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Investigating the Characteristics of Basil Seed Gum-based Film Enriched with *Echinophora platyloba* Extract and Its Preservative Effect on the Quality of Silver Carp

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Abstract

This research aimed to examine the potential of increasing the shelf life of *Hypophthalmichthys molitrix* (silver carp) fillets using basil seed gum-based film enriched by various concentrations (0, 0.5, 1, 1.5, and 2% /dry matter) of *Echinophora platyloba* extract (EPE) during a 16-day storage period. Each characteristic of basil seed gum-based films enriched by EPE followed a specific trend. The results showed that with increasing EPE, the film thickness, length to the rupture point, and antioxidant activity of film increased; however, the film with the highest concentration of EPE had the lowest solubility (19%) and tensile strength (15 MPa). The film loaded with 2% EPE overall presented acceptable physicchemical properties and was used for wrapping (optimum film). The fillets wrapped by the optimum film on the 16th day of storage indicated a lower total volatile basic nitrogen (TVB-N) value (36 mg N/100 g meat) compared to the nonenriched film (60 mg N/100 g meat) as well as the control sample (65 mg N/100 g meat). The fillet sample wrapped by basil seed gum-based film enriched with 2% EPE had lower pH and thiobarbituric acid index, peroxide index, and microbial load after 16 days of storage compared to the basil seed film sample without the extract and control sample. The obtained results encourage the use of basil seed gum-based film containing EPE in active food packaging systems to extend the shelf life of silver carp fillets.

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Keywords
Active film
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Silver carp fillet

Introduction

The positive effects of long-chain omega-3 polyunsaturated fatty acids on human health have led to a significant increase in seafood cravings. However, seafood is very sensitive in terms of quality (Jeon *et al.*, 2002). Various techniques are being developed to reduce the decay of meat products. These methods include vacuum packaging, modified atmosphere packaging, refrigeration, and active packaging (Daneshniya *et al.*, 2020; Lin & Lin, 2005). Packaging materials improve the shelf life of food by creating appropriate physicochemical states. In this context, newer methods such as coating/wrapping food with edible films consisting of natural polymers extracted from various sources are also used. Biodegradable films that have been prepared to alter the food surface properties are continuous matrices of proteins, polysaccharides, or lipids (Rhim & Ng, 2007).

Ocimum basilicum L. is one of the native plants of Iran, which is widely used as a medicinal plant. This plant grows in many parts of the world, especially in the tropics of Asia, Africa, and Central and South America (Bahramparvar & Goff, 2013). The outer layer of basil seeds swells rapidly when it is in contact with water and forms a gelatinous substance. Previous studies have shown that the hydrocolloid extracted from basil seeds has two main components, the main skeleton or core of glucomannan, which is resistant to acid (43%) and has glucose to mannose ratio of 10 to 2, and the acid-soluble part, which has xylan with 1 to 4 bonds (24.29%) (Abdolmaleki *et al.*, 2014; Javidi *et al.*, 2016; Safarkhanloo & abdolmaleki, 2022). Basil seed gum has good functional properties compared to some commercial hydrocolloids used in food industry (Bahramparvar & Goff, 2013).

Recently, researchers have centered on producing new samples of active packaging containing antimicrobial compounds (Wu *et al.*, 2014), and natural antimicrobials have attracted significant notice due to the difficulties caused by the use of chemical preservatives (Daneshniya *et al.*, 2021; Davidson & Zivanovic, 2003; Latifi *et al.*, 2019). Plant extracts such as *Echinophora platyloba* are a group of natural antimicrobials. *Echinophora platyloba* is a perennial herbaceous plant of the genus *Echinophora* (30-100 cm high) covering the short, curved trichome and a hard, thick stem. *Echinophora* has four species of aromatic herbs, two of which are exclusively indigenous to the Iranian plateau. This plant is known for its local names Khosharizeh, Tigh Toragh, Kashandar, and Toluq Otto. Previous studies have shown that this plant contains saponins, flavonoids, and alkaloids, and one of the most critical terpene compounds identified in it includes trans-β-ocimene with a concentration of 67.9% (Mahboubi *et al.*, 2009). The antibacterial effects associated with its extract on *Staphylococcus aureus* and *Streptococcus faecalis* (Avijgan *et al.*, 2006) *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* (Kalhor *et al.*, 2022)) have also been proven

The use of natural materials that are also degradable is necessary to maintain quality and increase shelf life due to the problems caused by qualitative changes in fish during cold storage and the utilization of synthetic preservatives. In a study regarding shelf life extension of refrigerated chicken fillets, it was reported that basil seed gum-based coatings incorporated with *Zataria multiflora* (Shirazi thyme) essential oil and summer savory essential oil could extend the shelf-life of fresh chicken fillets through retarding the adverse chemical reactions and microbial growth (Daneshniya *et al.*, 2020). In another study where the effect of basil seed gum coating containing *Elwendia persica* (black cumin seed) essential oil on the quality and shelf life of rainbow trout fillet during refrigerated storage was investigated, it was reported that the coating could slow down the increasing process of oxidation and microbial indices compared to the control treatment (Sayari *et al.*, 2021). Considering the abundance of cyprinid fish, mainly silver carp, which has the highest amount of products in the world as well as accounting for about 60% of domestic production of cyprinid fish in Iran (Kamkar *et al.*, 2014).

Therefore, the present study aimed to prepare an edible film based on basil seed gum enriched with *Echinophora platyloba* extract (EPE) and its utilization as a preservative film to increase the shelf life of silver carp fish at refrigerator temperature.

Materials and methods

Materials and apparatuses

To prepare *Echinophora platyloba* extract (EPE), the dried herb of *Echinophora platyloba* was purchased from a reputable grocery in Hamadan (Iran). The extraction was then performed by soaking and concentrating the extract with a rotary apparatus connected to a vacuum pump (Karami Moghadam & Emam-Djomeh, 2017). The purchased basil seeds were mixed with deionized water in a ratio of 1.50

(dry matter/water) at a temperature of 50 °C and a pH of 7. The resulting mixture was centrifuged (Universal 320, Arteco, Tehran, Iran) for 20 min at 5000 rpm to separate the gum from the basil seeds. The obtained gum was dried in an oven at 50 °C and stored in impermeable receptacles (Razavi *et al.*, 2009). Fresh silver carp were purchased from local markets and transported to the laboratory in a wholly frozen form. After abdominal emptying and cutting, the prepared fillets were thoroughly washed and placed under the hood to remove excess water. Other chemicals with analytical grades were prepared and supplied from Merck (Germany).

Preparation of basil seed gum film containing *Echinophora platyloba* extract

In this study, samples of basil seed gum film were produced based on the method of Hashemi & Mousavi Khaneghah (2017). In the initial stage, 5% (w/w) basil seed gum solution was prepared by dissolving 5 g of basil seed gum in 100 mL of deionized water and adding 30% (w/w) glycerol at 35 °C with the speed of 600 rpm for 15 min. In the next step, EPE was added to basil seed gum solution at concentrations of 0, 0.5, 1, 1.5, and 2%. The obtained solution was poured onto the mold to prepare the films and dried at room temperature for 18 h. Finally, the films were separated from the molds and placed in a desiccator at a temperature of 25 °C and relative humidity of 53% to adjust the humidity until utilization for wrapping the samples (Hashemi & Mousavi Khaneghah, 2017).

Determining the thickness

Digital micrometers with an accuracy of nearly 0.01 mm were used to determine the thickness of the samples at least 10 random points in each film. The average thickness was calculated and used to determine the mechanical properties.

Determination of solubility in water

The degree of solubility in water for different samples was determined according to the method of Zolfi *et al.* (2014). Strips on the scale of 4×1 cm were dried in an oven at 105 °C for 24 h and then weighed to determine the films' initial dry weight (A). To determine the films' final dry weight, 4×1 cm strips were immersed in 50 mL of distilled water for 6 h at 25 °C, then in an oven at 105 °C were dried for 24 h, and finally weighed (B). Solubility in water was calculated according to the following equation:

$$\text{solubility percentage in water} = \frac{A-B}{A} \times 100 \quad (1)$$

Measurement of mechanical properties

Tensile strength and strain to breakpoint were determined using a tissue measuring apparatus (QTS 25, Avantor, Vienna, Austria), (ASTM, 2002). All samples were adjusted for moisture before the test. The films were cut into rectangles measuring 6×1 cm^2. The initial distance between the two jaws of the device was 4 cm, the movement speed of the upper jaw was 50 mm/min, and the lower jaw was constant.

Measurement of antioxidant properties

The percentage of free radical scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was performed using Siripatrawan & Harte (2010) method. For this purpose, 25 mg of the film was gently stirred for 5 min in 3 mL of distilled water. Then, 3 mL of the film extract was added to the test tubes containing 1 mL of a 1 M solution of DPPH in methanol and kept at room temperature for 30 min. The absorbance of test and control tubes was measured by spectrophotometer (EV-2200, Onlab Instruments, Shanghai, China) at the wavelength of 517 nm. The decolorization degree of these compounds indicates the ability to trap free radicals by the relevant antioxidant. Finally, the DPPH free radical scavenging activity was determined using the formula below.

$$\text{DPPH free radical scavenging activity} = \frac{A-B}{A} \times 100 \quad (2)$$

Where *A* is the absorption rate of the control sample and *B* is the absorption rate of the film extract sample.

Different treatments of silver carp fillets

The fillets were placed in special containers, refrigerated, and evaluated for chemical and microbial properties for 16 days at 4-day intervals. It should be mentioned that these fish fillet samples included the sample wrapped by basil seed gum film, basil seed gum film containing the optimal concentration of EPE, and the unwrapped sample.

pH measurement

5 g of each homogenized tissue sample was added to 45 mL of distilled water and mixed for 30 seconds using a homogenizer (Royaniran, Tehran, Iran). Then a digital pH meter (HI981204, Hanna instruments, Tehran, Iran) was used to measure the pH of the samples (AOAC, 1982)

Peroxide index measurement (PV)

In order to measure the peroxide index, 20 cc of oil extracted from different samples was poured into a 250 mL flask with a decanter, and about 25 mL of chloroform acetic acid solution was added to the contents. Then, 0.5 mL of saturated iodide potassium solution, 30 mL of distilled water, and 0.5 mL of 1% starch solution were added to the solution. The amount of released iodine was titrated with 0.01 N sodium thiosulfate solutions (Kirk & Sawyer, 1991). The amount of peroxide was calculated based on Eq. (3):

(3)

$$\mathrm{Pv} = \frac{\text{The consumption volume of thiosulfate} \times \text{Normality} \times 1000}{\text{Meat sample weight}}$$

Measurement of total volatile bases of nitrogen (TVB-N)

10 g of fish sample with 2 g of magnesium oxide and 300 mL of distilled water were poured into a Kjeldahl balloon. The balloon was then attached to the apparatus and heated from below. A 250 mL Erlenmeyer flask containing 25 cc of 2% boric acid solution (2 g of boric acid per 100 cc of distilled water) with a few drops of methyl red reagent (0.1 g of methyl red per 100 cc) was placed at the bottom of the apparatus. Distillation lasted for 45 min from when the material was boiled in a balloon or about 100±5 cc of liquid accumulated in the Erlenmeyer flask. Upon alkalinization by volatile nitrogenous bases, the boric acid solution was turned yellow and distilled. The titration of this solution by 0.1 N sulfuric acid was resumed until the boric acid turned red again. The quantity of TVB-N in mg/100 g of meat was obtained according to Eq. (4), (Goulas & Kontominas, 2005).

(4)

$$\mathrm{TVB-N} = \text{Consumption volume of sulfuric acid} \times 14$$

Measurement of thiobarbituric acid (TBA)

The TBA was measured by the colorimetric method (Natseba *et al.*, 2005). 200 mg of meat sample was transferred to a 25 mL balloon and then brought up to the volume with 1-butanol. After filtration, 5 mL of this mixture was introduced into dry and capped tubes, and 5 mL of TBA reagent was added to it (TBA reagent by dissolving 200 mg of TBA in 100 mL of 1-Butanol solvent was obtained after filtration). The capped tubes were placed in a water bath at 95 °C for 2 h and then cooled to ambient temperature. Afterward, the absorbance (*As*) was read at the wavelength of 532 nm in front of the control distilled water (*Ab*). The quantity of TBA (mg malondialdehyde per kg of fish tissue) was estimated based on Eq. (5):

(5)

$$TBA = \frac{(\mathrm{As} - \mathrm{Ab}) \times 50}{200}$$

Microbial analysis

For microbiological experiments, 5 g of fillet meat sample was mixed with 45 mL of physiological serum solution and homogenized, followed by the preparation of drafts. 1 mL of each dilution was cultured in bacteria by the pour-plate method in plate count agar medium. Samples were placed in a 37 °C incubator for 48 h to identify the total bacterial load and in a 7 °C incubator for 10 days to distinguish psychrotrophic bacteria. After incubation, the colonies were counted (Abdollahi *et al.*, 2014).

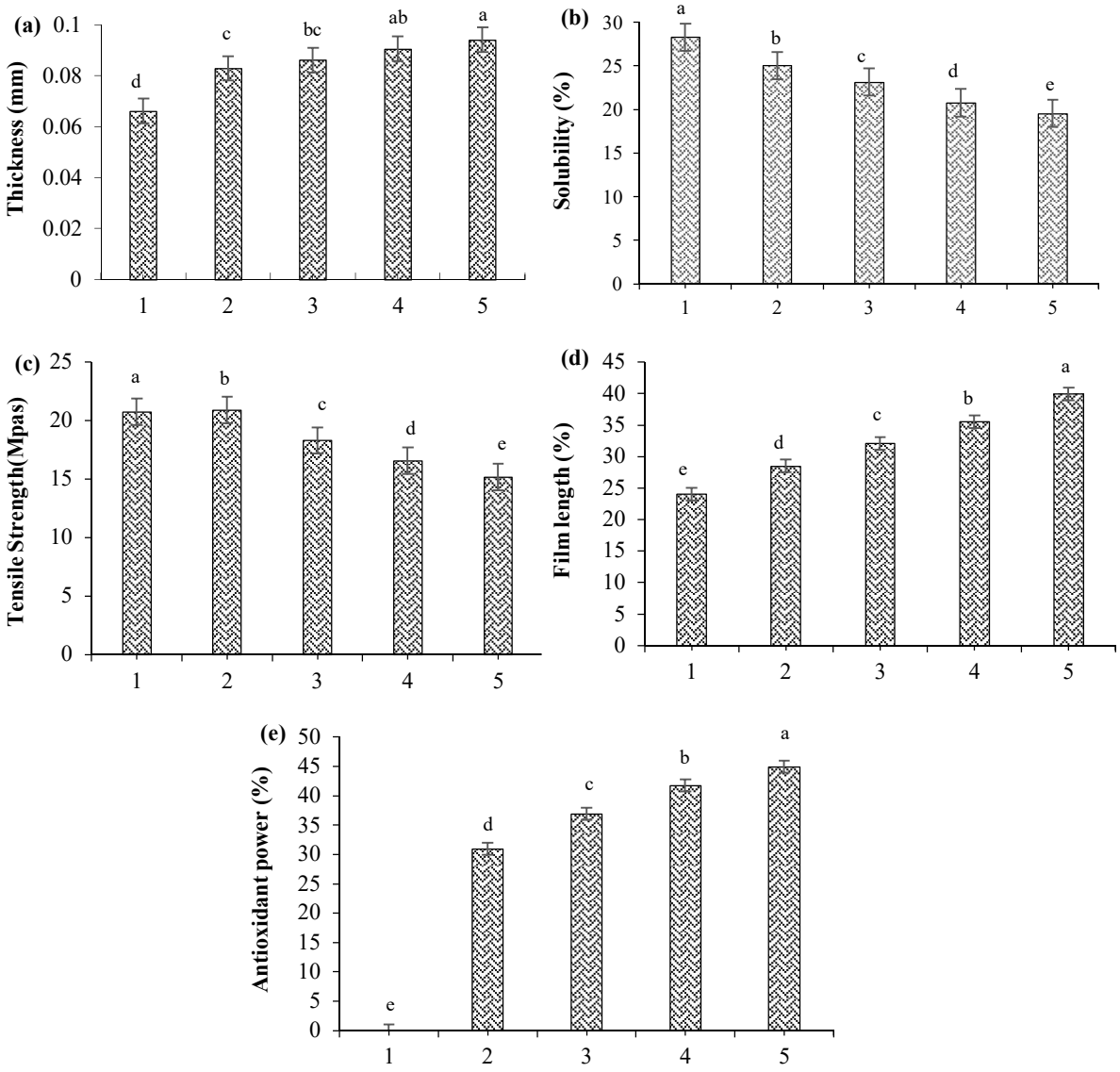

Fig. 1. Variations in film a) thickness, b) solubility, c) tensile strength, d) elongation at break, and e) antioxidant power at different concentrations of *Echinophora platyloba* extract. 1: Film containing 0% EPE, 2: Film containing 0.5% EPE, 3: Film containing 1% EPE, 4: Film containing 1.5% EPE, 5: Film containing optimum concentration -2% EPE. Different English letters indicate statistically significant differences at the 95% confidence level.

Statistical analysis methods

In this study, a completely randomized plan was used to compare the results of different properties of produced active films and investigate fish fillets' chemical and microbial changes. The results were analyzed using the GLM program from SAS (Version 9.1; USA). Duncan's test was used to compare the means of different characteristics, and the values were evaluated at a significance level of $P<0.05$. All measurements were performed in three replications, and Microsoft Office Excel 2013 software was used to draw the graphs.

Results and discussion

Film properties

Thickness

Thickness is one of the critical determinants in the preparation of edible films, which can directly impact the features of the film, including permeability to water vapor and oxygen, as well as the mechanical properties of the film (Emam-Djomeh *et al.*, 2015). Based on the result of the thickness evaluation shown in Fig (1), with increasing the amount of EPE, the film thickness increases significantly ($P\leq0.05$), so that unenriched film and the film containing 2% EPE had the lowest and highest thickness,

respectively. The increase in thickness of film samples as the direct outcome of the EPE addition can be due to the presence of hydrophilic and hydrophobic compounds in the extract, which lead to the formation of a porous structure. Another reason could be the entrapment of water molecules in the pores of the film structure, which increases moisture and swelling and thus increases the film thickness (Emam-Djomeh *et al.*, 2015). A study aligned with current research reported that adding pomegranate peel extract to the film with sodium caseinate substrate increases the film thickness (Emam-Djomeh *et al.*, 2015). Another study also reported that adding Shirazi thyme essential oil to carboxymethyl cellulose-based film traps micro-drops of essential oil in the film and increases its thickness (Dashipour *et al.*, 2015). The results of a study on κ-carrageenan film containing savory essential oil were also consistent with the results of this study (Shojaee-Aliabadi *et al.*, 2014). In this regard, it was also reported that adding the savory essential oil to agar films increases the thickness (Atef *et al.*, 2015).

Solubility

Based on these results of the film solubility, with increasing the concentration of EPE in the film formulation, the solubility decreased significantly ($P \leq 0.05$). While most of the samples had a solubility above 20%, the film enriched with the optimum concentration of EPE had a solubility below the mentioned percentage. The highest solubility among samples is related to unenriched film, with a solubility of 28%. In contrast to the thickness, elongation, and antioxidant activity of the films, the addition of EPE reduced the solubility samples. The continuous decreasing trend in solubility of film samples followed by the addition of different concentrations of EPE is related to the existence of water-repellent compounds in EPE. Similar to the findings of this study, in a study, a decrease in the solubility of agar film as a result of the addition of savory essential oil was observed (Atef *et al.*, 2015). In this regard, other similar results have been reported (Abdollahi *et al.*, 2012; Ojagh *et al.*, 2010).

Mechanical properties

Tensile strength

Tensile strength is the maximum tensile stress that a material can withstand without permanent strain (Ghanbarzadeh *et al.*, 2010), which depends on the chemical structure of the molecules or the interconnection of the polymer chains in the film matrix (Krotchta & De Mulder-Johnston, 1997). The results of the tensile strength showed that the addition of EPE had a significant reduction effect ($P \leq 0.05$) on the tensile strength of basil seed-based film. With 21 MPa unenriched sample had the highest tensile strength. Although the lowest EPE concentration did not bring the tensile strength below 20 MPa, concentration surge led to the decrease of tensile strength, so that film with optimum EPE concentration had the lowest tensile strength equal to 15 MPa. The reason for the reduction in tensile strength with the addition of EPE is the partial displacement of strong polymer-polymer bonds with weak polymer-oil bonds in the film network in the presence of the extract, which reduces the polymer network connection and, consequently, the tensile strength of the films. In a study consistent with the findings of the present study, it was reported that the addition of pomegranate peel extract reduces the tensile strength of sodium caseinate film (Emam-Djomeh *et al.*, 2015). Another study has revealed that tensile strength in chitosan film was reduced by adding orange essential oil (Sánchez-González *et al.*, 2010). The addition of thyme essential oil to chitosan has also been reported to reduce tensile strength (Hosseini *et al.*, 2016).

Strain percentage in breakpoint

The percentage of elongation indicates the maximum increase in film length compared to the initial length of the film until the rupture point due to tensile stress, which is an indicator of the edible film flexibility. The strain to breakpoint results of different films based on basil seed gum indicates that increasing the amount of the extract in the

film formulation has led to an increase in the percentage of elongation until the breakpoint ($P \leq 0.05$). By adding an optimum concentration of EPE to the film, the elongation percentage elevated more than 15% compared to unenriched film and reached 40%. Consistent with the mentioned findings of the current research, which revealed the elongation of films would gradually increase by enhancing the EPE concentration, (Hashemi & Mousavi Khaneghah, 2017) reported that adding thyme essential oil to the film based on basil seed gum will increase the strain percentage. In justifying their results, the researchers acknowledged that the extracts could act as emollients and increase the flexibility of the polymer chains, thereby increasing the elongation percentage. Also, the liquidity of the extracts at room temperature and their presence in the form of oil droplets in the film can cause its structural deformation and increase the flexibility of the films. In a study consistent with the results of the current research, adding oregano essential oil to chitosan and gelatin-based films resulted in an increase in the elongation percentage (Hosseini *et al.*, 2016). Another research associated with hydrocolloid films increase the flexibility of the films (Mohammad Amini *et al.*, 2015). The researchers attributed this phenomenon to the plasticizing effect of oregano essential oil, enhancing the film's elasticity. In another study, the elongation percentage of chitosan film increased with the addition of orange essential oil was revealed (Sánchez-González *et al.*, 2010).

Antioxidant activity

DPPH free radical scavenging activity can be considered as an indicator of the antioxidant activity of the film. According to the results of the percentage of free radical scavenging of DPPH by different film samples, free radical scavenging increased ($P \leq 0.05$) with increasing EPE. While the antioxidant activity of the unenriched film was evaluated below 5%, the addition of the lowest concentration of EPE significantly increased the antioxidant activity of the film, so that film enriched with 2% of EPE had the highest antioxidant activity equal to 45%. The antioxidant activity of essential oils and oil extracts in edible films and coatings has been investigated by previous studies, for which two mechanisms can be summarized; Improving the barrier capacity property against oxygen, which acts as an oxygen trap, and so-called oxygen scavenger and the specific antioxidant activity is released when the compounds are coated inside the product (Bonilla *et al.*, 2012). The antioxidant capacity of edible films naturally correlates with their phenolic composition (Shojaee-Aliabadi *et al.*, 2014). EPE with high compounds of α-Flanders, Limonene, *p*-Cymene, α-Pinene, Carvacrol, and β-Myrcene have shown high antioxidant effects (Pass *et al.*, 2012). Therefore, the percentage of free radical scavenging increases by adding the extract to the film formulation. A study showed that elevation of the cress extract combined with hydroxypropyl methylcellulose-based film significantly affects the antioxidant potential of the wrapping so that the film containing the highest concentration of the extract possesses the highest antioxidant activity (Latifi *et al.*, 2021).

Chemical and microbial characteristics of silver carp wrapped by the basil seed gum-based film enriched with optimum concentration of EPE

pH change

The results of examining the pH alterations of different fish samples during a 16-day refrigeration period were obtained. The pH of all samples increased significantly ($P \leq 0.05$) with increasing storage time. As it can be seen in Fig. (2), in each checkpoint, during the storage period, unwrapped fillets and samples wrapped by the basil seed gum-based film enriched with EPE had the highest and lowest pH, respectively. The pH of the fillets was measured 6.3, though it increased to 6.8 in the unwrapped sample. In general, the typical pH of live fish muscle is close to 6.9, which varies from 6 to 7 after death depending on the season, species, and other factors (Arashisar *et al.*, 2004).

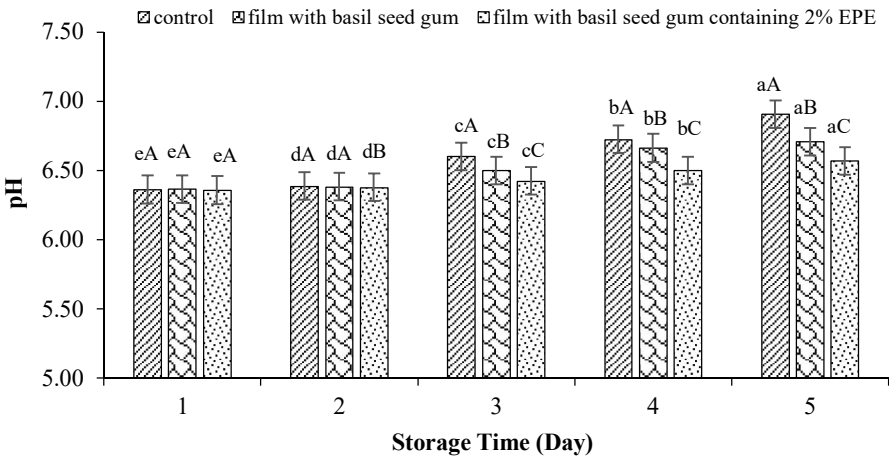

Fig. 2. pH variations of different fish samples during storage at refrigerator temperature. Different lowercase letters indicate significant changes in the pH of each treatment during the storage period at a significance level of $P<0.05$. Different uppercase letters indicate a significant difference in the pH of different treatments in the same storage period at a significant level of $P<0.05$.

The value of this index increased during the period based on the results of Fig. (2), which can be attributed to the activity of autolytic enzymes and proteolytic bacteria that spoil fish (Arashisar *et al.*, 2004). Lower pH in fish samples wrapped by film enriched with EPE can be related to the potential of inhibiting the activity of bacteria and enzymatic proteases by coatings (Fan *et al.*, 2009). Proteases can produce basic components (ammonia, trimethylamine, biogenic amines, etc.) from fish proteins. The results of this study complied with the findings of Hosseini *et al.* (2016), where fish gelatin-based coating enriched with essential oregano oil showed to be effective in slowing the pH elevation rate of refrigerated rainbow trout fillet. Comparing the treatments, it was observed that same as pH, the peroxide index of the control treatment was higher than other samples throughout the storage periods.

Peroxide index

The peroxide index is a measure of hydroperoxides. Hydroperoxides are the primary oxidation products in oils and fats that can be disintegrated into volatile and non-volatile by-products. The peroxide index is a good indicator for detecting the early stages of oxidation (Lin & Lin, 2005). Fig. (3) shows the results of measuring the peroxide number of different fish treatments during the 16-day refrigeration period. Based on these results, peroxide of all samples soared with increasing storage time up to the 12th day, which was statistically significant in all samples ($P\leq0.05$). The highest and lowest peroxide index were measured in unwrapped fillet and wrapped by the enriched film with the indexes of 5.8 and 4.3 meq/kg, respectively.

The peroxide index surge is due to the oxidation of fats, but the process decreased from the 12th day to the end of the period, which is probably due to the decomposition of hydroperoxides into by-products of oxidation (Gómez-Estaca *et al.*, 2007). In other words, the formation rate of hydroperoxides was higher than their decomposition rate at the beginning of the period. Therefore, we see an increase in the amount of peroxide, and after reaching the maximum amount due to the decrease in the amount of substrate or raw material and the instability of peroxide molecules, the decomposition rate increases (Mohan *et al.*, 2012).

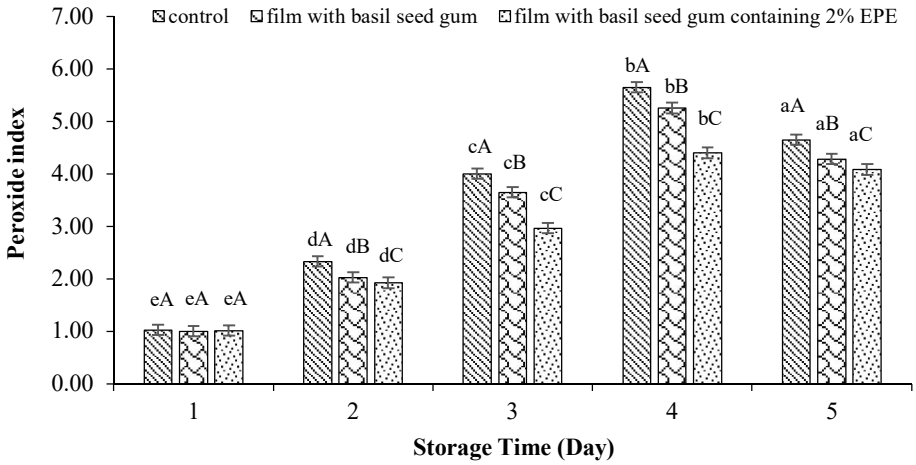

Fig. 3. Peroxide number variations of different fish samples during storage at refrigerator temperature. Different lowercase letters indicate significant changes in the peroxide number of each treatment during the maintenance period at a significant level of $P<0.05$. Different uppercase letters indicate a significant difference in the peroxide number of different treatments in the same storage period at a significance level of $P<0.05$.

The peroxide index of the samples decreased from the 12th day until the end of the storage period. Comparing the peroxide number of different samples at the same points of the storage period shows a significant surge ($P\leq0.05$) in the control sample's peroxide number compared to the other samples. Although there was no notable difference between the peroxide indexes of the samples on the first day of storage, the enriched film performed effectively in controlling the peroxide index. In general, biodegradable films such as basil seed gum film have a very low permeability to oxygen and carbon dioxide than synthetic films such as polyethylene. Therefore, coating the fish fillets with basil seed gum film significantly reduces the product's contact rate with oxygen and lessens the rate of initial oxidation of fats and subsequent formation of hydroperoxides (Mohan *et al.*, 2012). The reduction in fat oxidation by EPE may be due to its phenolic compounds. Although the antioxidants in the extract do not act as an oxygen absorber, they prevent the formation of fatty free radicals that react with or absorb oxygen during the oxidation process. In this way, they delay the auto -oxidation of fats (Turhan *et al.*, 2009). The results of this study were consistent with the results of other researchers, including Hosseini *et al.* (2016), where it was shown that fish gelatin-based coating enriched with essential oregano oil reduced the peroxide value of the refrigerated rainbow trout fillet. In another study, the lowest peroxide index was observed in the common carp fish fillet coated by hydroxypropyl methylcellulose-based coating enriched with cress extract (Latifi *et al.*, 2021).

Total volatile basic nitrogen (TVB-N)

The TVB-N is used as a chemical indicator for quality assessment, spoilage measurement, and marine products' shelf life (Rodríguez *et al.*, 2008). High bacterial activity levels break down compounds such as trimethylamine oxide, peptides, and amino acids into volatile bases (López-Caballero *et al.*, 2005). As indicated in Fig. (4), with increasing storage time, the amount of TVB-N in all samples has increased significantly ($P\leq0.05$) and reached its highest level at the end of the storage period due to spoilage bacteria's activity. There was no significant difference between the amounts of TVB-N on the first day, though in all other checkpoints throughout the storage periods except, the highest and lowest TVB-N levels were related to the control sample and the sample wrapped by basil seed gum film containing EPE, respectively.

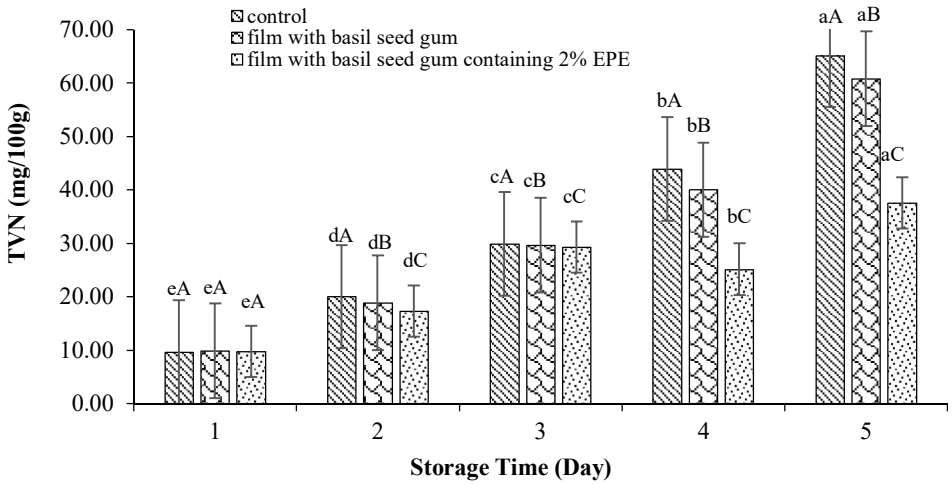

Fig. 4. Variations in the number of volatile nitrogen bases (TVB-N) of different fish samples during storage at refrigerator temperature. Different lowercase letters indicate significant changes in the number of volatile nitrogen bases of each treatment during storage at a significant level of $P<0.05$. Different uppercase letters indicate a significant difference in the number of volatile nitrogen bases of different treatments in the same storage period at a significance level of $P<0.05$.

While enriched film maintained the amount of TVB-N in fillet below 40 mg/100 g fillet sample, this quantity reached above 65 mg/100 g fillet sample. The amount of total volatile nitrogen in the wrapped samples was always less than the control samples throughout the storage period. The reason for this higher value was the higher bacterial count in the control treatment. This study showed that the total volatile nitrogen in the sample wrapped by basil seed gum film containing EPE was less than the control sample and the sample wrapped by basil seed gum film. The lower value of total volatile nitrogen in the samples wrapped by basil seed gum film containing EPE was due to the plant's antibacterial properties, mainly phenolic compounds. It should be noted that the hydrophobic nature of these phenolic compounds disrupts the lipid phase or the phospholipid phase of the bacterial cell membrane and increases the permeability and loss of cell contents. Interruption of the enzymatic system and its inactivation or degradation of genetic material is another mechanism that has been mentioned for the antibacterial activity of phenolic compounds in various resources (Mahmoud *et al.*, 2004). The results of the current study have been approved by the results of Hosseini *et al.* (2016), so that used a Persian gum-based coating containing clove and Shirazi thymol essential oils and a fish gelatin -based coating containing marjoram essential oil used to increase the life of rainbow trout fillets under freezing conditions. Moreover, in the study of Latifi *et al.* (2021), coating common carp fish fillet with hydroxypropyl methylcellulose-based coating reduced the total volatile basic nitrogen amount in the samples and a synergistic effect addition of *Lepidium sativum* extract significantly enhanced the reduction.

Thiobarbituric acid (TBA)

Thiobarbituric acid is broadly used to evaluate the degree of fat oxidation in fish. Oxidation by-products such as aldehydes and ketones cause an unpleasant taste and odor in the product (Kostaki *et al.*, 2009). The statistical analysis of changes in the TBA index for different fish samples during the storage period is shown in Fig. (5). The amount of this parameter increased in all samples with increasing storage time ($P\leq0.05$), and among them, the highest increase was related to the control sample.

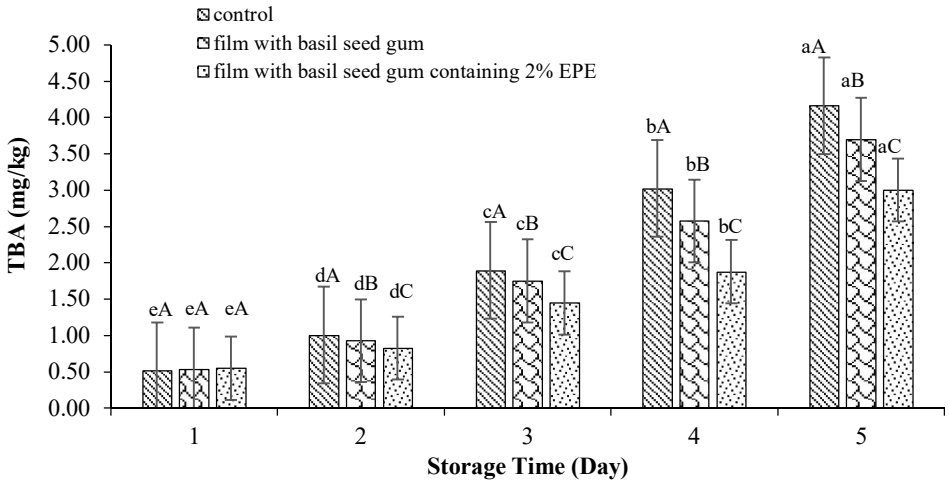

Fig. 5. Variations in the thiobarbituric acid (TBA) index for different fish samples during storage at refrigerator temperature. Different lowercase letters indicate significant changes in the thiobarbituric acid index of each treatment during the maintenance period at a significant level of $P<0.05$. Different uppercase letters indicate a significant difference in the thiobarbituric acid index of different treatments in the same storage period at a significance level of $P<0.05$.

Nevertheless, using a basil seed gum wrapping with and without EPE reduced the TBA index's increasing rate. On the first day of the storage period, there was no remarkable difference between the amount of TBA in the samples, and all had an approximate TBA of 0.5 mg/kg. At the end of the storage period sample wrapped by enriched film had the lowest TBA, equal to 3 mg/kg, and the unwrapped fillet had the highest with the quantity of 4.3 mg/kg. The same trend as total volatile nitrogen was observed for the thiobarbituric acid parameter. In a study that *Ferulago angulata* essential oil nanoemulsion was combined in chitosan to prepare an active coating for rainbow trout fillets preservation, thiobarbituric acid reactive substances were lower than other samples in each checkpoint during 16 days storage period (Shokri *et al.*, 2020). In the study of Jouki & Khazaei (2022), synthesized active batter coatings enriched by quince seed gum and carvacrol microcapsules reduced thiobarbituric acid reactive substances in r deep-fat fried chicken nuggets. In another study, carrageenan coating incorporated with lemon essential oil reduced thiobarbituric acid reactive substances in trout fillets during the storage period (Volpe *et al.*, 2019). All mentioned studies are consistent with the present study.

Microbial quality

The Figs. (6) and (7) present the statistical findings of the total viable count (TVC) and psychrophilic bacterial counts (PTC) of the fillet samples. The results show that with increasing storage time, both parameters increased significantly ($P\leq0.05$). From Fig. (6), it is evident that TVC grew gradually during the storage period. On the first day of this period, there was no noticeable difference between the samples, and the TVC of all was measured at roughly 4 log CFU/g. In all other checkpoints throughout the storage, the unwrapped and wrapped by enriched film samples were revealed to possess the highest and lowest TVC; so that, at the end of the storage period, sample wrapped by film enriched by the optimum concentration of EPE had 7.3 log CFU/g of TVC, while this quantity for the unwrapped sample was equal to 9.3 log CFU/g. Regarding psychrophilic bacterial counts, it can be perceived that a similar trend to TVC was observed. However, according to Fig. (7), an exception in this trend can be seen, where PTC of fillet wrapped by enriched film reached 4.5 log CFU/g on day 4 and decreased to below 4 log CFU/g on day 8.

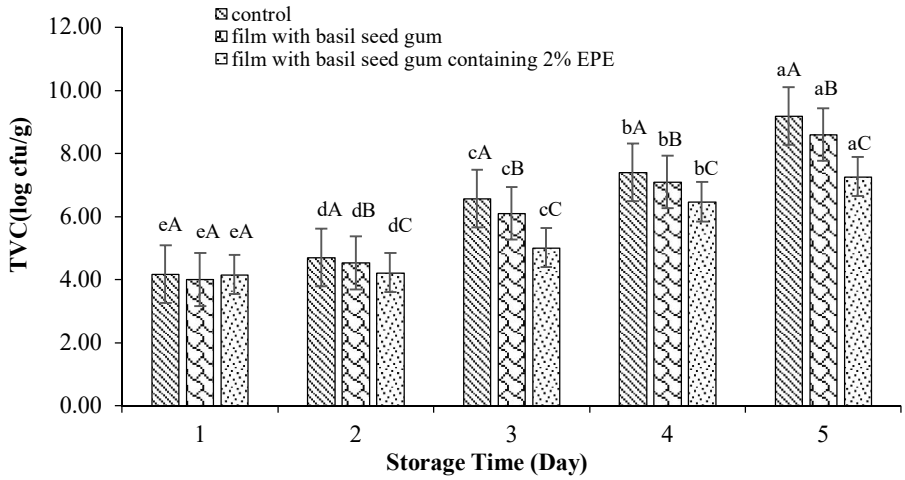

Fig. 6. Variations in the total viable count (TVC) of all different fish samples during storage at refrigerator temperature. Different lowercase letters indicate significant changes in the total bacterial count of each treatment during the maintenance period at a significant level of $P<0.05$. Different uppercase letters indicate a significant difference in the bacterial count of all different treatments in the same storage period at a significance level of $P<0.05$.

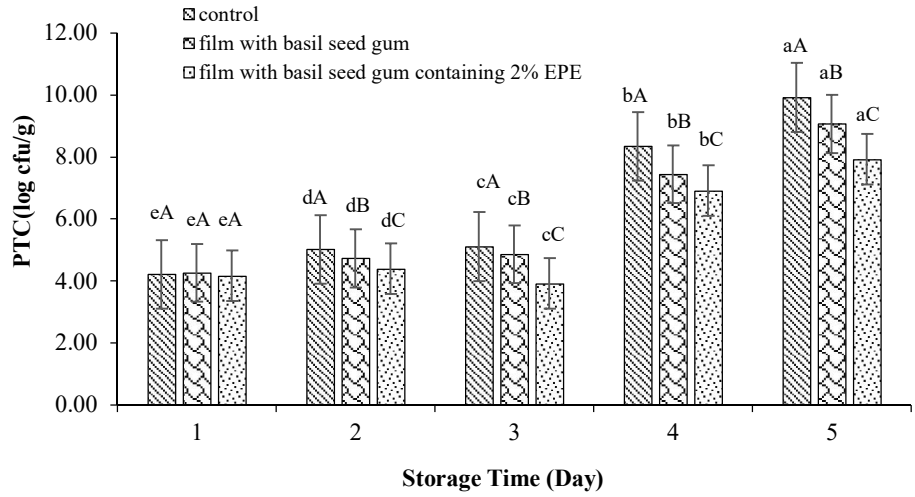

Fig. 7. Variations in the psychrophilic bacterial counts (PTC) in different fish samples during storage at refrigerator temperature. Different lowercase letters indicate significant changes in the number of cryogenic bacteria in each treatment during the maintenance period at a significance level of $P<0.05$. Different uppercase letters indicate a significant difference in the number of cryogenic bacteria of different treatments in the same storage period at a significance level of $P<0.05$.

Ultimately, at the end of the storage period, the sample wrapped by enriched film had a PTC of 8 log CFU/g, while this quantity for the unwrapped fillet was equal to 10 log CFU/g. Same as in the previous parameters, the lowest increase rate of the microbial load was related to the sample wrapped by a film containing EPE. Meat contains proper compounds for the growth of microbes, so the presence of bacteria is one of the reasons for the decrease in the quality of fish fillets during the storage period. The International Commission on Microbiological Specifications for Foods (ICMSF) has set a licit limit for the total bacterial load in raw fish, which is 7 log CFU/g (Guillerm-Regost *et al.*, 2006). The initial value of bacterial load depends on several factors such as processing during the fillets preparation, contamination of equipment used, and people's health involved in the work. During the storage period, the total bacterial load in all samples increased, so that at the end of the 12th day for the control treatment was 7.43, for the sample wrapped by basil seed gum and the

sample wrapped by basil seed gum containing EPE was 7.01, and 6.43 log CFU/g, respectively. Inhibition of bacterial growth by wrapping based on basil seed gum can be attributed to its coating effect and oxygen penetration inhibition. Similar results have been reported on the effect of biodegradable coatings on the bacterial nature of different species of fish during storage (Fan *et al.*, 2009; Ojagh *et al.*, 2010). Moreover, the antibacterial activity of EPE has been reported by Pass *et al.* (2012), as a result of which, the sample coated by the film containing the extract had the lowest microbial count.

Conclusions

The current study can be divided into two parts: The impact of different concentrations of EPE on different film properties based on basil seed gum was investigated in the first part of this study. The results revealed that the film thickness increased with increasing EPE in basil seed gum-based film while the film solubility decreased. Despite the increase in length, the tensile strength decreased with increasing the extract concentration. The antioxidant activity of basil seed gum film increased with the increasing concentration of EPE. Considering all mentioned parameters, basil seed gum film containing 2% EPE was selected as the best active film sample. Different characteristics of unwrapped silver carp fillets, wrapped by a basil seed gum-based film and a basil seed gum-based film containing 2% EPE during storage for 16 days in the refrigerator was investigated in the second part of this study. This study showed that the quality characteristics of all samples changed with increasing storage time, and the values of parameters such as pH, total volatile nitrogen bases, thiobarbituric acid index, peroxide number, and microbial load increased. The results also showed that the utilization of film containing basil seed gum enriched with EPE increases the shelf life of fish and prevents rapid spoilage. In general, the results of this study indicated that the use of a film based on basil seed gum containing EPE increases the shelf life of silver carp fish during storage at refrigerated temperatures.

Author contributions

Farzaneh Abdolmaleki: Presenting the research idea and study design, Supervising the study, Approval of the final version; **Shayesteh Shirkhorshidi**: Data collection, Data analysis and interpretation, Writing the draft of the manuscript; **Milad Daneshniya**: Data analysis, Revising and editing the manuscript.

Conflicts of Interest Statement

There is no conflict of interest based on the writers.

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بررسی ویژگی‌های فیلم صمغ دانهٔ ریحان غنی‌شده با عصارهٔ خوشاریزه و اثر نگهدارندهٔ آن بر کیفیت ماهی کپور نقره‌ای

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چکیده

این پژوهش با هدف بررسی پتانسیل افزایش ماندگاری فیله‌های کپور نقره‌ای با استفاده از فیلم صمغ دانهٔ ریحان غنی‌شده با غلظت‌های مختلف (۰، ۰/۵، ۱، ۱/۵، و ۲ درصد/مادهٔ خشک) عصارهٔ خوشاریزه (EPE) در یک دورهٔ نگهداری ۱۶ روزه انجام شد. هریک از ویژگی‌های فیلم‌های صمغ دانهٔ ریحان غنی‌شده توسط EPE روند خاصی را دنبال می‌کند. نتایج نشان داد با افزایش EPE، ضخامت لایه، طول تا نقطهٔ گسیختگی و فعالیت آنتی‌اکسیدانی فیلم افزایش یافت. بااین‌حال، فیلم با بالاترین غلظت EPE کمترین حلالیت (۱۹ درصد) و استحکام کششی (۱۵ مگاپاسکال) را داشت. به‌طورکلی فیلم حاوی ۲ درصد EPE ویژگی‌های فیزیکی و شیمیایی قابل‌قبولی را ارائه داد و برای بسته‌بندی استفاده شد (فیلم بهینه). فیله‌های پوشش‌داده‌شده توسط فیلم بهینه در روز ۱۶ انبارش مقدار TVB-N کمتری (۳۶ میلی‌گرم نیتروژن/۱۰۰ گرم گوشت) در مقایسه با فیلم غنی‌نشده (۶۰ میلی‌گرم نیتروژن/۱۰۰ گرم گوشت) و همچنین نمونهٔ شاهد (۶۵ میلی‌گرم نیتروژن/۱۰۰ گرم گوشت) داشتند. نمونهٔ فیلهٔ پوشش‌داده‌شده توسط فیلم صمغ دانهٔ ریحان غنی‌شده با غلظت ۲ درصد EPE در مقایسه با نمونهٔ فیلم دانهٔ ریحان بدون عصاره و نمونهٔ شاهد دارای pH و شاخص اسید تیوباربیتوریک، شاخص پراکسید و بار میکروبی کمتری پس از ۱۶ روز نگهداری بود. نتایج به‌دست‌آمده، تشویق به استفاده از فیلم مبتنی بر صمغ دانهٔ ریحان حاوی EPE را در سیستم‌های بسته‌بندی فعال مواد غذایی نمود که می‌تواند ماندگاری فیله‌های کپور نقره‌ای را افزایش دهد.

واژه‌های کلیدی: صمغ دانهٔ ریحان، عصارهٔ خوشاریزه، فیلم فعال، فیلهٔ کپور نقره‌ای