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## Evaluation Of Some Chemical, Microbial And Sensory Properties Of Silver Carp Fillets (*Hypophthalmichthys Molitrix*) Coated By Chitosan-Chia Gum Enriched With Nanoencapsulated Bay Leaf Essential Oil: Stored At Refrigerator Temperature

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### Abstract

*This study was conducted with the aim of using 6 types of coatings to increase the shelf life of silver carp fillets. All treatments were contained of 2% chitosan + 1.5% chia gum that enriched with 4 levels of encapsulated bay leaf essential oil (0, 0.5, 1 and 2 %) and/or a level of free essential oil of bay leaf (0.5 %) and/or a concentration of BHA antioxidant. The chemical, microbial analyses and sensory evaluations of samples were done on days 0, 3, 6, 9 and 12 on fillets kept in the refrigerator. The results were shown that the amount of phenolic compounds of bay leaf essential oil was 54.25 (mg gallic acid/g), flavonoids (8.58 mg/g) and the ability to inhibit DPPH free radicals was 51.45%. The power of iron ion reduction was 0.47 mmol Fe/g. The results also showed that the amounts of free fatty acids, peroxide index, thiobarbituric acid, the total volatile basic nitrogen and the number of Escherichia coli bacteria in the samples escalated with the increase in the storage time of fish fillets. This increase was less intense in the samples containing 2% of the encapsulated essential oil of bay leaves and BHA. The results also showed that the best antioxidant for fish fillets at refrigerator temperature was the synthetic antioxidant BHA, but considering the toxic effects of synthetic antioxidants, it is possible to use a coating containing chitosan-chia seed gum with 2% encapsulated bay leaf essential oil in order to increase the shelf life of fish.*

**Keywords:** Bay leaf Essential oil, Chia seed gum, Chitosan, Silver carp, Shelf life, Encapsulation

## 1- Introduction

More than 196 million tonnes of fish will be produced and processed yearly by 2025, making fish one of the major aquatic species whose production is a booming business[1]. Fresh fish has a good and pleasant taste, but due to the presence of nutrients, neutral pH, and high water activity, it spoils quickly [2]. Silver carp (*Hypophthalmichthys molitrix*) is one of the most important economic freshwater fish species cultured in eastern countries due to its fast growth rate, easy cultivation, high feed efficiency ratio and high nutritional value. However, silver carps as all type of fish are highly susceptible to quality deterioration caused by the activity of microorganisms, enzymatic autolysis and oxidation of lipids and proteins[3-5].

Various natural edible coatings are used alone or as carriers of active substances (antimicrobial substances, antioxidants, flavors, nutrients and colors) to increase the shelf life and improve the quality of fresh and frozen fish [6, 7]. Therefore, the use of natural antibacterial of plant extracts in combination with edible films obtained from natural polymers is a solution to limit bacterial spoilage. One of the advantages of coatings containing antibacterial substances is the gradual release of antibacterial compounds on the surface of the food [8-10]

In terms of natural abundance, chitosan is the second most significant polysaccharide after cellulose. Because of its anti-bacterial and anti-mold properties, it is widely used in the preparation of edible films and coatings. Also, the presence of fatty acids in chitosan increases its antimicrobial properties [11]. Several studies on the use of chitosan to coating of fish fillets have been conducted such as on silver carp [12], salmon fillets [13], trout fillets [14], golden pomfret fillet[15], snakehead fish [16], Horse mackerel fillets[17] and rainbow trout [18]. Pure chitosan coatings alone do not show good mechanical properties, resistance to moisture and good appearance characteristics. Also, the high price of chitosan compared to other biopolymers has led to finding a suitable solution for these coatings. Mixing chitosan with other polymers or gums can be considered a suitable method to improve its properties [19].

Chia seed (*Salvia hispanica L.*) is a plant belonging to the mint family that contains 5% mucilage and can act as a food coating. On the other hand, chia seeds are a rich source of natural antioxidants such as flavonols, chlorogenic acid, caffeic acid, myrstein, quercetin, kaempferol, as well as tocopherols and carotenoids, and the presence of these compounds

plays an important role in keeping the level of autoxidation low and increasing shelf life [20, 21]. Chia seed gum mucilage has been used to coat meat products, including chicken [22] and quail fillet [23] and sea bass fillets [24].

Essential oils are lipid extracts of plants that are used as additives in films and coatings to replace artificial preservatives, and due to the presence of biologically active compounds such as phenols and terpenoids, they naturally have antioxidant and antimicrobial properties. These compounds lead to the release of cell compounds and the death of microorganisms and are highly demanded due to their antioxidant and antimicrobial properties, protecting food from spoilage. [25]. Bay leaf (*Laurus nobilis*) is an evergreen tree or shrub with a height of 15-20 meters and two legs. The essential oil of this plant has antibacterial and antifungal properties and has strong antibacterial activity against a wide range of spoilage detected through food and pathogenic bacteria [26, 27]. However, their incorporation into low-fat foods is a challenge due to their hydrophobic nature, strong aroma, and flavor. Encapsulation is an effective approach to overcome these drawbacks [28]. Nanoencapsulation is a method that causes more protection of sensitive substances, more of their bioavailability, dissolution of hydrophobic substances in water environments, protection of taste and color effects of bioactive substances and controlled release of these substances[29].

Although various essential oils and plant compounds have been used in combination with edible coatings in meat and fish products, no study has yet focused on the simultaneous effect of coatings and gums enriched with essential oils and in meat products. Hence the purpose of this study is to use a combination of chitosan coating-chia seed gum enriched with nanoencapsulated bay leaf essential oil to increase the storage life of silver carp fillets at refrigerator temperature.

## **2- Materials and Methods**

### **2-1- Materials**

The fish purchased from the local market (Noor, Iran) and transported to the laboratory in insulated containers, next to the tank, and in polyethylene bags for filleting. Bay leaves and chia seeds used were also obtained from local market (Noor, Iran). Chitosan with a degree of solubility of 75-85% and a molecular weight of 760 kDa was purchased from Merck (Germany).

## **2-2- Preparation of essential oil of bay leaf and chia seed gum**

To prepare bay leaf essential oil, first, the excess parts of the plant were washed and dried immediately after preparation. After that, they were dried in a vacuum oven at a temperature of 50° C for 45 minutes and completely powdered by a grinder, and essential oil extraction was done by the water distillation method. For the complete extraction of the essential oil, a Clevenger apparatus (Shimi Azma Gostar, Iran) was used, and after extracting the essential oil, the water in the essential oil was extracted with sodium sulfate and the essential oil was stored in a closed container in the refrigerator [30]. To prepare chia seed gum, the seeds were mixed with distilled water in a ratio of 40 to 1 (the desired ratio was chosen based on the research done by other researchers). Stirring was done for two hours at a temperature of 80°C and a constant pH below 8, and then a centrifuge (Thermo, Japan) was used at a temperature of 20°C for 20 minutes. The supernatants (mucilage) were transferred to high-density polyethylene bags and then stored at 4°C until use [31].

## **2-2- Preparation of nanoliposomes containing essential oil**

Nanoliposomes were produced according to the method of Sarabandi et al. 2019 with a slight modification [32]. First, 2 grams of lecithin and 2 grams of Tween 80 were mixed in 38 ml of distilled water and shaken for 5 hours. In the next step, 4 grams of the essential oil of bay plant leaves were added to the aqueous dispersion of lecithin and the whole mixture was subjected to sonication (Hielscher, Germany) for 10 minutes (1 minute on and 1 minute off) at a frequency of 40 kHz and 40% of the device's power.

## **2-3- Preparation of combined coating (chitosan + chia gum) enriched with free and nanoencapsulation bay leaves` s essential oil**

Chitosan solution was obtained by dissolving 2% w/v chitosan in 1% v/v acetic acid. For better dissolution of chitosan, the solution was stirred for 3 hours at room temperature with a magnetic stirrer (Alpha, America) and to prepare a 2% w/v solution of chitosan coating, the first 20 grams of chitosan powder was added to one liter of distilled water and the stirring process at 1200 rpm and then it was heated at 70 °C for 30 minutes. Chia gum solution at 1.5% by weight was prepared by dissolving the gum in distilled water and vigorously stirring at a speed of 1200 rpm with a magnetic stirrer for 24 hours at ambient temperature. In the next step, 200 ml of chitosan solution was slowly added to the gum solution and stirring continued for 4 hours. After this period, 0.2% by volume compared to the amount of essential

oil, Tween 80 as an emulsifier was mechanically mixed with chitosan-chia seed gum and mixed with the help of a homogenizer for two minutes.( SR30, South Korea) and it was done at 9000 rpm so that the essential oils are uniformly distributed in the coating matrix [33].

#### 2-4-Morphology evaluation

The morphology of the samples was observed by scanning electron microscopy (Model and country ?) For this purpose, 1 mg of prepared sample was poured on the laboratory slide and dried at ambient temperature for 1 h and then the sample was coated with gold layer using an ion sputtering device to be viewed under electron microscopy. Then, the size of the particles was determined with a specific voltage and magnification using a scanning electron microscope [34].

#### 2-4- Preparation of fish samples

First, the body surface of the fish was disinfected with 70% alcohol and with the help of a scalpel, after emptying the intestines and viscera, skinning was done under the laminar hood. To reduce the microbial load to zero, the meat samples were irradiated using gamma rays with an intensity of 8 KGy. Phytophagous fish fillets were cut into pieces of 5 ×5 cm<sup>2</sup>and immersed in the desired coating for 1 minute according to the treatments given in Table 1 [35, 36]. Afterwards, samples were stored in the refrigerator and were analyzed for further chemical and microbial analyzes during time intervals of 3 days (0, 3, 6, 9 and 12 days.

Table 1- Treatments used in this study

Treatment Number	Chitosan (%)	Chia seed gum (%)	Type and amount of essential oil of bay leaves	BHA
1	2	1.5	0	0
2	2	1.5	0.5 %free essential oil	0
3	2	1.5	0.5 %encapsulated essential oil	0
4	2	1.5	1%encapsulated essential oil	0
5	2	1.5	2 %encapsulated essential oil	0
6	2	1.5	-	200 ppm

## **2-5- Characteristics of Bay leaf essential oil**

### **2-5-1- Measurement of total phenolic compounds**

To measure the amount of phenolic compounds in bay leaf essential oil, the method provided by Hossein et al. (2021) and a spectrophotometer (Biochrom, England) at a wavelength of 765 nm were used [37].

### **2-5-2- Determination of radical scavenging activity by DPPH method**

In this reductive method, (hydrogen-donating ability or radical-accepting power) of the extracted essential oil was checked on the stable DPPH radical and its color change from purple to yellow at 517 nm wavelength in such a way that 5 ml of the extract solution extracted with 1 ml Methanolic solution of DPPH (concentration 1 mM) was mixed and then kept for 30 minutes at ambient temperature and dark conditions, and finally, the absorbance of the samples was read at a wavelength of 517 nm [38].

### **2-5-3- Determination of total flavonoids in the essential oil**

Colorimetric aluminum chloride method was used for flavonoid determination. Briefly, 0.5 ml solution of each plant extracts in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (PG-instrument-Ltd, USA). Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg/ ml in methanol [39].

### **2-5-4- Iron-reducing power (RP)**

In this method, the reductive power of essential oil was evaluated based on the reduction of potassium ferric cyanide and the change of color spectrum from yellow to green or blue and the method of Fu et al. (2011) [40].

## **2-6- Characteristics of nanoliposomes**

### **2-6-1- Encapsulation efficiency (EE)**

To determine the EE, 2 ml of the freshly prepared sample was poured into a 100 kDa Amicon filter and centrifuged with a 3000 g centrifuge for 10 minutes (Sarabandi et al., 2019) and the

amount of phenolic compounds in the phase passed, according to the method Hosseini et al. (2021) was calculated. Finally, from equation 1, the EE of phenolic compounds was determined [37].

$$(\%)EE = \frac{E - D}{E} \times 100 \quad \text{eq. (1)}$$

where E is the phenolic compounds of the initial extract, D is the phenolic compounds present in the transitional phase.

### **2-6-2- Determination of particle size**

The measurement of the encapsulated sample was determined 30 minutes after production using the particle size scanning method (3DLS). The measurement basis of the device was based on the diffraction resolution of laser light [39]. In conducting this test, the refractive index was 1.33 and the dielectric constant was 78.5, the temperature was 25°C, and the measurements were made at the scattering angle of 173°.

### **2-6-3-Determination of zeta potential**

The surface electric charge of particles, which is called zeta potential, is one of the reliable indicators for predicting the stability of colloid dispersions under storage conditions. In order to determine this quantity, a Zeta Sizer device (Malvern, England) was used, which works based on the amount of electrophoretic displacement of surface electric charge particles. In performing this test, the refractive index was 1.33, the dielectric constant was 78.5, and the temperature was 25 °C [32, 39].

## **2-7- Analysis of fish fillets**

### **2-7-1- Physicochemical properties**

AOCS Cd 3-63 (1993) method was used to measure free fatty acids [41].The amount of peroxide in the samples was measured according to the method of AOCS Cd 8-53 (1993).The thiobarbituric acid index shows the amount of milligrams of malonaldehyde present in one kilogram of the sample and indicates the secondary stages of fat oxidation and the presence of secondary oxidation compounds in the sample, and Seabury [42] method was used to measure this index.The Kjeldahl apparatus and the method described by Goulas and Kontominas [43] were used to measure total volatile basic nitrogen (TVBN).

### **2-7-2- Microbial analyses**

ECC chrome agar culture medium was used to count *Escherichia coli*. To count bacteria at each sampling time, 5 grams of sample was added to 45 ml of physiological serum and then homogenized. Depending on the type of sample, the dilutions ranged from 2-10 to 4-10. 0.1 ml of the diluted sample was placed on ECC chrome agar surface culture medium and incubated at 44°C for 24 hours. [44].

### **2-7-3-Sensory evaluation**

The sensory evaluation of samples was conducted by a trained panel of 15 panelists. Each panelist was given a set of fried filler using sunflower oil at a temperature of 170 °C. Each sample was evaluated for overall acceptance. Samples were assessed organoleptically using a 5-point hedonic scale, where 5 correspond to “like extremely” and 1 corresponds to “dislike extremely [45].

### **2-8- Statistical Analysis**

Data analysis was done in the form of factorial experiments and a simple completely randomized design with three replications. SAS software was used for data analysis and Duncan's multiple range test at the 5% level was used to compare the mean of the data.

## **3-Results and Discussion**

### **3-1- The antioxidant properties of bay leaf essential oil**

As shown in Table 2, the ability to inhibit DPPH free radicals and iron reduction power in the synthetic antioxidant BHT was more than the extracted bay leaf essential oil. In terms of chemical composition, according to the conducted research, it has been determined that alpha-tocopherol is a medicinal plant of native leafy plants, and there are flavonoids, terpenoid lactones, alkaloids, isoquinolinic acids, and phenols in the leaves. The amount of alpha-tocopherol in the leaves of the hibiscus plant was very high, and its leaves contain a high amount of flavonoids [46]. Phenolic substances present in food indicate antioxidant properties. Bera et al (2018) stated that the advantages of natural antioxidants compared to synthetic antioxidants can be pointed to their high acceptability by consumers and their safety[47]. Dhifi et al. (2018) reported the amount of total phenol and flavonoids in the essential oil of bay leaf plant as 174.1 (mg gallic acid / g) and 149.2 (mg / g) respectively, which can be attributed to the type of plant and extraction method [48]. But Dobroslavic et al.



(2021) reported the amount of phenolic compounds in the essential oil of bay leaf plant as 42.35-42.21 (mg gallic acid / g)[49].

Table 2- Comparison of antioxidant properties of Bay Laurel essential oil with BHT

Type of composition	Total phenol (mg gallic acid / g)	Flavonoid compounds (mg / g)	DPPH (%)	ferric-reducing power (mmol Fe/g)
Bay Laurel Essential Oil	54.25±0.12	8.58±0.123	51.45±0.13 <sup>b</sup>	0.47±0.01 <sup>b</sup>
BHT	-	-	83.29±0.04 <sup>a</sup>	1.49±0.04 <sup>a</sup>

Data are the mean of three repetitions ± SD and numbers with different letters in column indicate significance at the 5% level.

### 3-2-Characteristics of encapsulated essential oil of Bay leaf

The most important point in using essential oils in the formulation of food is to protect them from evaporation and destruction so that the essential oil can be released and perform its own function at the right time. Natural essential oils usually have a high price and their transportation and storage in liquid form is not cost-effective, so different encapsulation methods are used to protect and increase stability, ease of storage and transportation [50]. Some properties related to the non-encapsulated essential oil of Bay leaf are given in Table 3. As it is known, the particle size of the prepared samples was 114.22 nm. In this study, the size of nanoparticles was also considered. Generally, particles with a zeta potential of ±30 mV are considered as stable colloids. Therefore, they are less sensitive to non-steady aggregation forces such as Van der Waals forces, Brownian motion, or particle-particle interaction; therefore, the surface zeta potential of nanoparticles is an important parameter in the process of nanoencapsulation, which indicates their stability [51], and the number obtained from the zeta potential of this study also indicated the stability of the prepared samples.

Morphological characteristics of nanoparticles were performed by high-resolution microscopic methods, which shows the effect of the production process, and the type and composition of the formulation of these materials [52]. The morphological characteristics of the nanoliposome containing the extract were investigated and it was determined that the particles were oval and sticky with smooth surfaces (Figure 1). Due to less surface contact with the environment, spherical particles have a greater ability to control the release of compounds, hence these particles are more stable [53].

Table 3- Some characteristics of the encapsulated essential oil of Bay leaf

Characteristics	Encapsulation Efficiency (%)	Particle size (nm)	Zeta Potential (mv)
Encapsulated essential oil of Bay leaf	57.81±0.2	114.22±0.2	-53.35±0.3

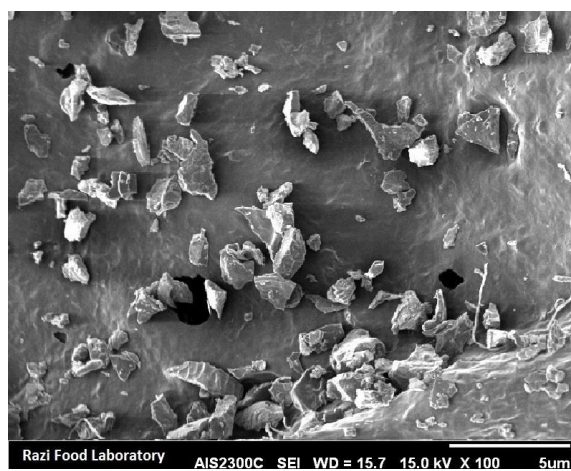


Figure 1- Morphology of encapsulated bay leaf essential oil

### 3-3- The effect of the studied parameters on the amount of free fatty acids

The results of the interaction effect of treatments and storage time on the fatty acids of the samples are given in Table 4. As it is clear, the maximum amount of free fatty acids of the samples belongs treatment 1. It was stored for 12 days and with increasing storage time, the amount of free fatty acids in the samples increased. On the other hand, it was found that with the increase in the use of micro-encapsulated essential oil, the increase in the amount of free fatty acids in the samples was less, and also the efficiency of the micro-encapsulated essential oil of bay leaf was higher than the free essential oil of this plant in reducing free fatty acids. After the death of fish, fat hydrolyzing enzymes can increase the amount of free fatty acids over time, so the measurement of free fatty acids can be considered a good indicator for showing the effect of lipolytic enzymes on fish fat and other meat products. In the study of Hassanpour et al. (2015) on the effect of using dill extract and plant powder during the storage of silver camphor fish burgers in the refrigerator, the results showed that the amount

of free fatty acids in all treatments increased over time [52]. Treatments containing plant essential oils had the lowest amounts of free fatty acids compared to treatments without plant extracts. The cause of this phenomenon is related to the antibacterial effect of essential oils, thus, essential oils control the activity of microbes and microbial enzymes, resulting in the production of free fatty acids [54, 55]. The reason for the decrease in acidity with the increase in the concentration of nanoliposomes containing the extract can be attributed to the ability of phenolic compounds to interrupt the process of formation and release of free radicals as well as free fatty acids [56]. Free fatty acids are considered the cause of cognitive impairment because they react with protein and cause protein denaturation and tissue changes [57]. But the formation of free fatty acids alone does not cause the nutritional value of the products. It has been proven that the accumulation of free fatty acids is related to the increase of fat oxidation and also affects the taste [58]. Gharibzahedi and Mohammadnabi (2017) by examining the effect of edible coatings based on jujube gum and nettle oil-loaded nanoemulsions on the shelf-life of Beluga sturgeon fillets, stated that the use of this coating leads to a decrease of fish's free fatty acids of were in line with the results of this section [59]. Xin et al. (2020) developed a zein/potato starch (PS) film based on chitosan nanoparticles incorporated with curcumin (CCN) and reported that CCN/zein/PS composite film decrease in free fatty acids for *Schizothorax prenati* fillets [60].

**Table 4- The effect of coating type and storage time on the amount of free fatty acids (% Oleic acid)**

Treatment number	Storage time (day)				
	0	3	6	9	12
1	0.157±0.000 <sup>n</sup>	0.181±0.002 <sup>h</sup>	0.193±0.001 <sup>c</sup>	0.196±0.001 <sup>b</sup>	0.201±0.001 <sup>a</sup>
2	0.157±0.000 <sup>n</sup>	0.178±0.001 <sup>i</sup>	0.188±0.001 <sup>e</sup>	0.191±0.001 <sup>d</sup>	0.197±0.001 <sup>b</sup>
3	0.157±0.000 <sup>n</sup>	0.175±0.001 <sup>j</sup>	0.183±0.002 <sup>g</sup>	0.186±0.001 <sup>f</sup>	0.190±0.001 <sup>d</sup>
4	0.157±0.000 <sup>n</sup>	0.171±0.002 <sup>l</sup>	0.179±0.001 <sup>i</sup>	0.182±0.001 <sup>gh</sup>	0.185±0.001 <sup>f</sup>
5	0.157±0.000 <sup>n</sup>	0.168±0.001 <sup>m</sup>	0.173±0.001 <sup>k</sup>	0.175±0.001 <sup>j</sup>	0.179±0.001 <sup>i</sup>
6	0.157±0.000 <sup>n</sup>	0.167±0.001 <sup>m</sup>	0.170±0.002 <sup>l</sup>	0.173±0.001 <sup>k</sup>	0.170±0.001 <sup>l</sup>

Data are the mean of three repetitions ± SD and numbers with different letters indicate significance at the 5% level

### 3-4- Peroxide value

Hydroperoxides are the main products of lipid oxidation and therefore, their measurement is very useful to indicate oxidative spoilage [61]. The results of the interaction effect of coating type and storage time on the peroxide value are given in Table 5. The highest amount of peroxide of the samples belonged to the sample containing 2% chitosan and 1.5% chia gum after 12 days of storage. During storage, the amount of peroxide of the samples increased. With the increase of encapsulated essential oil of bay leaf in the coating used for fish fillets, the amount of peroxide of the samples decreased. The most effective for reducing the amount of peroxide of the samples was related to the coating containing BHA. Furthermore, the encapsulated essential oil was more effective in reducing the amount of peroxide in the samples than the free essential oil of bay leaves. In general, with the increase in the storage period, the oxidation process of lipids is done and the amount of peroxide increases. The reason for the reduction of peroxide with increasing the concentration of the extract can be attributed to the antioxidant property of nanoliposomes containing phenolic compounds and giving hydrogen atoms to the free radicals produced during the process. On the other hand, with increasing storage time, the amount of peroxide in the samples increases due to the oxidation of fatty acids [62]. Bagheri et al. (2015) investigated the antioxidant effects of free and encapsulated fennel extract in preventing the oxidation of kilka fish and reported that the extract prevents fat oxidation due to the presence of phenolic compounds, and the antioxidant effect of the encapsulated extract was higher [63]. These results are consistent with the results of the present study. This indicates the capsule's ability to improve the antioxidant activity of essential oils and increase the availability of essential oils, which has been shown in various studies. In agreement with the results of this section, some researchers reported the use of nanoparticles containing essential oils in reducing the primary products of oxidation [45, 64]. Pei et al., (2022) reported that by using active coatings supplemented with epigallocatechin gallate (EGCG), the oxidation rate reduced due to the reduction of oxygen availability [65]. Nanoparticles with antioxidant properties protect food from oxidation of lipids (the most common cause of food spoilage) and hence increase the shelf life of food [66]. Fan et al. (2009) investigated effects of chitosan coating on quality and shelf life of silver carp during frozen storage. They reported the effect of chitosan coating on fish samples was to retain their good quality characteristics and extend the shelf life during frozen storage, which due to the

binding of chitosan with iron in iron-containing proteins in fish and prevention of free radical production [12].

**Table 5- The effect of coating type and storage time on the amount of peroxide (meqo2/kg sample)**

Treatment number	Storage time (day)				
	0	3	6	9	12
1	0.140±0.001 <sup>s</sup>	0.173±0.002 <sup>n</sup>	0.212±0.001 <sup>h</sup>	0.253±0.002 <sup>d</sup>	0.315±0.005 <sup>a</sup>
2	0.140±0.001 <sup>s</sup>	0.167±0.003 <sup>o</sup>	0.197±0.002 <sup>j</sup>	0.223±0.002 <sup>f</sup>	0.297±0.002 <sup>b</sup>
3	0.140±0.001 <sup>s</sup>	0.162±0.001 <sup>p</sup>	0.191±0.001 <sup>k</sup>	0.217±0.002 <sup>g</sup>	0.272±0.001 <sup>c</sup>
4	0.140±0.001 <sup>s</sup>	0.158±0.001 <sup>q</sup>	0.179±0.001 <sup>m</sup>	0.204±0.001 <sup>i</sup>	0.254±0.001 <sup>d</sup>
5	0.140±0.001 <sup>s</sup>	0.155±0.003 <sup>qr</sup>	0.173±0.003 <sup>n</sup>	0.196±0.002 <sup>j</sup>	0.232±0.002 <sup>e</sup>
6	0.140±0.001 <sup>s</sup>	0.151±0.001 <sup>r</sup>	0.164±0.001 <sup>p</sup>	0.182±0.001 <sup>l</sup>	0.211±0.002 <sup>h</sup>

Data are the mean of three repetitions ± SD and numbers with different letters indicate significance at the .5% level

### 3-5- The effect of the studied parameters on the amount of Thiobarbituric acid

The thiobarbituric acid index is used to evaluate the degree of fat oxidation in fish, and it measures the amount of malondialdehyde, which is a secondary product of the oxidation of polyunsaturated fatty acids [67]. The primary products of fat oxidation are hydroperoxides. In the second stage of autoxidation, where hydroperoxides are oxidized to aldehyde and ketone, malondialdehyde is formed. Secondary oxidation products cause an unpleasant taste and smell in the product [68]. The results showed that with the increase in storage time, the amount of thiobarbituric acid index increased, and this increase was more in the samples without antioxidants and also the essential oil of bay leaf (treatment 1) than in other treatments, so that the highest amount of thiobarbituric acid index belongs to the sample of

treatment 1 after 12 days. Samples containing the encapsulated essential oil of bay leaves had a lower thiobarbituric index than samples containing the free essential oil of bay leaves,, except for the day of production of fish fillets. Increasing in the amount of encapsulated essential oil of bay leaves in the coating of fish fillets led to a decrease in the index of thiobarbituric acid ( Table 6). The use of essential oils and natural extracts prevents the formation of free radicals due to the content of groups of phenolic compounds that act as antioxidants and preserves the characteristics of fish meat with the protective role of fat oxidation. [69]. Bay leaf essential oil was able to suppress free radicals or reduce the speed of their formation due to its strong antioxidant properties. Akbari et al. (2013) showed that with the increase in the storage time of fish fillets, the amount of secondary oxidation products, especially aldehydes, increases, which can be due to the increase of free iron and other prooxidants, and also aldehydes are created as a secondary oxidation product from the breakdown of hydroxides [70]. In a study conducted on the effect of nano-encapsulated cannabis extract in controlling the oxidation stability of hemp seeds, the authors stated that encapsulated extracts are more effective than free extract in inhibiting the thiobarbituric acid index and this index increases with increasing time, which was in line with these results [71]. The reason for the increased efficiency of nanoparticles in reducing the index with increasing storage time, thiobarbituric acid can be attributed to the increase in the level of these antioxidants compared to the free extract, as well as the destruction of free antioxidants, which play an essential role in neutralizing free radicals. The results of Li et al. (2012) and Alboghbeish and Khodanazary (2018) also confirm the results of this research, which indicate the positive effect of chitosan coating in combination with plant extract in increasing antioxidant properties and reducing bacterial enzyme reactions related to fat oxidation [72, 73].

**Table 6- The effect of coating type and storage time on the amount of Thiobarbituric acid (mg Malon de Aldehyd/kg sample)**

Treatment number	Storage time (day)				
	0	3	6	9	12
1	0.063±0.001 <sup>r</sup>	0.102±0.002 <sup>l</sup>	0.118±0.001 <sup>k</sup>	0.258±0.002 <sup>c</sup>	0.302±0.007 <sup>a</sup>
2	0.063±0.001 <sup>r</sup>	0.097±0.001 <sup>m</sup>	0.116±0.002 <sup>k</sup>	0.248±0.002 <sup>d</sup>	0.272±0.001 <sup>cb</sup>
3	0.063±0.001 <sup>r</sup>	0.092±0.001 <sup>n</sup>	0.105±0.001 <sup>l</sup>	0.218±0.002 <sup>g</sup>	0.244±0.002 <sup>e</sup>
4	0.063±0.001 <sup>r</sup>	0.087±0.002 <sup>p</sup>	0.097±0.001 <sup>m</sup>	0.195±0.001 <sup>ki</sup>	0.225±0.002 <sup>f</sup>
5	0.063±0.001 <sup>r</sup>	0.081±0.003 <sup>q</sup>	0.093±0.002 <sup>mn</sup>	0.167±0.002 <sup>j</sup>	0.211±0.003 <sup>h</sup>
6	0.063±0.001 <sup>r</sup>	0.078±0.001 <sup>q</sup>	0.088±0.001 <sup>p</sup>	0.116±0.001 <sup>k</sup>	0.194±0.001 <sup>i</sup>

Data are the mean of three repetitions ± SD and numbers with different letters indicate significance at the .5% level

### 3-6- The effect of the studied parameters on the amount of the total volatile basic nitrogen (TVBN)

The total index of TVBN is considered one of the main indicators of degradation, breakdown and quality determination of seafood, which includes trimethylamine (produced by bacterial spoilage), dimethylamine (produced by autoleptic enzymes) during storage), ammonia (produced by the deamination of amino acids and nucleotides) and other volatile basic nitrogen compounds are associated with the spoilage of marine products [74]. The comparison of averages using Duncan's test method (Table 7) showed that with the increase of the encapsulated essential oil of bay leaves in the coating used in fish fillets, the amount of TVBN decreased. On the other hand, it was found that the amount of TVBN increased with the increase of storage time, and the increase of the amount of TVBN in the samples without antioxidants as well as bay leaves essence (treatment 1) was more than other treatments. The highest acceptable level of TVBN in fish meat has been suggested as 25 mg of nitrogen per 100 g of sample [75]. The increase in the amount of TVBN during the storage period may be due to the activities of enzymes, for example, deamination of free amino acids, degradation

of nucleotides and oxidation of amines. The studied samples were acceptable until the end of the storage day, but the lowest amount of total volatile nitrogenous bases was observed in treatment 6. The use of chitosan coating containing antioxidant compounds such as essential oils is more effective than when these compounds are used alone in food due to the fact that these compounds are released over time [76]. Bay leaf essential oil prevents the increase of free fatty acid, the activity of internal proteases and the production of volatile nitrogenous compounds due to its strong antioxidant activity [77]. The amount of nitrogen-containing bases in this study gradually increased with the increase in storage time, which can be attributed to the increase in the number of bacteria, and the use of different coatings leads to a decrease in this characteristic due to the decrease in the growth of bacteria [78]. Majidiyan et al. (2022) also used a protein coating containing horse chestnut essential oil to reduce the amount of TVBN in rainbow trout fillets [79]. Volpe et al (2015) stated that the use of edible coatings on the surface of fish fillets leads to a decrease in the amount of volatile nitrogenous bases due to the reduction of bacterial growth and the decreased oxygen intake [80]. The findings of Pourkargar and Rafati (2020) were also in agreement with the results of this section [81].

**Table 6- The effect of coating type and storage time on the amount of Thiobarbituric acid (mg Malon de Aldehyd/kg sample)**

Treatment number	Storage time (day)				
	0	3	6	9	12
1	0.063±0.001 <sup>r</sup>	0.102±0.002 <sup>l</sup>	0.118±0.001 <sup>k</sup>	0.258±0.002 <sup>c</sup>	0.302±0.007 <sup>a</sup>
2	0.063±0.001 <sup>r</sup>	0.097±0.001 <sup>m</sup>	0.116±0.002 <sup>k</sup>	0.248±0.002 <sup>d</sup>	0.272±0.001 <sup>cb</sup>
3	0.063±0.001 <sup>r</sup>	0.092±0.001 <sup>n</sup>	0.105±0.001 <sup>l</sup>	0.218±0.002 <sup>g</sup>	0.244±0.002 <sup>e</sup>
4	0.063±0.001 <sup>r</sup>	0.087±0.002 <sup>p</sup>	0.097±0.001 <sup>m</sup>	0.195±0.001 <sup>ki</sup>	0.225±0.002 <sup>f</sup>
5	0.063±0.001 <sup>r</sup>	0.081±0.003 <sup>q</sup>	0.093±0.002 <sup>mn</sup>	0.167±0.002 <sup>j</sup>	0.211±0.003 <sup>h</sup>
6	0.063±0.001 <sup>r</sup>	0.078±0.001 <sup>q</sup>	0.088±0.001 <sup>p</sup>	0.116±0.001 <sup>k</sup>	0.194±0.001 <sup>i</sup>

Data are the mean of three repetitions ± SD and numbers with different letters indicate significance at the .5% level



### **3-7- The effect of the studied parameters on the number of *Escherichia coli* bacteria**

The results of the interaction effect of the type of coating and storage time on the number of *Escherichia coli* in the samples showed that the highest number of this bacteria was related to the samples obtained from the treatment of 1 and 12 days of storage, and with the increase of the storage time and the decrease in the concentration of the essential oil of bay leaves in the coating of fish fillets. The number of these bacteria increased and the samples containing the synthetic antioxidant BHA had less *Escherichia coli* than the other samples on all days of storage except the day of fish fillet production (Table 8). Chitosan's antibacterial properties are a result of the presence of amine groups, or positively charged molecules, which bond with negatively charged molecules on the surface of bacterial cells to cause the bacterial cell membrane to rupture, allowing intracellular substances to leak out and ultimately causing the bacterial cell to die. In addition, its function can be a barrier against the penetration of oxygen [82]. Bay leaf essential oil has high amounts of phytochemical compounds including flavonoids, anthocyanins and phenols, and these compounds have led to its high antioxidant properties [49]. In addition to the antioxidant properties of these compounds, the investigation of the antimicrobial effect of the flavonoids of bay leaf essential oil has also shown a significant ability against human pathogenic pathogens such as *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*[83]. The compounds present in the essential oil of bay leaves cause the release of enzymes and different nutrients by changing the structure of the cell membrane of bacteria or by scratching the lipopolysaccharide layer of the outer membrane of the bacteria [84]. The results of the present study are consistent with the studies of Gomez-Estaca et al. (2007) on the effect of chitosan-containing mint plant extract on sardines [76] and Wu et al. (2013) on the effect of gelatin with chitosan and oregano essential oil on salmon fillets [85]. It has been shown in the studies that the use of coatings containing antibacterial compounds such as extracts, due to the fact that these compounds are gradually released in the food, compared to the time when these compounds are added alone in the food are more effective. Due to its strong antimicrobial activity, the essential oil of bay leaf prevents the increase of free fatty acids and the production of volatile nitrogenous compounds. Plant extracts with antioxidants or used alone to increase the shelf life of aquatic products are compatible. The reason for the agreement of these researches may be due to the antibacterial property of chitosan coatings and the prevention of nutrients such as amines from reaching the bacterial cell membrane and the binding of the phenolic compounds of the

antioxidants of bay leaf essential oil with the proteins in the bacterial cell wall, which leads to The lysis and destruction of the cell wall [77].

**Table 8- The effect of coating type and storage time on the number of *Escherichia coli* (Log cfu/g)**

Treatment number	Storage time (day)				
	0	3	6	9	12
1	2.491±0.12 <sup>m</sup>	4.953±0.02 <sup>hi</sup>	5.530±0.31 <sup>d</sup>	7.379±0.04 <sup>b</sup>	8.280±0.06 <sup>a</sup>
2	2.491±0.12 <sup>m</sup>	4.493±0.36 <sup>hi</sup>	5.067±0.08 <sup>fgh</sup>	6.349±0.04 <sup>c</sup>	7.322±0.08 <sup>b</sup>
3	2.491±0.12 <sup>m</sup>	3.726±0.47 <sup>sh</sup>	4.068±0.44 <sup>ij</sup>	6.257±0.07 <sup>cd</sup>	6.488±0.07 <sup>c</sup>
4	2.491±0.12 <sup>m</sup>	3.521±0.23 <sup>jk</sup>	3.857±0.25 <sup>jk</sup>	5.700±0.22 <sup>def</sup>	5.967±0.03 <sup>cde</sup>
5	2.491±0.12 <sup>m</sup>	3.324 ±0.06 <sup>kl</sup>	3.590 ±0.16 <sup>kl</sup>	5.224 ±0.10 <sup>efg</sup>	5.700±0.22 <sup>def</sup>
6	2.491±0.12 <sup>m</sup>	3.145±0.08 <sup>l</sup>	3.369±0.02 <sup>kl</sup>	4.723±0.13 <sup>hi</sup>	5.283±0.03 <sup>efg</sup>

Data are the mean of three repetitions ± SD and numbers with different letters indicate significance at the .5% level

### 3-8- The effect of the studied parameters on the overall acceptance of fillets

The results showed that with the increase of the encapsulated essential oil of bay leaves in the coating used in fish fillets, except for the first day of storage, the points given by the panelists for the overall acceptance of the samples increased, and with the increase of the storage time, the overall acceptance of the samples decreased, as well as the range of changes for the scores obtained for overall acceptance ranged from 1.67 to 5.00 (Table 9). Volatile nitrogenous compounds and the end products of fat oxidation (hydroperoxides, aldehydes, ketones, and fatty acids), change the smell, taste, color, value and quality of food and cause fish to be undesired [86]. Preventing the entry of oxygen and moisture into the texture of the product is one of the important and effective features of chitosan, which prevents the occurrence of adverse enzymatic and non-enzymatic reactions that lead to changes in the quality of the product [87]. The results of the sensory evaluation of the samples were consistent with the results of the chemical tests. The high oxidation of fat and the growth of microbes in the fish

fillet in the control sample show signs of spoilage in the form of color change, bad smell and taste change. Probably, because of its strong antioxidant activity and inhibition of free radicals, the essential oil of bay leaves prevents chemical spoilage and preserves sensory indicators. Therefore, it can be stated that the use of chitosan and bay leaf essential oil in combination with the food coating used, due to its antioxidant activity, preserves the quality of the sensory indicators of fish. These results are in agreement with Mexis et al. (2009) who studied combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets [68].

**Table 9- The effect of coating type and storage time on the general acceptance**

Treatment number	Storage time (day)				
	0	3	6	9	12
1	5.00±0.00 <sup>a</sup>	4.00±0.05 <sup>bcd</sup>	3.00±0.11 <sup>efg</sup>	2.50±0.08 <sup>ghi</sup>	1.67±0.02 <sup>j</sup>
2	5.00±0.00 <sup>a</sup>	4.00±0.05 <sup>bcd</sup>	3.17±0.07 <sup>efg</sup>	2.67±0.06 <sup>fghi</sup>	2.00±0.01 <sup>ij</sup>
3	5.00±0.00 <sup>a</sup>	4.33±0.03 <sup>abc</sup>	3.33±0.03 <sup>def</sup>	3.00±0.01 <sup>efgh</sup>	2.00±0.01 <sup>ij</sup>
4	5.00±0.00 <sup>a</sup>	4.67±0.04 <sup>ab</sup>	3.67±0.04 <sup>bcd</sup>	3.17±0.02 <sup>efg</sup>	2.33±0.04 <sup>hij</sup>
5	5.00±0.00 <sup>a</sup>	4.76±0.33 <sup>a</sup>	3.73±0.04 <sup>cde</sup>	3.33±0.03 <sup>def</sup>	2.67±0.06 <sup>fghi</sup>
6	5.00±0.00 <sup>a</sup>	4.83±0.17 <sup>a</sup>	3.70±0.03 <sup>cde</sup>	3.33±0.02 <sup>def</sup>	2.83±0.04 <sup>fgh</sup>

Data are the mean of three repetitions ± SD and numbers with different letters indicate significance at the 5% level

#### 4- Conclusion

Various natural food coatings are used alone or as carriers of active substances (antimicrobial substances, antioxidants, flavors, nutrients and colors) to increase shelf life and improve the quality of fresh and frozen food. Therefore, the use of natural antibacterial of plant extracts in combination with edible films obtained from natural polymers is a solution to limit bacterial spoilage. Therefore, in this study, it was tried to increase the shelf life of silver carp fish

fillets in the refrigerator by using a combined chitosan-chia gum coating enriched with the encapsulated essential oil of bay leaves. According to the chemical, microbial and sensory results of the fillets kept in the refrigerator, it can be stated that the use of encapsulated bay leaf essential oil containing chitosan-chia seed gum is very useful for improving the chemical, microbial and sensory properties of silver carp fish, and the encapsulated essential oil is more effective than free essential oil.

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