and benzoic acid. Mechanisms explaining the antimicrobial activity of such compounds include inhibition of extracellular microbial enzymes, deprivation of substrates required for microbial growth, or direct action on microbial metabolism through inhibition of oxidative phosphorylation or iron deprivation [34]. Remya et al. (2017) used chitosan-based film containing ginger essential oil as an active antimicrobial agent to prevent fish spoilage. They found that the film was a good inhibitor against lactic acid bacteria and*Brochothrix thermosphacta*It was [35].

**3-3-Chemical parameters**

Use ofK-valueAs an indicator of freshness, it has been controversial due to its significant dependence on the type of muscle food, species, and external factors such as handling

conditions, season, and slaughter method. Regardless of the type of food and species containing meat, endogenous enzymesATPafter deathAMP، NOW، Hxand finally decompose uric acid [36]. The freshness of fishery products can be assessed based onK-valueclassified into three categories: (1) products with values of 20K≤ Very high percentage(2) those in the range of 20 to 50 percent are classified as semi-fresh and (3) products with values of 70 to 80 percent are classified as fresh.K≥ It indicates the stage of corruption [37]. Figure 3-A-F Product changes related toATPIn shrimp samples packaged in two-layer films without extract and containing different percentages of extract, the initial concentrationATPIn shrimp samplesμmol/ gIt was 0.99.

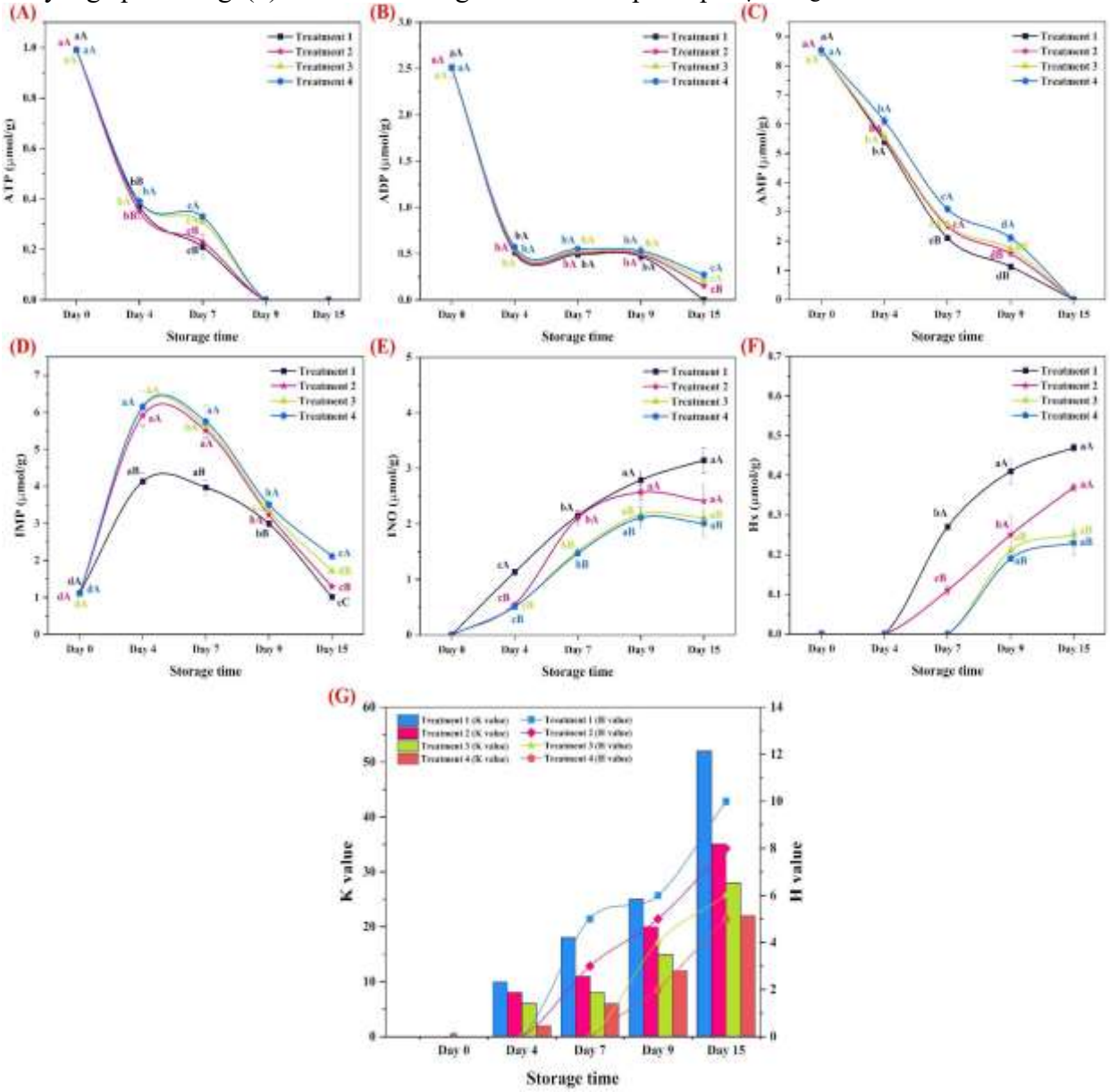


Figure 3- Changes in ATP-related products (A–F), K-value, and H-value of shrimp samples over the storage period. Different letters within each parameter represent statistically significant differences ($p < 0.05$).

From day zero to day four of storage, the amountATPandADPShrimp samples showed a decrease (Figure 3).AMPBy the seventh day of storage, the rate of decline was steep and then the rate of decline slowed. On the fifteenth day, the rate ofAMPIt reached zero. ConcentrationIMPIn shrimp of different treatments, it increased from day 0 to day 4 and then decreased and reached zero on day 15. At the end of the storage period, the highest amount was in the fourth

treatment.IMP μmol/ g11.2 and the lowest value in the control treatmentμmol/ g1.01 was observed. With increasing holding time, the amountNOWincreased and the highest amount was in the control treatmentμmol/ g3.15 was observed. In treatments 2, 3 and 4, theNOWshowed a decrease from the ninth day onwards. Lee et al. (2022) and Seki et al. (2017) showed that the decompositionATPToIMPIt takes place in less than a few hours to two days and is mainly related to the activity of endogenous autolytic enzymes rather than the activity of microorganisms, while the decompositionIMPby bacteria [38,39]. During storage, the amount ofHxIt increased in all treatments.*Pseudomonas* spp.and*Shewanella putrefaciens*They can produce inosine nucleosidase, whichNOWtoHxConverts [40]. FigureGIt shows that the amountKShrimp samples in treatments 2, 3, and 4 were semi-fresh by the end of the storage day, and the amountKShrimps in the control treatment exceeded 50%. The study by Mohammad Alinejad et al. (2024) showed that the rateHxIn shrimp samples up toμmol/ g2.07 increased during 7 days of storage [41]. The amountHAnother quality parameter for assessing seafood spoilage is hypoxanthine accumulation, and the higher its amount, the poorer the quality of seafood products [36].ParameterHAt the beginning of the storage period, it was close to zero in the film-packed shrimp sample and reached 10.5% in the control treatment and 5.2% in the fourth treatment at the end of the period. The amountHIt increased continuously, followed by a sudden increase on the last day in four treatments (Figure 3).

Edible films in the form of active packaging have been shown to prevent the formation of biogenic amines, including histamine. Changes in biogenic amines are closely related to the safety and quality of aquatic animals, including shrimp [42,43]. Histamine is the most important biogenic amine in aquatic animals, which increased during storage (Figure 4). On day 0, the initial histamine level was 17 mg/kg, which increased to 71.19 and 53.67 mg/kg on day 15 in the control and treatment 4, respectively. This result indicated that the applied films not only did not prevent the formation of histamine in shrimp samples, but even increased its formation. However, in none of the shrimp samples did its level exceed 10–50 mg/100 g, which is the risk range for histamine [44]. Histamine production is attributed to the number of bacteria capable of synthesizing histidine decarboxylases. Histamine formation is usually due to Enterobacteriaceae [45].

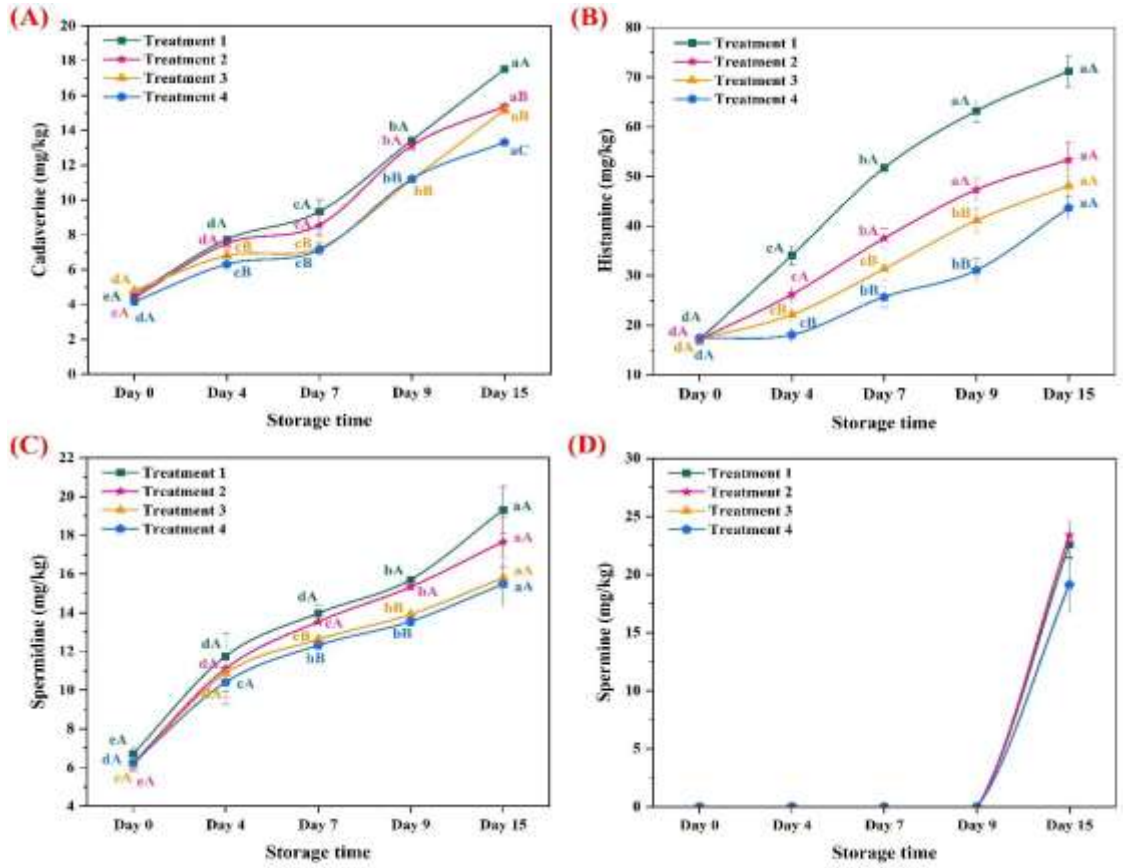


Figure 4- Changes in biogenic amine content (mg/ kg) of shrimp during storage at 4 °C for 15 days.

Cadaverine is associated with off-flavors in aquatic products [45]. As shown in Figure 3, cadaverine levels were low throughout the storage period. Spermidine and spermine levels increased during storage, but spermine levels were undetectable until day 9. Spermine levels were increased in treatments 2 and 3 compared to the control. Since biogenic amines in food products are mainly produced by decarboxylation of the corresponding amino acid precursors, either by bacterial decarboxylases or endogenous enzymes, the availability of free amino acids is essential for the formation of biogenic amines [42]. According to Tohidinsab and Roumian (2023), hydrolyzed protein, which is the main component of the films in the present study, is a good source of free amino acids, 3.396 g free amino acids per 100 g hydrolyzed protein [46]. Part of the hydrolyzed protein in a non-bound form could be a source of free amino acids, which leads to an increase in the production of biogenic amines in the film-coated samples. However, it should be noted that despite 15 days of storage, the levels of all biogenic amines in the samples treated with the film containing 20 and 40% of the extract frommg/kg 50 (the permissible level of histamine in seafood) [44]. In the study of Pongstkol et al. (2022), an increase in biogenic amines was observed in shrimp packaged in polyethylene, glass, vacuum-sealed nylon, and polypropylene containers during storage, with the highest and lowest levels measured in vacuum-sealed nylon and glass packaging, respectively [47]. In the study of Li et al. (2021), histamine and tryptophan were identified as the most abundant biogenic amines in farmed shrimp [48]. The type and amount of biogenic amines formed during storage depend on many factors such as shrimp species, microbial flora, packaging type, temperature, and other factors [41, 43]. In this study, shrimp stored in different film treatments, which can have different permeability to water and gas, may lead to different formation and accumulation of biogenic amines. In the study by Pongstkol et al.

(2022), the histamine level in packaged shrimp samples was 27.79-48.14 mg/kg [47]. ChangespH ، TVB-NandTBARSIn shrimp samples wrapped with a two-layer film containing hydrolyzed protein and orange extract, it is shown in Figure 5.TVB-NIt is formed from the breakdown and decomposition of proteins and their conversion to trimethylamine oxide, dimethylamine, trimethylamine, formaldehyde, and from the deamination of adenine nucleotides. This process causes an increase inpHand the activity of endogenous enzymes and microorganisms that target methylated amines [42]. The amountTVB-NIt varies based on the type of food, microbial contamination, and storage conditions [27]. Bakhit et al. (2021) Acceptable limitTVB-NFor raw shrimpmg N/ 100g < 12, 20-12 edible, 25-20 for border line andmg N/ 100g > 25 were determined to be inedible [49]. The amountTVB-N، mg N/ 100g9.07 was obtained in shrimp samples on the first day of storage. With increasing storage time, the amountpHandTVB-NThe highest values of these two indices were in the control treatment (7.47,mg N/ 100g21.43) and the lowest in the fourth treatment (24.7,mg N/ 100g13/25) was measured. The amountpHThere was a significant difference (0.05) in different days of storage in a treatment.P<In this study, on day zero, the rate ofpHwas higher than shrimp samples in other studies. This could be partly due to their high content of non-protein nitrogenous compounds and free amino acids. In addition, their low carbohydrate content leads to limited lactic acid production and a significant reduction inpHPrevents [50,51]. Initial valueTBARSIn shrimp samples, 0.51-0.91 mg/kg was obtained (Figure 5). From day 0 to day 4, the changes in this parameter were low and decreased in the control and treatment 2, and had a statistically significant difference with treatment 3 and 4 (P<0.05).P<From the fourth day onwards, the amountTBARSIt increased, which was more steep in the control or 1 treatment.

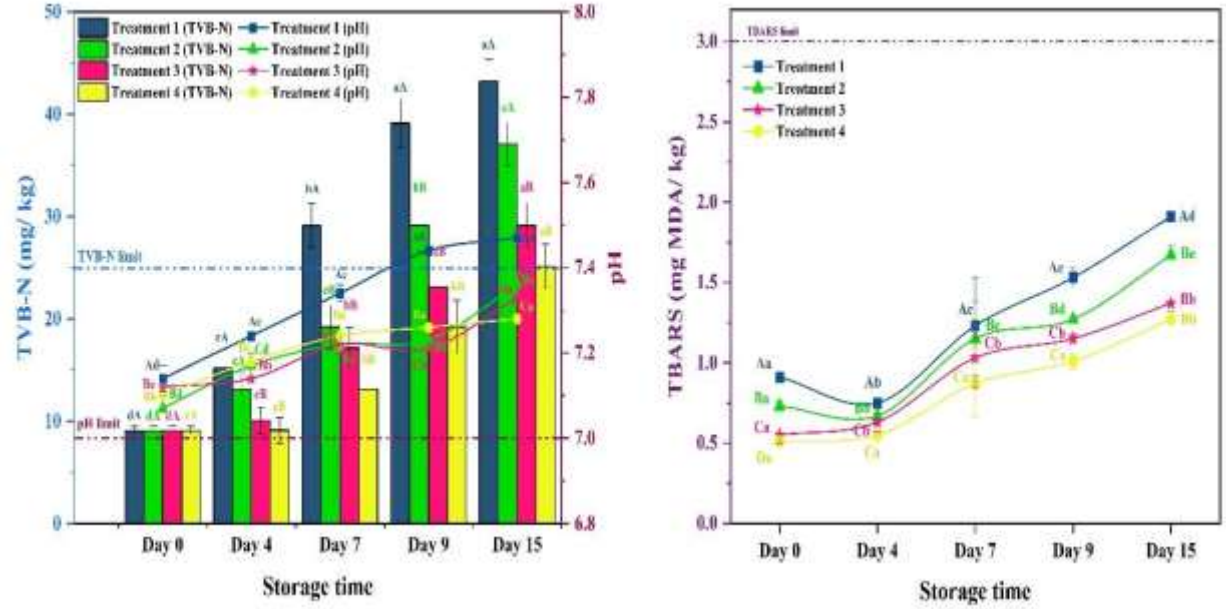


Figure 5- Changes in pH, TVB-N and TBARS level of shrimp during storage at 4 °C for 15 days.

The lowest on day zero and the highest on day fifteenTBARS It was observed in shrimp samples wrapped in a two-layer film containing orange extract. In the third and fourth treatments, no statistically significant difference was observed in shrimp samples on different days of the study (0.05).P>). A similar result was obtained in a study by Diabro et al. (2011) who showed that in blue shark fillets packaged in low-density polyethylene film containing natural antioxidants, a reduction in the rate of lipid oxidation occurred, however, this effect did

not persist in subsequent days of storage [52]. Hydrolyzed proteins are a mixture of active and inactive peptides [46]. In another study, it was shown that shrimp packaged in low-density polyethylene with nanoparticles$Not._2$caused a decrease in the oxidation rate compared to the control treatment [53], which was consistent with the present study. They also showed that lipid oxidation in shrimp is catalyzed by microbial activities. In another study, a similar result was observed, where the rate ofTBARSA two-layer film containing hydrolyzed gelatin from carp skin with the addition of peptide as an antioxidant, compared to the control treatment without peptide, failed to show a significant difference with the control treatment [25]. These results can be explained by the delayed release of active molecules from the film matrix or interactions between the film matrix and the active molecules in the film.

**4-3-Color changes**

In Figure 6, the color parameter changes$a^*$، $b^*$ and$L^*$ Samples of packaged shrimp are shown in four film treatments. Maintaining color stability for fishery products that are sensitive to$pH$are very important. This is essential to ensure that the indicator maintains its color throughout the shelf life of the product and provides consumers with reliable and accurate visual feedback [25]. According to Figure 6, different trends were observed in the color components of the shrimp samples of the 4 treatments studied. The degree of whiteness or$L^*$ In the studied shrimps, it increased until the seventh day and then decreased on the ninth day and increased again. The highest whiteness parameter was observed in shrimps wrapped with double-layer film containing orange extract on day zero. Between the parameter values$a^*$ No statistically significant differences were observed in the 4 treatments studied until the end of the experiment period. (05/0P>)Amount$a^*$In shrimp samples, it was between 7.97 and 9.04. The pattern of changes in the index$b^*$In shrimp samples, it was different from other color parameters. The amount $b^*$In shrimps wrapped with a two-layer film containing hydrolyzed protein and orange extract, it did not follow the same pattern. In treatment 2, it decreased until the seventh day, then increased and decreased again. In treatments 3 and 4, the amount $b^*$Shrimp samples decreased until the fourth day, then increased and then decreased. In the control treatment, the amount$b^*$It showed a decrease from day zero to the end of the storage period.

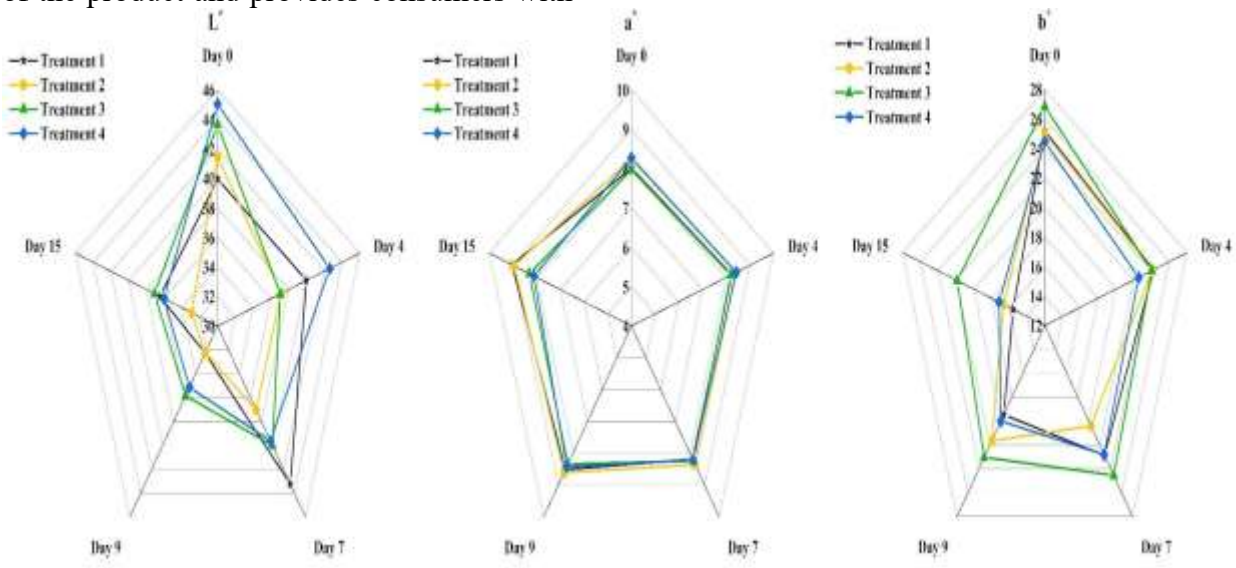


Figure 6- Changes in color parameters of shrimp during storage at 4 °C for 15 days.

Lin et al. (2022) stated that astaxanthin and anthocyanin are naturally found as pigments

in shrimp and are the source of the red color in shrimp [54], so the darker color of shrimp could be a reason for the reduction of the factor.$b^*$ Or$L^*$ During storage. Other studies conducted on shrimp and shrimp products concluded that storage time causes browning and darkening of shrimp color [48,50]. On the other hand, The mechanism of color change in active films may be based on the fact that$NH_3$Escape with$H_2$THEIn the combined film, which leads to the production of ions$NH^{4+}$and$OH^-$Hydroxyl ions create an alkaline environment in which the anthocyanin structure is converted to chalcone, which in turn changes its color [54]. The amount$\Delta E$Above 5 means that the color change can be detected by eye, while values$\Delta E$Above 12 allows for the detection of differences by untrained members [41].

Principal component analysisPCA[3]To identify microbial, chemical and color parameters, understand the relationships between them and detect patterns of changes during storage of shrimp samples wrapped in a two-layer film containing hydrolyzed protein and different concentrations of orange extract during 15 days of storage at refrigerated temperature (Figure 7). Multivariate analysis of the quality properties of shrimp samples allowed the variables to be reduced to two principal components. As shown in Figure 7A-The components of treatment 1 or control showPC1(85.58 percent) andPC2(58.6%) explain a total of 92.16% of the variance. Figure 7B-In the second treatment, the componentsPC1 (88.67 percent) andPC2(63.5%) 30.94% variance, Figure 7C-Related to the third treatment of componentsPC1(81.14 percent) andPC2(11.53 percent) 67.92 percent of variance and Figure 7D-(Fourth treatment) ComponentsPC1(81.68 percent) andPC2(42.9%) explain 10.91% of the variance. Although the percentage of variance of the second treatment orBIt is more than other treatments, but the third and fourth treatments show a more balanced distribution between the components. Treatments 3 and 4 showed a more effective relationship between microbial, chemical and color parameters, which can be a good separation of the relevant time changes. On the other hand, the relationship between the components was higher in the last days of storage, which indicated shrimp spoilage. In all four treatments, color parameters had no correlation with other microbial and chemical parameters.

3- Principal Component Analysis

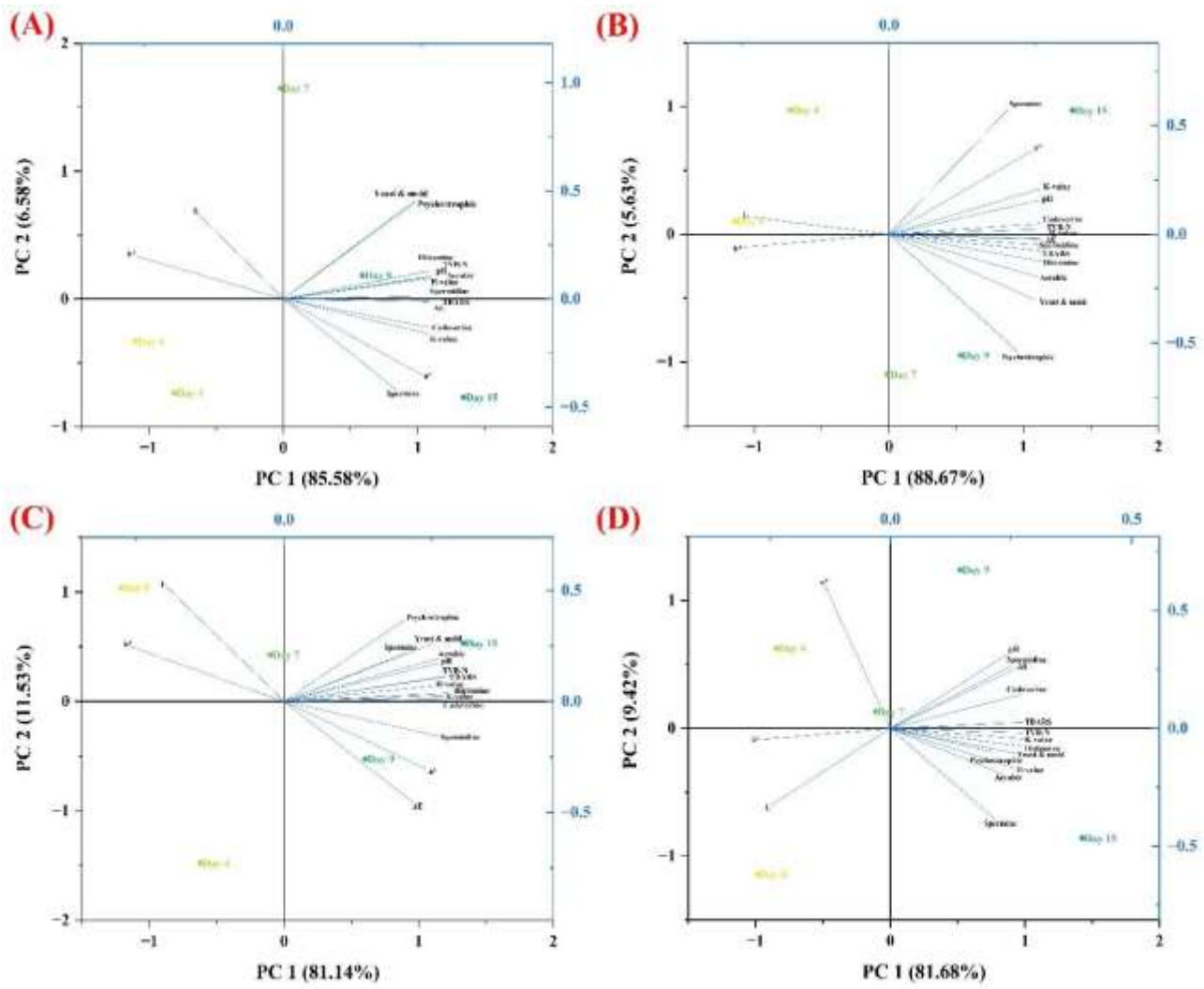


Figure 7- PCA score quality parameters (black color), plots of shrimp stored in different film containers for 15 days (other colors)

From the seventh day of storage, the amount of psychrophilic bacteriaandTVB-N(Given the close relationship between these two indicators), increased. So, considering the onset of spoilage from the seventh day to the end of the storage period, the quality indicators were more closely related to each other, and this coherence is better seen in the third and fourth treatments. In the four treatments studied, the color components had a positive correlation with the second component, which was quite clear in the fourth treatment, while the microbial and chemical parameters showed a positive correlation with the first component and a negative correlation with the second component. In the four treatments studied, the color parameter$L^*$and $b^*$showed a high correlation with each other. Also, the relationship between yeast and mold and psychrophilic bacteria was well shown in the 4 treatments. As Figure 8 shows, the parameter$L^*$and $b^*$in the four treatments studied, they had negative coefficients with other quality parameters. In the fourth treatment, all three color parameters$a^*$، $L^*$and $b^*$showed negative correlation with all microbial, chemical and color parameters. Microbial and chemical parameters had positive correlation coefficients. The results obtained from the correlation coefficients confirmed the results obtained from the principal component analysis.

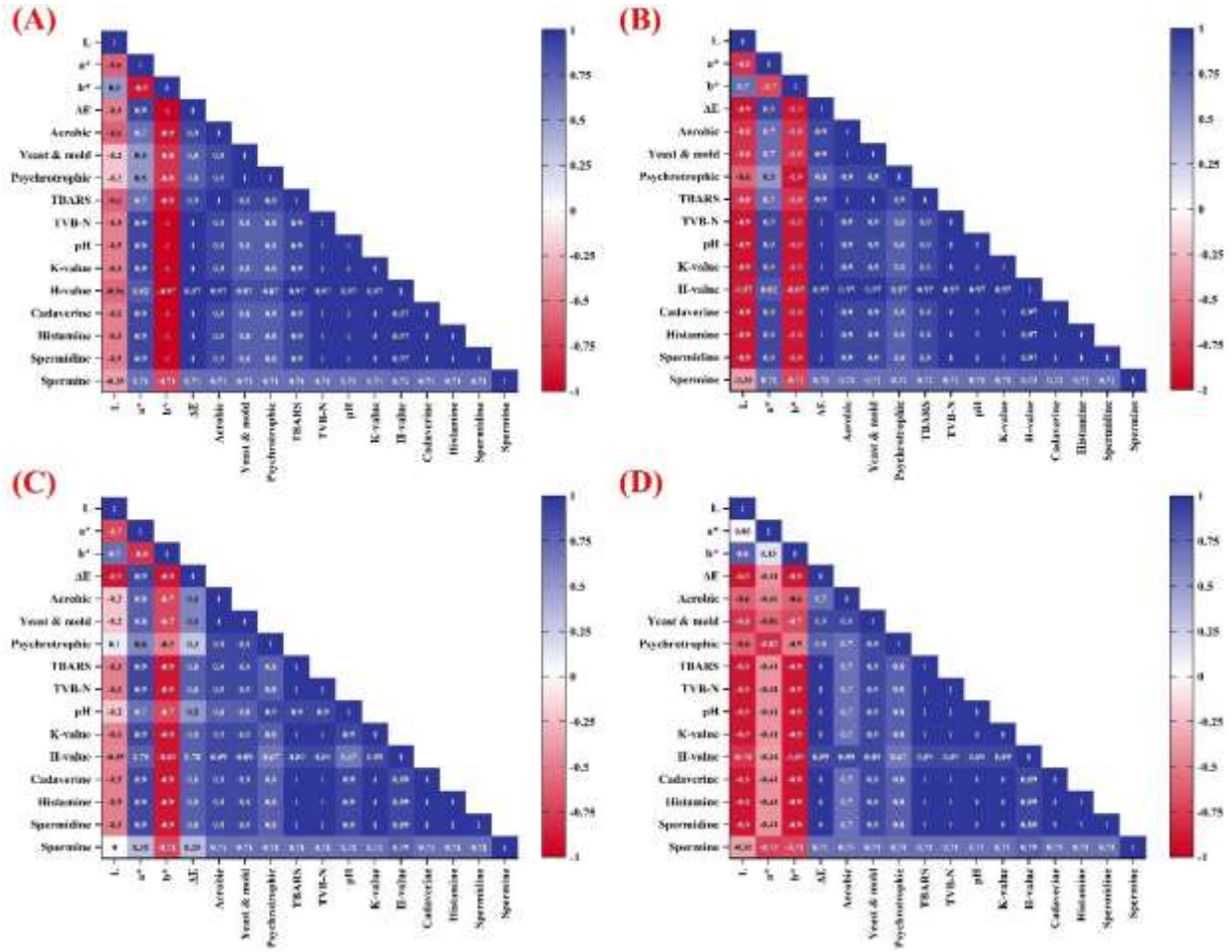


Figure 8- Correlation coefficients between microbial, chemical and color characteristics of shrimp samples in the four studied treatments

## 4- General conclusion

Different levels of changes in microbial, chemical and color characteristics of shrimp samples packaged in different film treatments were measured. Results: The two-layer film containing hydrolyzed protein and carboxymethylcellulose (treatment 1) was able to preserve shrimp shelf life for 8 days (compared to treatment 4) less than other treatments containing orange leaf extract at different concentrations. Hydrolyzed protein from white tuna waste and the use of orange extract were able to reduce microorganisms and shrimp oxidation. The results obtained showed the developmental nature of the work. Improving the protective effect along with reducing the amount of environmental pollution can develop the field for the effect of such bioactive compounds in the packaging and preservation of other types of food products.

## 5-Resources

[1] Mozuraityte, R., Rodríguez-Turienzo, L., Requena, R. and Slizyte, R. 2024. Valorisation of salmon backbones: Extraction of gelatine and its applicability in biodegradable films. Heliyon. 10: e34373.

[2] Mishra, P.K., Gautam, R.K., Kumar, V., Kakatkar, A.S. and Chatterjee, S. 2020. Synthesis of biodegradable films using gamma irradiation from fish waste. Waste and Biomass Valorization. 12: 2247-2257.

[3] Tsang, Y.F., Kumar, V., Samadar, P., Yang, Y., Lee, J., Ok, Y.S., Song, H., Kim, K.H., Kwon, E.E. and Jeon, Y.J. 2019. Production of bioplastic through food waste valorization. Environment International. 127: 625-644.

[4] Gautam, R.K., Kakatkar, A.S. and Karani, M.N. 2016. Development of protein-based biodegradable films from fish processing waste. International Journal of Current Microbiology and Applied Sciences. 5: 878-888.

[5] Sasidharan, A. and Venugopal, V. 2020.Proteins and co-products from seafood processing discards: Their recovery, functional properties and applications. Waste and Biomass Volarization. 11: 5647-5663.

[6] Romani, V.P., Martins, P.CH., da Rocha, M., Bulhosa, M.C.S., Kessler, F. and Martins, V.G. 2024. UV Radiation and Protein Hydrolysates in Bio-Based Films: Impacts on Properties and Italian Salami Preservation. Antioxidants. 13: 1-13.

[7] Kunjiraman, S., Singh, I.S.B., Sarasan, M. and Puthumana, J. 2024. Immobilized microbial consortia: An eco-friendly and sustainable solution for aquaculture waste management. The Microbe. 4: 100100.

[8] Eranda, D.H.U., Chaijan, M., Panpipat, W., Karnjanapratum, S., Cerqueira, M.A. and Castro-Munoz, R. 2024. Gelatin-chitosan interactions in edible films and coatings doped with plant extracts for biopreservation of fresh tuna fish products: A review. International Journal of Biological Macromolecules. 280: 135661.

[9] Tahmasebi, M., Moazzezi, S. and Labeiki, G. 2024. Investigating the effect of lamb meat coating with flax seed mucilage containing *Echinophora platyloba* nanoemulsion on its shelf life. Research in Plant Metabolites. 2: 83-110.

[10] Tadayoni, M. and Cheragi, P. 2024. The effect of aqueous and ethanolic extract of acorn (*Peach oak*) on shelf life of prebiotic and egg-free salad sause. Research in Plant Metabolites, 1: 17-32.

[11] Lima, D.A.S., Grisi, C.V.B., Florentino, G.I.B., Santos, M.M.F., Madruga, M.S., da Silva, F.A.P. 2024. Preparation and characterization of sustainable active packaging based on myofibrillar proteins and protein hydrolysates from the cutting by-product of *Scomberomorus brasiliensis* filleting on the band saw machine. Food Chemistry. 460: 140490.

[12] Athanasopoulou, E., Bigi, F., Maurizzi, E., Karellou, E.I.E., Pappas, C.S., Quartieri, A. and Tsironi, T. 2024. Synthesis and characterization of polysaccharide and protein-based edible films and application as packaging materials for fresh fish fillets. Scientific Reports. 14:517

[13] Leite, L.O., Nogueira, S.M.S., da Silva, A.I.M., do Vale, D.A., Cerqueira, D.A., da Silva, A.L.C., Filho, M.S.M., de Souza, B.W.S. 2024. Development and characterization of myofibrillar protein film obtained from Nile tilapia mechanically separated meat (MSM). Revista Ciencia Agronomica. 55: e20238808

[14] Ali, M.S., Roy, V.Ch., Park, J.S., Haque, A.R., Mok, J.H., Zhang, W. and Chun, B.S. 2024. Protein and polysaccharide recovery from shrimp wastes by natural deep eutectic solvent mediated subcritical water hydrolysis for biodegradable film. Marine Biotechnology. 26: 876-890.

[15] Nelluri, P., Rout, R.K., Tammineni, D.K., Joshi, T.J. and Sivaranjani, S. 2024. Technologies for management of fish waste & value addition. Food and Humanity. 2: 100228.

[16] Fuentes, C., Verdú, S., Grau, R., Barat, J.M. and Fuentes, A. 2024. Impact of raw material and enzyme type on the physico-chemical and functional properties of fish by-products hydrolysates. Food Science and Technology. 201:116247.

[17] Garofalo, S.F., Cavallini, N., Demichelis, F., Savorani, F., Mancini, G., Fino, D. and Tommasi, T. 2023. From tuna viscera to added-value products: A circular approach to fish-waste recovery by green enzymatic hydrolysis. Food and Bioproducts Processing. 137: 155-167.

[18] Dehghan, H. and Roomiani, L. 2020. Antimicrobial activity of Nano-clay films enriched with *Citrus aurantium* essential oil against indicator food-borne pathogens in fishery products. Iranian Journal of Nutrition Sciences & Food Technology. 14: 103-111

[19] Fathimoghadam, F., Shahamirian, M., Roomiani, L. and Tadayoni, M. 2024. Investigating the properties of gelatin-based film activated with *Citrus aurantium*essential oil and their impacts on the quality and shelf life of Jinga shrimp (*Metapenaeus affinis*). Iranian Journal of Chemistry Engineering. 43: 302-313

[20] Eshaghi, R., Mohsenzadeh, M. and Ayala-Zavala, J.F. 2024. Bio-nanocomposite active packaging films based on carboxymethyl cellulose, myrrh gum, $TiO_2$ nanoparticles and dill essential oil for preserving fresh-fish (*Cyprinus carpio*) meat quality. International Journal of Biological Macromolecules. 263:129991

[21] Darvishi, Kh., Kamrani, E., Safaei, M., Daliri, M. and Owfi, F. 2024. Length-weight relationship, length abundance, maturity percentage and growth parameters of green tiger prawn (*Penaeus semisulcatus* de Haan, 1844) caught by trawl net in the waters of Bushehr province (in Mond and Dayyer-Nakhilo). Journal of Utilization and Cultivation of Aquatics, 12 (4):173-184.

[22] Lerfall, J., Thomassen, G.M.B. and Jakobsen, A.N. 2018. Quality of fresh saithe (*Pollack virens*) in

modified atmosphere packages as affected by the gas composition. Food Packaging and Shelf Life. 18: 147–156

[23] ¨Ozogul, F., Taylor, K., Quantick, P. and ¨Ozogul, Y. 2002. Biogenic amines formation in Atlantic herring (*Clupea herring*) stored under modified atmosphere packaging using a rapid HPLC method. International Journal of Food Science and Technology. 37: 515–522.

[24] Ocano-Higuera, V., Maeda-Martínez, A., Marquez-Ríos, E., Canizales-Rodríguez, D., Castillo-Yáñez, F., Ruíz-Bustos, E., Graciano-Verdugo, A.Z. andPlascencia-Jatomea, M. 2011. Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. The Scientific World Journal. 125: 49–54.

[25] Jamr´oz, E., Kulawik, P., Tkaczewska, J., Guzik, P., Zając, M., Juszczak, L.,Krzyściak, P. and Turek, K. 2021. The effects of active double-layered furcellaran/gelatin hydrolysate film system with Ala-Tyr peptide on fresh Atlantic mackerel stored at -18 °C. Food Chemistry. 338: 127867.

[26] EU2019/627. 2019. Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls. Official Journal of the European Union. L131: 51–100.

[27] Khalafpour, M.J. and Roomiani, L. 2022. Investigation of microbial, chemical and color changes of fish burgers in different storage conditions using artificial neural network. Research and Innovation in Food Science and Technology. 11: 67-82.

[28] Jamroz, E., Kulawik, P., Guzik, P. and Duda, I. 2019. The verification of intelligent properties of furcellaran films with plant extracts on the stored fresh Atlantic mackerel during storage at 2 °C. Food Hydrocolloids. 97:105211.

[29] Jamroz, E., Kulawik, P., Krzyściak, P., Talaga-Ćwiertnia, K. and Juszczak, L. 2019. Intelligent and active furcellaran-gelatin films containing green or pu-erh tea extracts: Characterization, antioxidant and antimicrobial potential. International Journal of Biological Macromolecules. 122: 745–757.

[30] Arafat, Y., Altemimi, A., Pratap-Singh, A. and Badwaik, L.S. 2021. Active biodegradable films based on sweet lime peel residue and its effect on quality of fish fillets. Polymers. 13, 1240.

[31] HPA, H. 2009. Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market; HPA: London, UK.

[32] Abdelhedi, O., Jridi, M., Nasri, R., Mora, L., Toldrá, F. and Nasri, M. 2019. Rheological and structural properties of *Hemiramphus far* skin gelatin: Potential use as an active fish coating agent. Food Hydrocolloid. 87, 331–341.

[33] Pane, K., Durante, L., Crescenzi, O., Cafaro, V., Pizzo, E., Varcamonti, M., Zanfardino, A., Izzo, V.; di Donato, A. and Notomista, E. 2017. Antimicrobial potency of cationic antimicrobial peptides can be predicted from their amino acid composition: Application to the detection of "cryptic" antimicrobial peptides. Journal of Theoretical Biology. 419: 254–265.

[34] Pedram Nia, A., Mortazavi, A. and Nemat Shahi, M.M. 2018. Study of chemical compounds and the antimicrobial effects of leaf extract of *Laurel* L on various microbial strains. Journal of food science and technology. 81: 217-226

[35] Remya, S., Mohan, C.O., Venkateshwarlu, G., Sivaraman, G.K. and Ravishankar, C.N. 2017. Combined effect of $O_2$ scavenger and antimicrobial film on shelf life of fresh cobia (*Rachycentron canadum*) fish steaks stored at 2 °C. Food Control. 71: 71–78

[36] Jakobsen, A.N., Gabrielsen, L., Johnsen, E.M., Rotabakk, B.T. and Lerfall, J. 2022. Application of soluble gas stabilization technology on ready-to-eat pre-rigor filleted Atlantic salmon (*Psalm of the Salt* L.). Journal of Food Science. 87(6): 2377–2390.

[37] Huang, Y.R., Zelaya, M.F.G. and Shiau, C.Y. 2016. Changes in biochemical compositions and quality of white shrimp (*Litopenaeus vannamei*) during storage. Journal of Aquatic Food Product Technology. 25: 35–45.

[38] Li, D., Zhuang, S., Peng, Y., Tan, Y., Hong, H. and Luo, Y. 2022. Mechanism of inosine monophosphate degradation by specific spoilage organism from grass carp in fish juice system. Foods. 11(17): 2672.

[39] Seki, H., Nakazato, K. and Hamada-Sato, N. 2017. Adenosine monophosphate degradation and inosinic acid accumulation in the shrimp *Penaeus japonicus*. International Aquatic Research. 9: 37–52.

[40] Surette, M.E., Gill, T.A. and LeBlanc, P.J. 1988. Biochemical basis of postmortem nucleotide catabolism in cod (Gadus morhua) and its relationship

to spoilage. Journal of Agricultural and Food Chemistry. 36(1): 19–22

[41] Mohammadalinejhad, S., Jensen, I.J., Kurek, M. and Lerfall, J. 2024. Novel colorimetric indicators based on alginate hydrogel beads containing anthocyanin for visual freshness monitoring of shrimp and minced chicken. LWT-Food Science and Technology. 199: 116127.

[42] Tkaczewska, J., Jamróz, E., Guzik, P. and Kope´c, M. 2022. Attempt to extend the shelf-life of fish products by means of innovative double-layer active biodegradable films. Polymers. 14: 1717.

[43] Fazlara, A., Peighan, R., Gharehchahi, M. and Ali Noori, S.M. 2020. Toxicity of biogenic amines and the chemical indices for spoilage in peeled white shrimp (*Litopenaeus vannamei*). Journal of Nutrition Fasting and Health. 8: 248- 253.

[44] Lehane, L. and Olley, J. 2000. Histamine fish poisoning revisited. International Journal of Food Microbiology. 58: 1-37.

[45] Alak, G., Hisar, S.A., Hisar, O. and Genççelep, H. 2011. Biogenic amines formation in Atlantic bonito (*Sardinian Sardinian*) fillets packaged with modified atmosphere and vacuum, wrapped in chitosan and cling film at 4 °C. European Food Research and Technology. 232: 23–28.

[46] Tohidi Nasab, M. and Roomiani, L. 2023. Optimizing the hydrolyzed protein process from *Metapenaeus affinis* shrimp wastes. Iranian Journal of Nutrition Sciences and Food Technology. 18: 93-104

[47] Pongsetkul, J., Benjakul, S. and Boonchuen, P. 2022. Changes in volatile compounds and quality characteristics of salted shrimp paste stored in different packaging containers. Fermentation. 8: 1-19.

[48] Li, W., Lu, H., He, Z., Sang, Y. and Sun, J. 2021. Quality characteristics and bacterial community of a Chinese salt-fermented shrimp paste. LWT-Food Science and Technology. 136: 110358

[49] Bekhit, A. E.-D. A., Holman, B. W., Giteru, S. G., & Hopkins, D. L. (2021). Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. Trends in Food Science & Technology, 109, 280–302.

[50] Ghorbani, M., Divsalar, E., Molaei, R., Ezati, P., Moradi, M., Tajik, H., et al. (2021). A halochromic indicator based on polylactic acid and anthocyanins for visual freshness monitoring of minced meat, chicken fillet, shrimp, and fish roe. *Innovative Food Science & Emerging Technologies, 74*, Article 102864.

[51] Zhang, K., Huang, T.-S., Yan, H., Hu, X., & Ren, T. (2020). Novel pH-sensitive films based on starch/polyvinyl alcohol and food anthocyanins as a visual indicator of shrimp deterioration. *International Journal of Biological Macromolecules, 145*, 768–776.

[52] de Abreu, D.A.P., Losada, P.P., Maroto, J. and Cruz, J.M. 2011. Natural antioxidant active packaging film and its effect on lipid damage in frozen blue shark (*Prionace glauca*). Innovative Food Science and Emerging Technologies. 12: 50–55

[53] Lue, Z., Xu, Y. and Ye, Q. 2015. Effect of nano-$SiO_2$-LDPE packaging on biochemical, sensory, and microbiological quality of Pacific white shrimp *Penaeus vannamei*during chilled storage. Fisheries Science. 81: 983-993.

[54] Lin, D., Sun, L., Chen, Y.L., Liu, G.M., Miao, S. and Cao, M.J. 2022. Shrimp spoilage mechanisms and functional films/coatings used to maintain and monitor its quality during storage. Trends in Food Science & Technology. 129: 25-37

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

# مطالعه فیلم دولایه زیست‌تخریب‌پذیر بر پایه پروتئین هیدرولیزشده و تاثیر آن در ماندگاری میگوی ببری‌سبز

امیر قلندری[۱] ، لاله رومیانی[۲*]

۱- کارشناسی ارشد گروه علوم و صنایع غذایی، واحد اهواز، دانشگاه آزاد اسلامی، اهواز، ایران

۲- دانشیار گروه شیلات، واحد اهواز، دانشگاه آزاد اسلامی، اهواز، ایران

**اطلاعات مقاله**

**تاریخ های مقاله :**

**تاریخ دریافت: ۱٤۰۳/۸/۲**

**تاریخ پذیرش: ۱٤۰٤/۳/۱۹**

**کلمات کلیدی:**

فیلم،

ماندگاری،

شاخص‌های میکروبی و شیمیایی،

میگو

DOI: 10.22034/FSCT.22.166.75.

* مسئول مکاتبات:

laleh.roomiani@iau.ac.ir

l.roomiani@yahoo.com

**چکیده**

در این مطالعه، هدف ما تولید فیلم دولایه سازگار با محیط‌زیست با پروتئین هیدرولیزشده بدست آمده از ضایعات هوورمسقطی فرآوری‌شده و عصاره برگ گیاه نارنج به عنوان افزودنی بود. فیلم‌های دولایه بر پایه کربوکسی‌متیل‌سلولز و پروتئین هیدرولیزشده (لایه اول) و بکارگیری عصاره نارنج در سه سطح ۱۰، ۲۰ و ٤۰ (وزنی/ حجمی) (لایه دوم) بودند. ارزیابی تاثیر فیلم‌ها بر ماندگاری میگوی ببری‌سبز در طول ۱۵ روز نگهداری در دمای ٤ درجه سانتی‌گراد، از طریق پارامترهای میکروبی، شیمیایی و رنگ مورد تجزیه و تحلیل قرار گرفت. نتایج به‌دست‌آمده نشان داد که نمونه‌های میگو بسته‌شده با فیلم‌های دولایه، سبب کاهش تعداد باکتری‌های هوازی کل، کپک و مخمر و باکتری‌های سرمادوست به میزان ۳، ۱ و ۳ لوگ نسبت به تیمار شاهد شد. فیلم دولایه حاوی ٤۰ درصد عصاره نارنج توانست نسبت به سایر تیمارها سبب کاهش K-value، H-value، TVB-N و TBARS در میگو شود. هیستامین، کاداورین، اسپرمیدین و اسپرمین با افزایش زمان نگهداری در تیمارها افزایش نشان داد. میزان $a^*$ در نمونه‌های میگو دارای تفاوت معنی‌داری بین تیمارها نبود (P>۰/۰۵) اما مقادیر $b^*$ و $L^*$ میگو اختلاف معنی‌دار آماری بین تیمارهای مطالعه نشان داد (P<۰/۰۵). نتایج ضرایب همبستگی، تاییدکننده و منطبق با نتایج حاصل از آنالیز مؤلفه‌های اصلی بود. نتایج این مطالعه نشان داد که فیلم‌های دولایه می‌توانند جایگزینی برای مواد پلاستیکی شوند.