



## Scientific Research

# Investigating The Performance Of The Film Containing Basil Stem Color Indicator Based On Rice Bran Fiber To Monitor The Spoilage Of Lion Fish (*Siganus commersonii*)

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## ARTICLE INFO

## ABSTRACT

## Article History:

Received:2024/8/3

Accepted:2024/12/22

## Keywords:

Color marker,  
Edible film,  
Spoilage monitoring

**DOI: 10.22034/FSCT.22.159.169.**

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The production this trend, the aim of this research is to design a sensor containing a basil stem color indicator based on rice bran fiber to determine the quality of lionfish (*Siganus commersonii*) kept for 1-30 days at refrigerator temperature. In this study, a factorial design was used to evaluate the effects of control treatments, 200 ppm, 400 ppm and 600 ppm. The results of scanning electron microscopy showed that basil stem anthocyanin extract causes changes in polymer chains and reduces film matrix porosity. Anthocyanin with many hydroxy groups as a plasticizer increased the free volume and interpolymeric macromolecular mobility due to the reduction of intermolecular forces and lower density, and as a result improved the stretchability and flexibility of the layers. In addition, the chemical properties (pH, thiobarbituric acid and nitrogen compounds) for all samples increased with increasing storage time up to 30 days. In FTIR spectroscopy, it was observed that on day zero, life of lionfish fillets. Basil stem anthocyanin extract and rice bran fiber can be used as a suitable indicator the removal of carbonyl in anthocyanin extract of basil stem increased the hydroxyl band and acidified the environment. Antioxidant properties of basil stem anthocyanin extract in the presence of phenolic compounds have prevented the initiation of free radical chain reactions and reduced it. The film containing the color indicator of anthocyanin extract of basil stem based on rice bran fiber has the potential to increase the quality and shelf to investigate changes in spoilage in packaged food products.

## 1-Introduction

The use of food coating protects the food product against physical, chemical or biological damage. Also, studies and reviews show that the addition of anthocyanin compounds as a powerful tool is easy and has countless potential advantages, including naturalness, ease of access, wide distribution in nature, high sensitivity, cheapness, non-toxicity, and dyeability. It is high on pH.

Change, they are a good alternative to artificial colors in the development of smart packaging to monitor the freshness of food products. But despite their numerous advantages, their use in the industry faces problems due to their low stability in harsh conditions such as light, temperature, oxygen, and enzymes. Lion fish (*Siganus commersonnii*) is a type of saltwater fish and is found in the waters of Southeast Asia, westward along the northern coastal areas to the eastern coast of Africa, the Middle East, and the Persian Gulf. And it is very valuable from an economic point of view, and its nutrition consists of small fish and, to a lesser extent, shrimp and squid (2020). Fish is one of the valuable sources of protein, fat and energy, and as a food product, it is an ideal environment for the growth of different groups of pathogenic bacteria and there is a possibility of contamination and spoilage by them.

Due to the presence of significant amounts of unsaturated fatty acids with double bonds (Polyunsaturated fatty acids) and omega-3 compounds, especially (Docosahexaenoic acid) and (Eicosapentaenoic Acid) in fish tissue, their oxidation happens faster after death and leads to its spoilage. Arora et al., 2022). Lipoxidases present in some microorganisms activate the reaction between fatty acids and oxygen, and the spicy taste of fats is created due to the presence of aldehydes and ketones resulting from these reactions (Omwange et al., 2021). In order to prevent fish spoilage, we can mention temperature control and its reduction, vacuum packaging and addition of antioxidants. The use

of suitable packaging and the use of suitable identifiers, which can provide the necessary information to the consumer about the quality of the product, can help to develop the export of these products (Ghiasi et al., 2020; Tongnuanchan et al., 2014). Anthocyanins, which are water-dissolved flavonoid pigments, are widespread in nature and compatible with biological systems. The characteristic of pH-sensitive color change of anthocyanins, and functions such as antioxidant activity and antimicrobial effect, are highly useful for the design of smart packaging films with natural color indicator. And they can be used in fish preservation as natural and preservative substances and because of their high anti-oxidation power, they prevent the oxidation process of fats and proteins in fish (Chandrasekhar et al., 2012; Khoo et al., 2017). Also, anthocyanins can play a role in improving the nutritional characteristics of fish. and act as useful factors in increasing the amount of vitamin C and omega-3 fatty acid in fish. These features make the fish maintain its quality and nutritional value until consumption (Andrade et al., 2017). Today, the recycling and use of food waste and agricultural industries is known as a new strategy for exploitation. This strategy, in addition to profitability, with regulations and the lowest cost, has also achieved a beneficial relationship and compatibility with the environment.

Protection of food products against environmental influences such as oxygen, water, light and chemical pests from the beginning of production to reaching the consumer is one of the main concerns of the food packaging industry.

The type of packaging that leads to reducing the quality of food products is very important in terms of efficiency in distribution and marketing. The use of biological substrates modified with natural pigments sensitive to spoilage effectively helps to reduce the spoilage of food products (Despoudi et al., 2021; Ravindran & Jaiswal, 2016). Basil (*Ocimum basilicum*) is a herb that belongs to the mint

family and is known as one of the most important leafy vegetables. It has biological properties such as antioxidant activity such as flavonoids and phenols and antimicrobial effect and is also used as a medicinal plant. It also has a good ratio of omega 3 to omega 6. In addition, the basil stalk contains significant amounts of vitamins C, K, minerals calcium, iron and fiber. And it can be effective for the treatment of some diseases of the central nervous system as well as in the control of some stomach infections such as stomach ulcers and *Helicobacter pylori* infection (Fernandes et al., 2019; Shahrajabian et al., 2020). Rice bran, which is a byproduct of rice milling, also has a significant percentage of protein. As an important source of fiber, it is rich in B vitamins and a good source of minerals such as calcium, magnesium, zinc, phosphorus and manganese (Gul et al., 2015). Fibers are a combination of indigestible carbohydrates, and they increase food volume and promote feelings of fullness, while reducing calorie intake. This can be effective in weight control. Fiber consumption can reduce the risk of some cancers, especially colon cancer, and type 2 diabetes (Sharif et al., 2014). Due to their kinetic stability, high solubility in water, transparency, protection against oxidation, light and heat, and controlling the release and protection of vitamins in food and the body, nanoemulsions are considered as a preservation method of interest in various industries. Also, having nanometer droplets in the range (between 20 and 100 nm), these systems have advantages such as preventing bad taste and ugliness in food and more resistance to the phenomenon of gravitational segregation and droplet accumulation (Aswathanarayan & Vittal)., 2019; Ozogul et al., 2022). By studying the pH detector film made of corn starch and glycerol, with the addition of a natural dye (anthocyanin) extracted from *Hibiscus sabdariffa* plant, the researchers reported that the indicator film turned pink and green respectively when in contact with rotten meat, which shows the acidic and basic characteristics of rotten food proved. In another study, a pH-sensitive smart

indicator based on the natural dye extracted from *Echium amoenum* flower (EAE) visually detected spoilage of shrimp by color change, and the suitability of *E. amoenum* anthocyanin as a pH-sensitive dye for package Intelligent classification of protein-rich foods showed. The results showed that packaging films made from tea waste CMC (carboxyl methyl cellulose) and forsaleran, packaging films prevent the growth of microorganisms and the accumulation of biogenic amines, and as a result, the shelf life of fillets They increase salmon (Jiang et al., 2020; López-Torres et al., 2023; Mohamed et al., 2020; Musso et al., 2019).

Therefore, the pH indicator film can be used as a sensor to know the quality of food. According to the studies, the high nutritional value of rice bran and basil plant and its positive effects on human health and the interest in smart biodegradable packaging in the current research based on the color change of the extract extracted from the basil stem with the change pH and also in the presence of biogenic amines, a biodegradable nanosensor based on rice bran fiber extracted by enzymatic method was designed and manufactured. And to monitor the freshness of milk fish meat, it was studied.

Investigating and developing edible films containing color markers from basil stalks as a natural tool for monitoring fish quality and spoilage, as well as evaluating the performance of these films in detecting and monitoring lionfish spoilage using the property of changing the color of the marker, so that with the change in fish quality, It is important to change the color of the film. Promoting the use of rice bran fiber and basil stem as natural and sustainable sources in the production of packaging materials and edible films and investigating the effect of these films in reducing food waste and increasing the shelf life of fish through accurate and timely monitoring of spoilage, analyzing the physical and chemical properties of films produced and their effect on the quality and safety of fish is one of the important goals of this research.

## 2-Materials and methods

## 2-1-Materials

Basil plant (*Ocimum basilicum*), lion fish (*Siganus commersonii*), rice bran were purchased from Bushehr city (Bushehr, South of Iran). Unsaturated vitamin E-rich sunflower oil with a total fat of 15 g was purchased (Shiraz, Iran). Petroleum ether (101769), hydrochloric acid (HCL37%) (Merc, Germany), acetone grade (Extra Pure), ethanol (96% v/v), ammonia from a chemical laboratory (Dr. Majalli, Tehran, Iran), alpha enzymes Amylase,  $\alpha$ -glucosidase (G5003-100UN), protease (Sigma Chemical, USA), Tween 80 (Merc, Germany), Culture medium (Kant agar) (Merc, Germany), double distilled water (DDW) with purity (18 M $\Omega$  cm) from water purification system (Nanopure Infinity, Barnstaeas International, Dubuque, IA, USA) were prepared and purchased.

## 2-2-Enzymatic extraction of rice bran fiber

First, the Soxhlet system was used to extract fat from rice bran (Wen et al., 2017), then the defatted bran was gelatinized in the presence of stable amylase at a temperature of 100 °C for 1 h. In the next step, rice bran is digested at 60 °C for 1 h using protease, and in order to separate protein and starch, using amyloglucosidase enzyme, incubation at 60 °C after 1 h, 4 V of 95% ethanol It was added to the solution and allowed to form a precipitate at a temperature of 60°C for 60 min. After filtration, the remaining materials were washed using 78%, 95% ethanol and acetone solutions. Finally, after packing, the extracted fiber was stored in a dry and cool place for further tests (Y. Liu et al., 2021).

## 2-3-Extraction of anthocyanin extract from basil stem (*Ocimum basilicum*)

In order to extract anthocyanin from the basil stem, first the desired layers were separated from the stem. Then, 50 grams of these dried layers were kept with 250 ml of 80% ethanol and 1% hydrochloric acid solution at 4°C for 24 hours. Then the obtained extract solution was centrifuged at a speed of 8000 rpm for 15 min (Germany, rpm/min 500), and the top layer of the solution was separated. Then, the amount of

anthocyanin in the extract was measured using the absorption difference method at different pH (2-12) (Kim et al., 2022).

## 2-4-Measurement of total phenolic content of basil stem (*Ocimum basilicum*)

The amount of total anthocyanin phenolic compounds produced was determined by Folin-Ciocalto method. The amount of 0.5 ml of the extract was mixed with 2.5 ml of 10% Folin Ciocalto solution and after 3 min, 2 ml of 5.7% sodium carbonate solution was added to it, then the absorption of the sample at 765 nm by UV-Vis device (Schimadzu) UV/Vis-240 IPC, Japan) was read. The results were reported in terms of DW (gAE/g) of gallic acid present in 100 ml of extracted anthocyanin. To draw the standard curve, dilutions of 100-700 standard gallic acid were used (Ahmed et al., 2019).

## 2-5-Investigating the antioxidant property of basil stem extract (*Ocimum basilicum*)

The effect of free radical inhibition was evaluated with the help of DPPH (diphenyl-1-picrylhydrazyl free radical). 4 ml of anthocyanin extracted from the basil stem was placed in 100 ml of methanol solution at 20 °C for 1 h to react. The absorbance of the reacted solution was measured at 517 nm by a UV-Vis device (Schimadzu UV/Vis-240 IPC, Japan). And the antioxidant power was calculated from the following equation (1) (Teofilović et al., 2017)

$$\text{Dpph radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

**A0**=The absorption rate of the sample

**A1**= the absorption rate of the control

## 2-6-Preparation of nanoencapsulation of anthocyanin extract (*Ocimum basilicum*)

In this research, o/w nanoemulsions were prepared using the optimization of ultrasonic conditions. The aqueous phase containing anthocyanin at a concentration of 5% w/w and the hydrophilic surfactant 80-Tween at a concentration of 2% w/w and the oil phase containing an emulsifier at a concentration of



3% w/w were used. Preparation of nanoemulsion was done using an ultrasonic device with a titanium prop (HD3200, Berlin, Germany) with a power of 300 W, a frequency of 24 kHz for 5 min, at a temperature of 25°C (Guan & Wang, 2021).

## 2-7-Edible film production

Preparation of stock solution: 10% w/v rice bran fiber, 10 g bran fiber and 90 g water were weighed. And then it was stirred for 10 min at 300 rpm and kept at 4 °C for 24 h to be hydrated. Preparation of edible film using 10% fiber stock solution and basil stem anthocyanin nanocapsules (*Ocimum basilicum*) and carboxyl methyl cellulose stock solution (0.2% w/v) with the addition of 0.2 gr of carboxyl methyl cellulose powder in 100 mm (DDW) mixed. And then glycerol was added to a W/W amount of 0.75% as a plasticizer and stirring

was done at a speed of 12000 rpm for 10 min at a temperature of 80°C (Yang et al., 2022). Then, after cooling, the temperature of the solution was brought to 37 °C and according to Table 1, the anthocyanin nanocapsule of basil stem mixed with the stock solution of rice bran fiber was added to the hydrated carboxyl methyl cellulose solution and to homogenize the solution at 40 °C for 30 min was stirred at 500 rpm by a roller mixer (Movil-Rod - JP SELECTA Spain). The film-forming solution was aerated using a vacuum pump under ambient conditions for 5 min. Then 100 ml of each film-forming solution was molded on the flat surface of laboratory plates and kept at 25°C until drying. The sample without anthocyanin nanocapsules was prepared as a control (D. Liu et al., 2021).

Table 1. Treatments produced from basil stem color marker and cmc based on rice bran fiber

Name	Sample(film)	CMC(g)	Glycerol %	10Stock souton %(g)	cmc	DDW (g)
Film C	-	0.2	0.75	-		75
Film 1	Fiber 100ppm+200ppm En anto	0.2	0.75	5		75
Film 2	Fiber 100ppm+400ppm En anto	0.2	0.75	5		75
Film 3	Fiber 100ppm+600ppm En anto	0.2	0.75	5		75

### 2-7-1-The effect of biogenic gases

Film samples were prepared and cut into 2 x 2 cm dimensions. In this experiment, 0.5 ml of ammonia gas was dripped on the film and the film was placed in a container with a labeled marker. Then, after one hour, the color change was recorded (Basumatary et al., 2022).

### 2-7-2-Moisture

To measure the moisture content, the samples were first weighed and then placed in an oven at 110 °C for 3 hours until a constant weight was reached, and finally the moisture content of the film was obtained from equation (2) (Tabari, 2018).

$$\% \text{ Moisture} = \frac{M_0 - M_1}{M_0} \times 100$$

$M_0$  = initial weight of the sample

$M_1$  = sample weight after drying

### 2-7-3-Measuring solubility in water

Solubility in water was expressed as the percentage of dry matter of the dissolved film after 24 h immersion in water. The dried films were immersed in 50 ml of DDW distilled water at a temperature of 25°C for 6 hours and stirred at a speed of 100 rpm. Then the pieces of the film were separated from the water and after drying at 50 °C for 24 hours, they were weighed. The solubility percentage of films was calculated from equation (3) (Silva et al., 2019)

$$\% \text{ Solubility} = \frac{w_1 - w_2}{w_1} \times 100$$

$w_1$  = initial dry weight

$w_2$  = final dry weight

### 2-7-4-Thickness

The thickness of the films was determined by a micrometer with an accuracy of 0.01 mm. For

the prepared films, in order to determine the mechanical properties, the thickness was measured at 8 points along the film (Acevedo-Fani et al., 2015).

#### 2-7-5-Opacity

The prepared films were cut into small pieces. Then it was placed in the cell of a spectrophotometer (Schimadzu UV/Vis-240 IPC) and their absorbance was read at a wavelength of 600 nm. Equation (4) will be used to determine turbidity (Zhao et al., 2022)

Opacity = Absorbance 600 nm/ Film thickness (mm)

#### 2-8-Fish coating with basil stem colored marker based on rice bran fiber

Lionfish with the scientific name (*Siganus commersonnii*) with a weight of about 400-500 gr was transported to the laboratory with a fiber box containing ice after it was purchased from Farash Gulf Wharf in Bushehr. And after decapsulating and separating the skin and bones of the fish at 25°C to reduce the microbial load, the prepared fillets were washed with 9% sodium chloride solution and then with DDW distilled water. In order to monitor spoilage in fish samples, 20 grams of fish samples were transferred into a sterilized plate and after placing the lid on the plate, which was previously attached to the film containing the concentrations of anthocyanin encapsulated from the basil stem, it was evaluated (Table 1). Then they were transferred to the refrigerator (4°C) (Umaraw et al., 2020) and chemical tests, and passing through the Ftir device at time intervals (0-10-20-30) were performed on the fish fillet containing the indicator film.

#### 2-8-1-pH measurement

2 grams of fish meat samples from each treatment were added to 10 ml of distilled water and homogenized for 1 minute by a homogenizer (HD3200 Bandelin, Germany), then the pH of the samples was measured with

a pH meter (Metrohm 713) (Ekrami et al., 2022).

#### 2-8-2-Measurement of fatty acid (FFA)

In order to determine the amount of free fatty acids index in fish meat sample, 10 g of homogenized fish meat sample was extracted with 60 ml of methanol and 60 ml of chloroform. After 24 h, 48 ml of distilled water was added to the solution. First, 50 ml of solvent (an equal mixture of 96% ethanol and diethyl ether) was added to the oil sample, then 1-2 drops of phenolphthalein were added to the mixture. The mixture was titrated using 0.1 N sodium titer (Wang et al., 2022).

#### 2-8-3-Measurement of Total Volatile Nitrogen Bases (TVB-N)

To determine the amount of volatile nitrogen in fish meat, 10 g of fish meat sample was transferred in a 1000 ml distillation flask, then 2 g of magnesium oxide, 300 ml of distilled water, a few boiling stones and a little antifoam were added to the flask. He heated the flask and reached the boiling temperature after 15 min. The vapors from the distillation flask were collected directly inside the Erlenmeyer. This Erlenmeyer flask contained 25 ml of 2% boric acid solution and a few drops of methyl red reagent.

The population of vapors and boric acid solution in Erlenmeyer reached 150 ml. The color of boric acid solution with methyl red reagent, which was initially red due to its acidic property, gradually became alkaline and green with the accumulation of vapors from distillation. At the end, the solution obtained from the accumulation of distillation vapors was titrated using 0.1 N sulfuric acid until it reached the color of onion skin, then the amount of volatile nitrogen substances was calculated in terms of milligrams per gram of the sample (Homayounpour et al., 2021).

$BNV \text{ T in } mgN.100g = 14.(150 + A).V$   
 $.F.N.100 / V a.P$

#### 2-8-4-Measurement of thiobarbituric acid index (TBARS)

A fish meat sample weighing 10g was placed in a 100ml distillation flask. 97.5 g of distilled water was added to it, and it was stirred for 2 min. Then 2.5 ml of 4M hydrochloric acid was added. After adding a few drops of anti-foam, the flask was heated and within 10 min of the boiling time, 50 ml of distilled liquid was obtained. Then 5 ml of distillation liquid was mixed with 4 ml of thiobarbituric acid reagent. The test tubes were placed in a bain-marie at a temperature of 100 °C for 35 min. Then, their absorption was measured by a spectrophotometer (Schimadzu UV/Vis-240 IPC) at a wavelength of 538 nm (Pabast et al., 2018).

$$\text{TBARS content} = 50 \times (\text{As} - \text{Ab}) / 200$$

AS= absorption of sample Ab= absorption of control

#### 2-8-5-FTIR spectroscopy test

To perform the FTIR test, a device (Spectrum Two, FTIR Perkin Elemer, USA) was used. Thin tablets of powdered fish samples containing basil stalk anthocyanin coating rich in rice seuss fiber with a thickness of less than 1 mm from mixing water and samples and coating with KBr and by freeze drying (operon, Korea) at -70 °C dry and then in a ratio of 1:20 and applying a pressure of about 60 Kpa for 10 min in a tablet press machine, and the transmission spectrum of the samples was obtained in the range of 400-400-400 cm<sup>-1</sup> wave number and with a resolving power of 5 cm<sup>-1</sup>. The analysis was done (Johnson et al., 2022).

#### 2-9-Statistic analysis

The statistical analysis of the data for the physicochemical and sensory characteristics of the treatments was performed by one-way analysis of variance (ANOVA) in the form of a completely randomized design with three replications. The comparison between means was done using Duncan's test at the 5%

probability level. Data analysis was done using SPSS software and curve drawing was done using EXCEL software.

### 3-Discussion and results

#### 3-1-The effect of pH on anthocyanin extracted from basil stem (*Ocimum basilicum*)

Anthocyanins show different colors in acidic and alkaline environments. This color change is known as pH effect or pH shift. These color changes are related to the change in oxidation state and electron charge difference in the structure of anthocyanins. pH has a direct effect on these variables, and with the change of pH, the balance between oxidized and purified states in anthocyanin changes. Therefore, pH can have a significant effect on the color and properties of anthocyanin, and this property can be used in various applications such as food industry, laboratory research, etc. (Khezerlou et al., 2023; Nogueira et al., 2024). Figure 1 shows the effects of pH (2-12) that anthocyanin changes color to pink in acidic pH and to yellow in alkaline pH. Ekrami et al. (2022) investigated the pH-responsive color indicator of saffron (*L. Crocus sativus*) salp mucilage edible film activated with anthocyanin, the pH sensitivity investigation showed that changing the pH of the solution changes the color of anthocyanin. When the pH increased from 3 to 11, the colors changed from pink to brown. Also Koshy et al. (2022) reported smart pH-sensitive films from whole arrowroot powder and isolated soy protein containing red cabbage anthocyanin. Films combined with anthocyanin showed significant color changes in different pH buffers and good sensitivity for ammonia vapor detection. By comparing the previous research and the current research, the results are consistent. Visible and significant color changes in anthocyanin occurred with pH change.

Table 2. Effects of pH (2-12) on anthocyanin

extracted from basil stem (*Ocimum basilicum*)

pH	Absorption
----	------------

control	0.295
7	0.2710
2	0.2369
4	0.3124
6	1.2188
8	0.1806
10	0.1782
12	0.2552

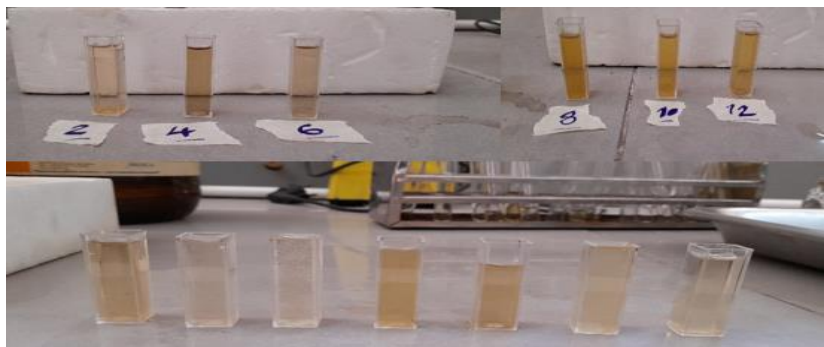


Figure 1. Examination of entocyanine extracted from basil stem (*Ocimum basilicum*) at pH (2-12)

### 3-2-Investigating the antioxidant properties and total anthocyanin phenol of basil stem (*Ocimum basilicum*)

Anthocyanins are a type of color pigment that are present in the basil plant. These compounds have very strong antioxidant properties due to the presence of phenolic groups in their structure. Antioxidants can protect against oxidative damage in the body and slow down anthocyanin extracted from basil stem (*Ocimum basilicum*)

the aging process of cells, their antioxidant properties make them effective in reducing inflammation and maintaining cardiovascular health. Regarding the basil stem, some research has shown that the basil stem contains anthocyanins and may have antioxidant properties (Garcia & Blesso, 2021; Tena et al., 2020).

Table 3. The amount of TFC, DPPH of

Property	Amount	Unit
TFC	365.32	GAE/g
DPPH	82.25	%

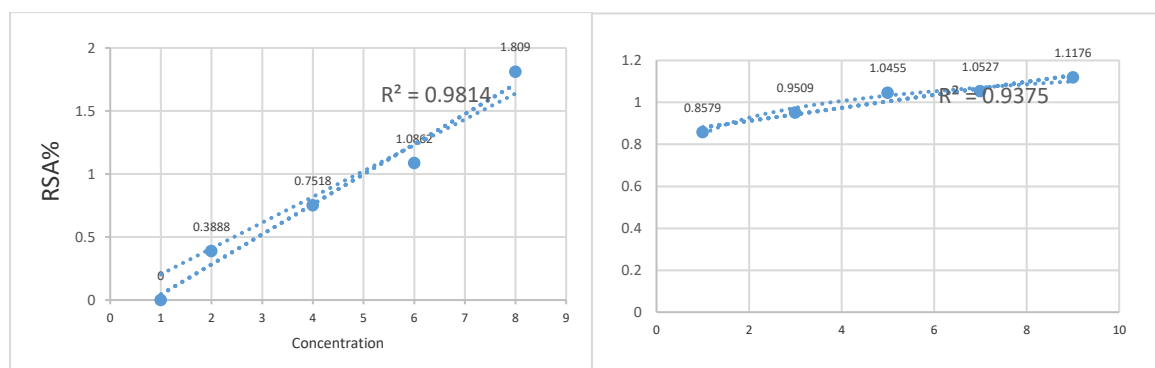




Figure 2. Calibration of TFC, DPPH of anthocyanin extracted from basil stem (*Ocimum basilicum*)

To check the antioxidant capacity of the anthocyanin sample extracted from the basil stem, a colorimetric assay method was used, which is performed based on the ability of the sample to remove free radicals and reduce radical cations. Table 3 shows the amount of TPC (GAE/g 365.32) and DPPH (82.25%). Anthocyanins extracted from basil stem had the highest antioxidant capacity. Phenols such as phenolic acids, flavonoids and anthocyanins, including their glycosylated and acylated derivatives, are considered in TPC calculation. However, the results of the present study were lower than the data reported by previous researchers (Chua et al., 2023; Flanigan & Niemeyer, 2014). In addition to the extraction technique, the solvent system also plays an important role in phenolic extraction. Previous researches have shown that acidic solvent is more effective for phenolic and anthocyanin recovery, respectively (Boulekbache-Makhlouf et al., 2013; Peanparkdee et al., 2019). In the DPPH assay, the presence of free radicals is significant and it is reasonable that the performance of this study is higher than the previously reported results (Samad et al., 2016; Wang et al., 2022; Wang et al., 2016). Donating hydrogen atom is to quench the radicals. Especially plants that contain anthocyanins, flavylum compounds (flavonoid cations) in the C ring of the flavonoid skeleton, promote hydrogen atom transfer for radical scavenging (Bendokas et al., 2020).

### 3-3-FTIR spectroscopic test of anthocyanin in basil stem

The spectra obtained from Fourier transform infrared spectroscopy related to the

anthocyanin sample of basil stem in optimal conditions are shown in figure (3). In figure (3), different absorption areas can be distinguished. The strong band in the range of 3300-3500  $\text{cm}^{-1}$  is related to OH functional groups in the anthocyanin structure. These OH functional groups are usually involved in the formation of polymer structure with hydrogen bonds (Gahruie et al., 2022; Turturică et al., 2015). Also, the peak that appears in the band at 2211.75  $\text{cm}^{-1}$  and 1460.88  $\text{cm}^{-1}$  is related to the aliphatic H-C stretching vibrations. These vibrations include bending and stretching vibrations of CH, CH<sub>2</sub> and CH<sub>3</sub> groups (Bhushan et al., 2023; Farooq et al., 2020). In a research, the use of FTIR spectroscopy was investigated to quantify the total anthocyanin content in ethanol extracts of plums, and it shows the wide potential of FTIR spectroscopy to ensure the quality of plum samples based on their solvent extracts (Johnson et al., 2022). In another study, the use of deep eutectic solvents as a green and biodegradable medium to extract anthocyanins from black carrots, FT-IR results showed that pure choline chloride shows several functional groups. And many of these functional groups coexisted after the formation of deep eutectic solvents (Türker & Doğan, 2021). By using the analysis of absorption bands and identification of different bands, it is possible to analyze and identify the structural characteristics and groups in the anthocyanin extract of basil stem.

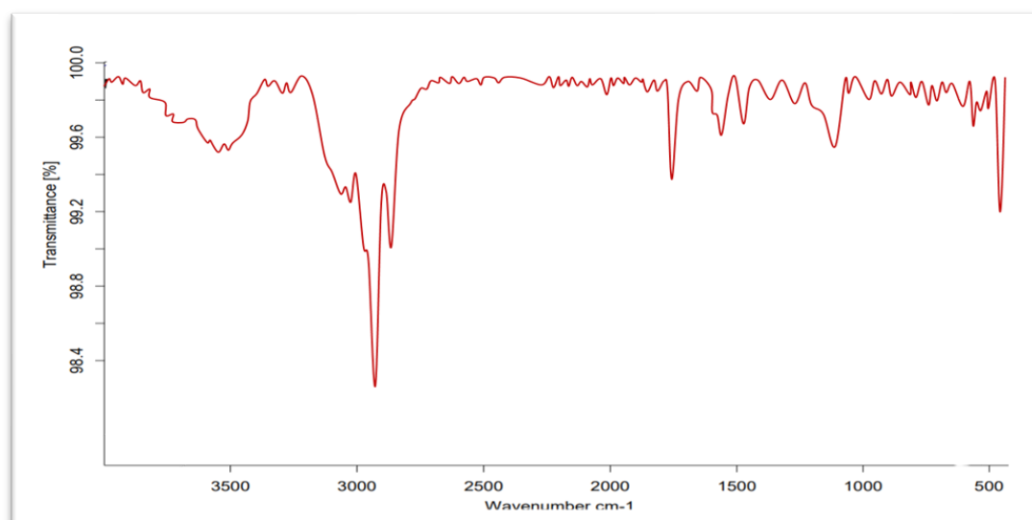


Figure 3. Fourier transform infrared spectroscopy in the identification of functional groups of anthocyanin extracted from basil stem from wavelength 400 to 4000  $\text{cm}^{-1}$  (*Ocimum basilicum*)

### 3-4-The effect of biogenic gases

Biogenic amines are considered as important biomarkers for controlling food quality and detecting the freshness of protein products. Biogenic amines are low molecular weight organic bases that are mainly produced by microorganisms and through enzymatic decarboxylation of foreign amino acids during food spoilage or endogenous tissue metabolism. Tyramine, tryptamine, putricine, cadaverine, spermidine and histamine are among biogenic amines (Shi et al., 2023; Tirtashi et al., 2019). These types of amines are found in various foods, including meat. According to its chemical structure, anthocyanin can bind to these amines. Figure 4

shows the color changes of the indicator in the presence of ammonia gas. After one hour of exposure to ammonia gas, a visible color change is observed in the indicator. Also, in a research for the development of simple sensors, to evaluate the freshness of meat and detect biogenic amines (Bas), a colorimetric sensor array was made by printing nine natural pigments on a hydrophobic nanoporous film. This research showed that pigments containing anthocyanin may be a very useful colorimetric sensor for meat quality evaluation (Zhang et al., 2023). This color change is due to the structural changes of anthocyanin in the vicinity of amine (Ameri et al., 2024).



Figure 4. Changes of biogenic gases on the film containing anthocyanin marker extracted from basil stem (*Ocimum basilicum*)

### 3-5-Investigating the morphology of the film containing the marker

In the obtained SEM image (Figure 5), it was observed that there is a good compatibility and miscibility between the components in the matrix of the film containing basil stem color

indicator based on rice bran fiber. The presence of hydrophilic surfactant 80-Tween at a concentration of 2% w/w and an oily phase containing nanoemulsified emulsifier in the film matrix caused uneven surfaces due to the accumulation of oil droplets. Encapsulated nanoemulsion may undergo some instabilities, phase separation and coalescence during the molding or drying process. However, with the addition of basil stem anthocyanin extract in the rice bran-based film, changes occurred in the polymer chains, which led to a decrease in the porosity of the film matrix. SEM results showed similar changes in sodium alginate/chitosan edible film containing red beetroot anthocyanin extract in the smart packaging study, which showed that the extract causes changes in polymer chains and increases the porosity of the film matrix (Ranjbar et al., 2023). Also, the present findings are similar to the reported findings, which observed similar changes in the microstructure of a gelatin-based film with the combination of curcumin extract. corresponds to (Bitencourt et al., 2014). The film samples containing the color indicator of basil stem (*Ocimum basilicum*) based on rice bran fiber have a porous structure and mostly have small pores. According to the SEM images, the holes in the sample have a spherical structure and the continuity It is clearly visible between them.

The images clearly show a fine structure of uniform and coordinated distribution of nanoemulsion particles of basil stem anthocyanin in nanometer dimensions between polymer chains or polymer matrix. The SEM images of the cross-section of the treatments show that the films containing basil stem color marker based on biodegradable rice bran fiber encapsulated nanoemulsion (Film 2) have high intermolecular density and the amount of voids in its structure is very low. The dots and protrusions in the film bed can be attributed to the rice bran fiber that causes the formation of insoluble particles (Rawdkuen et al., 2020). In a similar research on the synthesis and physicochemical properties of anthocyanin and starch based on polymer film, it showed that the morphologically developed film of the combination of anthocyanin and starch creates a smooth and homogeneous surface with intermolecular hydrogen bonding that increases the wavelength of the biopolymer (Erna et al. al., 2022). Shivangi et al. (2021); Yong and Liu (2020) reported that the biopolymer materials used to make the films, the source and amount of added pigment, and the methods used to make the films affected the microstructure of anthocyanin-rich films.

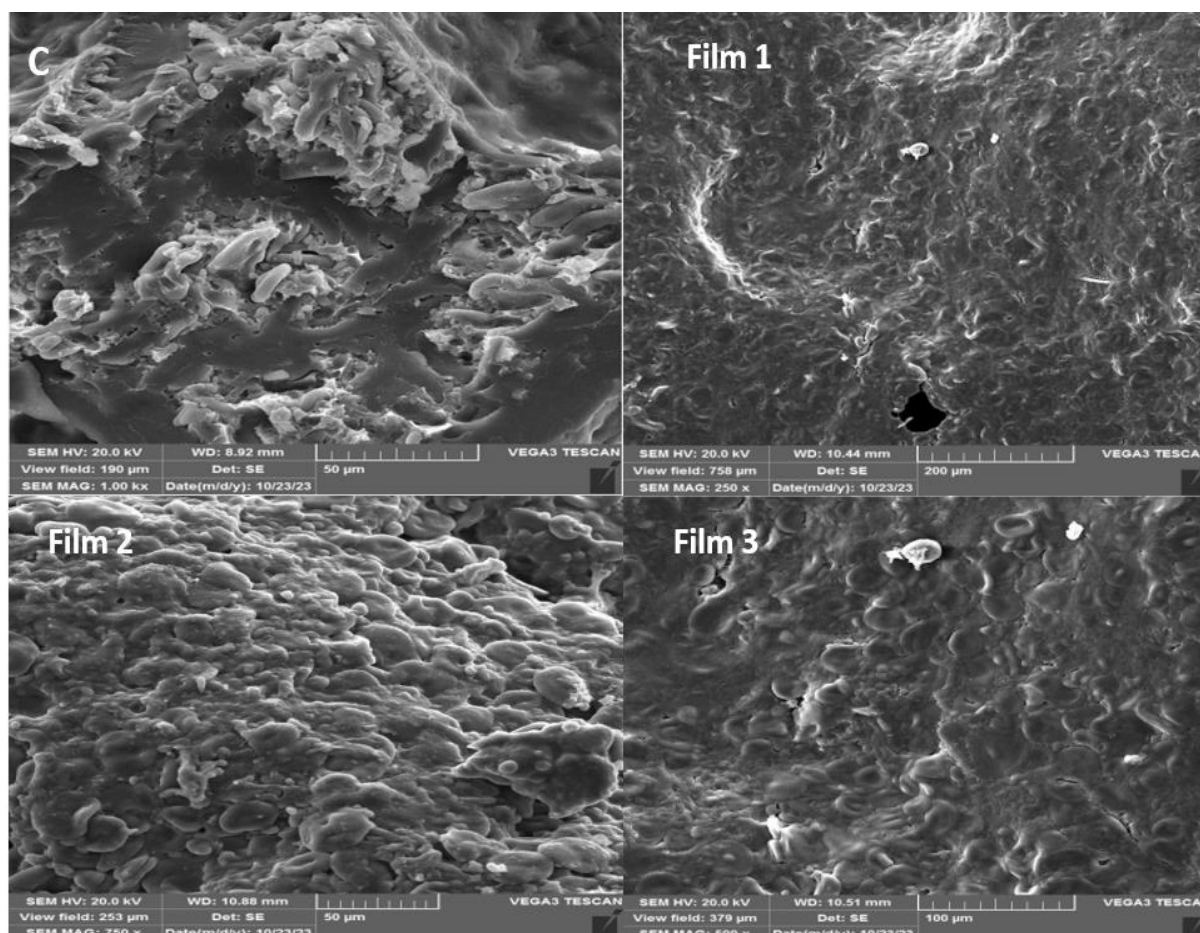


Figure 5. Morphology of the film containing endocyanine marker of basil stem (*Ocimum basilicum*)

### 3-6-The results of the physical characteristics of the film containing the color indicator of basil stem (*Ocimum basilicum*) based on rice bran fiber

By increasing the amount of anthocyanin in the basil stem (Table 4), the density increased. There was no significant difference between the control treatment and the treatment containing 200ppm basil stem anthocyanins. Also, there was no significant difference between the treatments containing 400ppm and 600ppm basil stem anthocyanins. There was no significant difference between the control treatments and the treatments containing 200ppm and 600ppm basil stem anthocyanins in thickness. The amount of thickness in the treatment containing 400ppm basil stem anthocyanin was significantly lower than other treatments ( $p \leq 0.05$ ). The highest amount  $1.70 \pm 0.05$  mm was observed in the treatment of 600ppm containing anthocyanin of basil stem. A significant difference between the treatments was observed in the solubility analysis. There

was no significant difference between the treatments containing 200ppm and 400ppm basil stem anthocyanins. The highest amount ( $46.53 \pm 1.00$ %) was observed in the treatment containing 600ppm basil stem anthocyanin and the lowest amount ( $23.14 \pm 1.00$ %) was observed in the treatment containing 200ppm anthocyanin basil stem. The results show that the treatment containing 400 ppm of anthocyanin extract had a lower thickness than other treatments. This may be due to the appropriate concentration of anthocyanin, which helped to improve the physical properties of the film. At higher concentrations (600 ppm), negative effects may have occurred on the film structure, leading to increased thickness. At higher concentrations, the viscosity may increase and this can lead to a decrease in film thickness upon drying, as the film spreads more uniformly and thinner. The presence of anthocyanins may promote hydrogen bonds and intermolecular interactions that can affect thickness. At certain concentrations, these bonds can lead to a tighter structure and thus



reduce the thickness of the film [71]. A significant difference between the treatments was observed in the moisture analysis ( $p \leq 0.05$ ). By increasing the percentage of anthocyanin in basil stem, a changing trend was observed in the amount of moisture. The highest amount ( $46.53 \pm 1.00$ ) percent was observed in the treatment containing 600 ppm anthocyanin of basil stem and the lowest amount ( $16.32 \pm 0.5$ ) % was observed in the treatment containing 200 ppm anthocyanin of basil stem. In the turbidity analysis, a significant difference was observed between the treatments, and with the increase in the anthocyanin percentage of the basil stem, a decreasing trend was observed in the turbidity level. There was no significant difference between the treatments containing 200ppm and 400ppm containing basil stem anthocyanin ( $p \leq 0.05$ ). Anthocyanin with hydroxy groups may act as a plasticizer, increase the free volume and mobility of macromolecules between polymers, and as a result, the polymer network becomes less dense due to the reduction of intermolecular forces, and as a result, the ability to stretch and It improves the flexibility of

layers (Cheng et al., 2022; Roy et al., 2021; Stoll et al., 2016). The relatively limited moisture content of ( $16.32 \pm 0.5$ ) % in the treatment of 200ppm in the films produced with anthocyanin from basil stem may be due to the interaction between the amine and OH groups of rice bran fiber and anthocyanins, which may limit the interaction of rice bran fiber with water. The slow composition and quantity of anthocyanins are the main factors in the moisture content of films (de Oliveira Filho et al., 2021; Yan et al., 2022; Zhang et al., 2019). In the research of Sohany et al. (2021) investigated the properties of purple sweet potato starch related to anthocyanin and skin-based pH indicator films and reported that the thickness, water solubility and degree of swelling of the films increased with the increase of anthocyanin. It has also been investigated in previous studies that the presence of anthocyanin changes the mechanical ability and the effects of moisture on the produced films, which is consistent with the research conducted (Capello et al., 2021; Etxabide et al., 2021; Merz et al., 2020)

Table 4. The results of the physicochemical characteristics of the film containing the color indicator of basil stem based on rice bran fiber

Treatment	Film C	Film 1	Film 2	Film 3
density(g/mm <sup>3</sup> )	$1.114 \pm 0.01^B$	$1.116 \pm 0.01^B$	$1.139 \pm 0.01^A$	$1.149 \pm 0.01^A$
Thickness (mm)	$1.62 \pm 0.02^A$	$1.67 \pm 0.05^A$	$1.10 \pm 0.05^B$	$1.70 \pm 0.05^A$
solubility (%)	$28.39 \pm 1.00^A$	$23.14 \pm 1.00^C$	$24.47 \pm 1.00^C$	$46.53 \pm 1.00^A$
Moisture (%)	$19.29 \pm 0.5^C$	$16.32 \pm 0.5^B$	$17.69 \pm 0.5^C$	$22.53 \pm 1.00^A$
Opacity (mm <sup>-1</sup> )	$43.26 \pm 1.00^A$	$22.45 \pm 1.00^B$	$20.92 \pm 1.00^B$	$18.34 \pm 0.5^B$

Different alphabets a,b indicates significant difference between groups

### 3-7-Investigating the chemical properties of fish samples coated with a film containing basil stem color indicator based on rice bran fiber

In examining the pH (Table 5), a significant difference was observed between the results of the treatments during the storage period ( $p \leq 0.05$ ). During the storage period in the control treatment and the treatment containing 400ppm basil stem anthocyanin, the pH trend increased. The amount of this factor in the treatments

containing 200ppm and 600ppm basil stem anthocyanin decreased on the tenth day and increased after that. Also, there was a significant difference between the pH of the samples prepared on each day ( $p \leq 0.05$ ). During the period, with the increase in the amount of anthocyanin in the basil stem, the pH value had a variable trend. On the day of production, the highest pH value related to the treatment containing 600ppm anthocyanin of basil stem. The highest value ( $10.30 \pm 0.05$ ) was observed in the control treatment on the 30th day and the



lowest value ( $6.90 \pm 0.05$ ) was observed in the treatment containing 400ppm basil stem anthocyanin on the production day. The increase in pH in the fish fillets containing the indicator was less due to the production of carbon dioxide from the fish tissue compared to the control fish under normal conditions. The increase in pH during the storage period is due to the denaturation of the protein leading to the release of amino compounds such as amines, di Methylamine and trimethylamine during the breakdown of amino acids and proteins. Also, the presence of antimicrobial and antioxidant

compounds in the film containing basil stalk color marker based on rice bran fiber affected the growth of spoilage bacteria and as a result the pH decreased (Bojorges et al., 2020; Marrone et al., 2021). P. You et al. (2022) investigated a fish packaging film, an antibacterial anthocyanin konjac glucomannan/carboxymethyl cellulose/black grape composite and found out the antioxidant and antibacterial properties of black grape anthocyanin and its inhibitory effect on food-borne pathogens.

Table 5. Examining the pH of fish samples coated with a film containing basil stem color indicator based on rice bran fiber

Treatment	Day 1	Day10	Day20	Day30
Control	$7.10 \pm 0.05$ <sup>Cd</sup>	$8.30 \pm 0.05$ <sup>Ac</sup>	$9.60 \pm 0.05$ <sup>Ab</sup>	$10.30 \pm 0.05$ <sup>Aa</sup>
200ppm	$7.40 \pm 0.05$ <sup>Bc</sup>	$7.10 \pm 0.05$ <sup>Dd</sup>	$8.30 \pm 0.05$ <sup>Cb</sup>	$10.10 \pm 0.05$ <sup>Ba</sup>
400ppm	$6.90 \pm 0.05$ <sup>Dd</sup>	$7.80 \pm 0.05$ <sup>Bc</sup>	$8.10 \pm 0.05$ <sup>Db</sup>	$9.70 \pm 0.05$ <sup>Ca</sup>
600ppm	$7.50 \pm 0.05$ <sup>Ac</sup>	$7.20 \pm 0.05$ <sup>Cd</sup>	$8.30 \pm 0.05$ <sup>Bb</sup>	$8.70 \pm 0.05$ <sup>Da</sup>

The results are presented in the form of average standard deviation for each treatment, all experiments were done in three repetitions. In each column, the values with different uppercase letters and in each row the values with different lowercase letters have a significant difference at the 5% level.

### 3-8-Fatty acid FFA

In the examination of fatty acid (Table 6), a significant difference was observed between the results of the treatments in all periods ( $p \leq 0.05$ ). The amount of this factor increased over time (1 to 30 days) in all treatments. Also, there was a significant difference between the amount of fatty acid of the samples prepared on each day ( $p \leq 0.05$ ). On the day of production, the amount of fatty acid increased with the increase of anthocyanin content of basil stem. In other days, with the increase in the amount of anthocyanin in the basil stem, the amount of fatty acid varied. The highest value

( $5.27 \pm 0.5$ )% was observed in the control treatment on the 30th day and the lowest value ( $0.015 \pm 0.001$ )% was observed in the control treatment on the first day. The amount of free fatty acids had an upward trend with increasing storage time until day 30. The final reduction of fat in the measured samples is probably due to the oxidation of fat and the effective effect of hydrolytic degradation of fat and its conversion into free fatty acids (Ganiari et al., 2017; Vlčko et al., 2022). In previous studies, it has been mentioned that adding plant extracts to fish can control the increasing trend of free fatty acids in fish (Gao et al., 2024). Roohinejad et al. (2017) reported in the previous study that the ability to inhibit free radicals or chelate metal ions by the phenolic compounds of the extracts was the reason for their effectiveness in controlling free fatty acids in fish treated with phenolic extracts of seaweed.

Table 6. Fatty acid results of fish coated with rice bran fiber-based basil stem color indicator

Treatment	Day 1	Day10	Day20	Day30
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Control	$0.015 \pm 0.001$ <sup>Dd</sup>	$2.43 \pm 0.05$ <sup>Ac</sup>	$3.87 \pm 0.05$ <sup>Bb</sup>	$5.27 \pm 0.5$ <sup>Aa</sup>
200ppm	$1.32 \pm 0.05$ <sup>Cd</sup>	$1.82 \pm 0.05$ <sup>Bc</sup>	$2.37 \pm 0.05$ <sup>Db</sup>	$4.65 \pm 0.5$ <sup>ABa</sup>
400ppm	$1.45 \pm 0.05$ <sup>Bb</sup>	$1.78 \pm 0.05$ <sup>Bb</sup>	$3.98 \pm 0.05$ <sup>Aa</sup>	$4.21 \pm 0.5$ <sup>Ba</sup>
600ppm	$1.72 \pm 0.05$ <sup>Ac</sup>	$1.81 \pm 0.05$ <sup>Bc</sup>	$3.65 \pm 0.05$ <sup>Cb</sup>	$4.54 \pm 0.5$ <sup>ABa</sup>

The results are presented in the form of average standard deviation for each of the treatments, all the experiments were done in three repetitions. In each column, the values with different uppercase letters and in each row the values with different lowercase letters have a significant difference at the 5% level.

### 3-9-Trimethylamine, protein, total volatile nitrogen

In the examination of trimethylamine (Table 7), a significant difference was observed between the results of the treatments in all periods ( $p \leq 0.05$ ). The amount of this factor varied over time (1 to 30 days) in the control treatment. But in other treatments, the amount of trimethylamine decreased on the 10th day and then increased. Also, there was a significant difference between the amount of trimethylamine in the samples prepared on each day ( $p \leq 0.05$ ). On the day of production, with an increase in the amount of anthocyanin in the basil stem, the amount of trimethylamine has increased significantly ( $p \leq 0.05$ ). The highest amount ( $3.31 \pm 0.05$ ) mg/100g was observed in the treatment containing 4% anthocyanin of basil stem on the 30th day and the lowest amount ( $0.03 \pm 0.01$ ) mg/100g was observed in the treatment containing 6% anthocyanin of basil stem on the 10th day. No significant difference was observed between the amount of protein in Table (8) in the control treatment and the treatment containing 600ppm basil stem anthocyanin during the storage period ( $p \leq 0.05$ ). However, in the treatment containing 200ppm basil stem anthocyanin, the amount of protein on the 10th day is significantly lower than other days ( $p \leq 0.05$ ).

In the treatment containing 400ppm basil stem anthocyanin, the amount of protein on the first and 30th days is significantly lower than other

days ( $p \leq 0.05$ ). Also, there was no significant difference between the amount of protein in the samples prepared on the first, 20th, and 30th days ( $p \leq 0.05$ ). The highest value ( $22.43 \pm 1.00$ ) percent and the lowest value ( $19.15 \pm 0.5$ ) percent were observed in the treatment containing 400 ppm anthocyanin of basil stem on the 20th and 30th days, respectively. In the analysis of total volatile nitrogen in Table (9), a significant difference was observed between the results of the treatments in all periods ( $p \leq 0.05$ ). The amount of this factor increased over time (1 to 30 days) in all treatments compared to the day of production. On the day of production, with an increase in the amount of anthocyanin in the basil stem, the amount of total volatile nitrogen decreased, but no significant difference was observed between the treatments ( $p \leq 0.05$ ). There was no significant difference between the total volatile nitrogen of the samples prepared on the 10th and 20th days of production ( $p \leq 0.05$ ). On the 30th day, a significant difference between the treatments was observed ( $p \leq 0.05$ ). The highest amount ( $14.81 \pm 0.5$ ) mg/100g was observed in the treatment containing 200ppm basil stem anthocyanin on the 30th day and the lowest amount ( $11.04 \pm 0.5$ ) mg/100g was observed in the treatment containing 600ppm basil stem anthocyanin on the 10th day. And it effectively controls the production of volatile nitrogen bases, a wide range of volatile base compounds including ammonia, methylamine, dimethylamine, trimethylamine and other similar compounds produced by microbial and enzymatic activities. are used under the title TVB-N to show meat spoilage (Huang et al., 2019; S. You et al., 2022). Figure 6 shows the trend of TVB-N and color change of the film sensors containing basil stem anthocyanin marker. In other studies, the positive and

significant effect of different plant extracts in controlling the TVB-N index of marine products has been mentioned. These data are similar to the findings of previous studies,

which means that the experiment shows strong results. Ghorbani et al., 2021; Sun et al., 2022; Zhai et al., 2020).

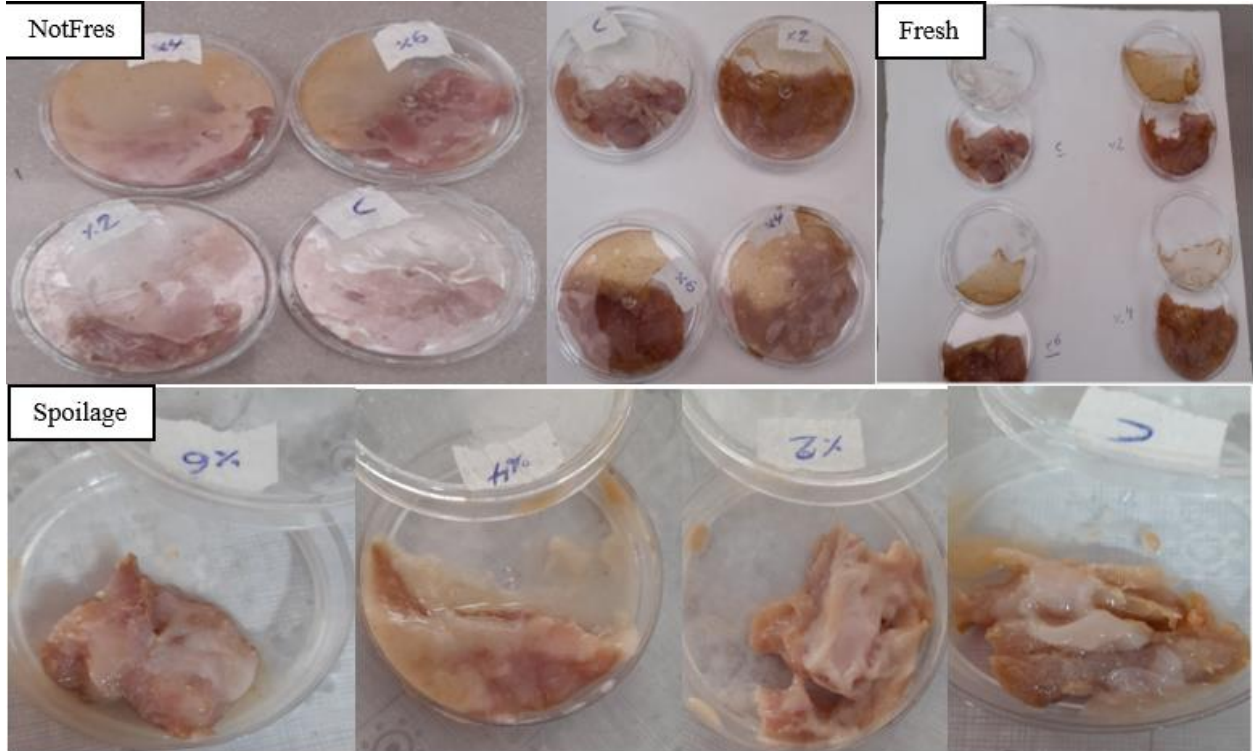


Figure 6. Color change of anthocyanin indicator extracted from basil stem on the freshness of lionfish (*Siganus commersonii*) at 4°C

The findings of this study with the results of Hui et al. (2023) in a research, inhibition of lipid oxidation, protein degradation and biogenic amine accumulation in postmortem fish with modified biopolymer functional enamel layers, the results indicated enamel storage, change in the degree of reflected spoilage of soluble peptides and total volatile nitrogen (TVB). -N) restrained. Glaze Table 7. The results of trimethylamine changes of fish coated with a film containing basil stem color marker based on rice bran fiber

also limited the biogenic content of major amines (histamine, putrescine, cadaverine, spermine, spermidine, tyramine, tryptamine) to relatively low levels. Similar chemical changes have been observed in fish after death. Tahir et al. (2022) in a research reported the positive effects of anthocyanins on meat and their use as indicators and inhibition of negative effects, which are consistent with our studies.

Treatment	Day 1	Day10	Day20	Day30
Control	0.59 ± 0.05 <sup>Cb</sup>	1.15 ± 0.02 <sup>Bc</sup>	0.71 ± 0.05 <sup>Da</sup>	0.06 ± 0.01 <sup>Dd</sup>
200ppm	2.43 ± 0.05 <sup>Bb</sup>	0.25 ± 0.02 <sup>Ad</sup>	1.65 ± 0.05 <sup>Cc</sup>	2.54 ± 0.05 <sup>Ca</sup>
400ppm	2.34 ± 0.05 <sup>Bb</sup>	0.12 ± 0.02 <sup>Bd</sup>	2.24 ± 0.05 <sup>Bc</sup>	3.31 ± 0.05 <sup>Aa</sup>
600ppm	2.56 ± 0.05 <sup>Ab</sup>	0.03 ± 0.01 <sup>Cc</sup>	2.54 ± 0.05 <sup>Ab</sup>	2.97 ± 0.05 <sup>Ba</sup>

The results are presented in the form of average standard deviation for each of the treatments, all

the experiments were done in three repetitions. In each column, the values with different

uppercase letters and in each row the values with different lowercase letters have a significant difference at the 5% level.

Table 8. The results of protein changes of fish coated with a film containing basil stem color marker based on rice bran fiber

Treatment	Day 1	Day10	Day20	Day30
Control	20.32 ± 1.00 <sup>Aa</sup>	21.34 ± 1.00 <sup>ABa</sup>	20.74 ± 1.00 <sup>Aa</sup>	20.54 ± 1.00 <sup>Aa</sup>
200ppm	21.32 ± 1.00 <sup>Aa</sup>	19.32 ± 1.00 <sup>Cb</sup>	21.47 ± 1.00 <sup>Aa</sup>	21.16 ± 1.00 <sup>Aa</sup>
400ppm	20.32 ± 1.00 <sup>Ab</sup>	22.34 ± 1.00 <sup>Aa</sup>	22.43 ± 1.00 <sup>Aa</sup>	19.15 ± 0.5 <sup>Ab</sup>
600ppm	20.16 ± 1.00 <sup>Aa</sup>	20.45 ± 1.00 <sup>BCa</sup>	21.27 ± 1.00 <sup>Aa</sup>	20.47 ± 1.00 <sup>Aa</sup>

The results are presented in the form of average standard deviation for each of the treatments, all the experiments were done in three repetitions. In each column, the values with different

uppercase letters and in each row the values with different lowercase letters have a significant difference at the 5% level.

Table 9. The results of changes in volatile nitrogen of whole fish coated with a film containing basil stem color marker based on rice bran fiber

Treatment	Day 1	Day10	Day20	Day30
Control	11.01 ± 0.5	11.3 ± 0.5 <sup>Ab</sup>	14.45 ± 0.5 <sup>Ab</sup>	12.43 ± 0.5 <sup>BCa</sup>
200ppm	11.06 ± 0.5 <sup>Ac</sup>	11.2 ± 0.5 <sup>Ac</sup>	12.65 ± 0.5 <sup>Ab</sup>	14.81 ± 0.5 <sup>Aa</sup>
400ppm	11.06 ± 0.5 <sup>Ac</sup>	11.11 ± 0.5 <sup>Ac</sup>	12.24 ± 0.5 <sup>Ab</sup>	13.5 ± 0.5 <sup>Ba</sup>
600ppm	11.06 ± 0.5 <sup>Ab</sup>	11.04 ± 0.5 <sup>Ab</sup>	12.32 ± 0.5 <sup>Aa</sup>	13.09 ± 0.5 <sup>Ca</sup>

The results are presented in the form of average standard deviation for each of the treatments, all the experiments were done in three repetitions. In each column, the values with different uppercase letters and in each row the values with different lowercase letters have a significant difference at the 5% level.

### 3-10-Examining the results of antioxidant characteristics of fish samples

#### Examining the results of the antioxidant level

The antioxidant activity of film composites plays an important role in food packaging. The use of natural antioxidant compounds in the food itself as well as in food packaging increases the ability to preserve food (Qin et al., 2020). Next, the antioxidant effect was evaluated using the radical reduction capacity (RSC) method with the help of 2,2-diphenyl-1-picrylhydrazyl DPPH radical. DPPH is a purple compound that easily becomes a radical due to

the presence of a phenyl group in its structure and is actually a source of free radicals (Arun et al., 2022). According to graph (3), a significant difference was observed between the results of the treatments in all periods when examining the amount of antioxidants ( $p \leq 0.05$ ). The amount of this factor changed over time (1 to 30 days) in the treatment containing 200ppm basil stem anthocyanin. But in the control treatment and treatments containing 400ppm and 600ppm anthocyanin of basil stem, the amount of antioxidant increased on the 10th day and then decreased. Also, there was a significant difference between the amount of antioxidants of the samples prepared on each day ( $p \leq 0.05$ ). In all days, with the increase in the amount of anthocyanin in the basil stem, the amount of antioxidant has increased. The highest value ( $56.83 \pm 1.00$ ) % was observed in the treatment containing 600ppm basil stem anthocyanin on the 10th day and the lowest value ( $10.32 \pm 0.5$ ) %

A significant difference in antioxidant activity, the positive effect of fish coating, from the film containing basil stem color indicator based on rice bran fiber on retaining a greater amount of anthocyanin nanoemulsion compounds of basil stem compared to a lower amount of nanoemulsion compounds. Anthocyanin of

basil stem was observed until the storage period of the sample. The results showed that in laboratory conditions, the antioxidant power has a direct relationship with the total phenolic content (Chaiyasut et al., 2016; Mary et al., 2020).

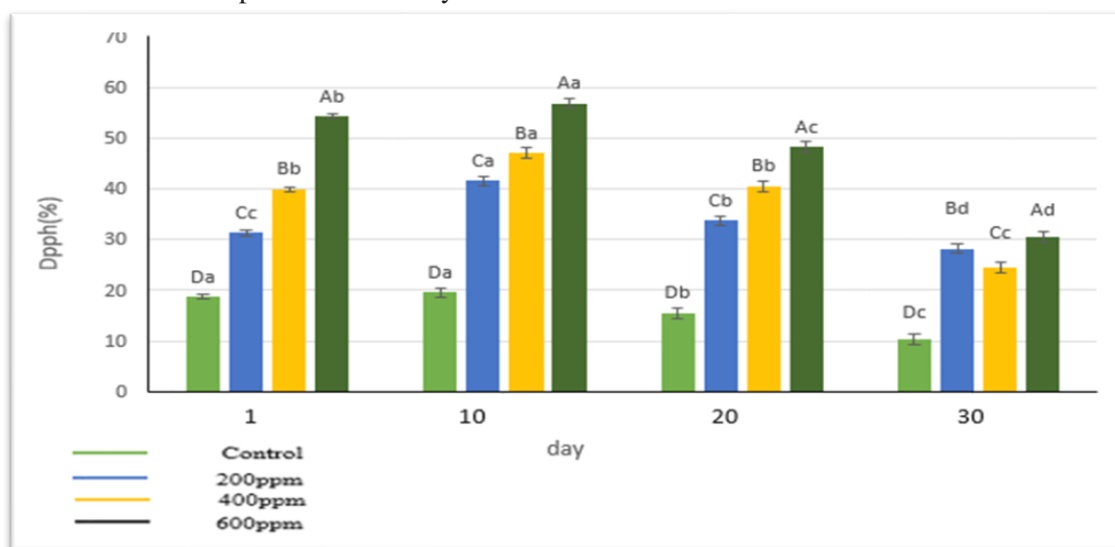


Figure 7. Comparison of antioxidant changes of fish coated with a film containing basil stem color marker based on rice bran fiber

### 3-11-Examining the changes of thiobarbituric acid

Malondialdehyde formed as a result of oxidation can react with other compounds of fish fillet such as nucleic acid, phospholipids, nucleotides, proteins, amino acids and other aldehyde compounds. The increase in TBA index during storage is attributed to lipid oxidation, production of volatile metabolites and tissue hydrogenation (Kanatt, 2020; Ranjbar et al., 2023). In the examination of thiobarbituric acid in Figure 8, a significant difference was observed between the results of the treatments in all periods ( $p \leq 0.05$ ). The amount of this factor increased over time (1 to 30 days) in all treatments. Also, there was a significant difference between the thiobarbituric acid samples prepared on each day ( $p \leq 0.05$ ). On the day of production, the amount of thiobarbituric acid in the treatments containing basil stem color marker based on rice bran fiber is significantly lower than the

control treatment ( $p \leq 0.05$ ). The highest amount ( $6.76 \pm 0.5$ ) percent was observed in the control treatment on the 30th day and the lowest amount ( $0.24 \pm 0.5$ ) % was observed in the treatment containing 600ppm basil stem anthocyanin on the first day. Thiobarbituric acid is used as an indicator of secondary fat oxidation. During this stage, peroxides are oxidized to substances such as aldehydes and ketones. Bioactive compounds of basil stem extract (such as flavonoids, phenolic compounds and anthocyanins) can effectively delay the oxidation of fat in fish fillet (Hosseini et al., 2022; Yin et al., 2023). A study by Bahrami Feridoni and Khademi Shurmasti (2020) The effect of nanoencapsulated sour tea extract (*L. Hibiscus sabdariffa*) with carboxymethyl cellulose on the quality and shelf life of chicken nuggets, then total volatile nitrogen (TVB-N), thiobarbituric acid (TBA) They were evaluated in the treatments stored in the refrigerator for 9 days. The results showed that sour tea extract has antioxidant properties



and the coating of the extract increased its antioxidant properties as the piece containing 100 ppm of nanoencapsulated sour tea extract with CMC delayed the oxidative spoilage and organoleptic changes of chicken nugget.

Therefore, it seems that basil stem anthocyanin extract nanoencapsulated with CMC can be used as a natural preservative in meat and meat and seafood products.

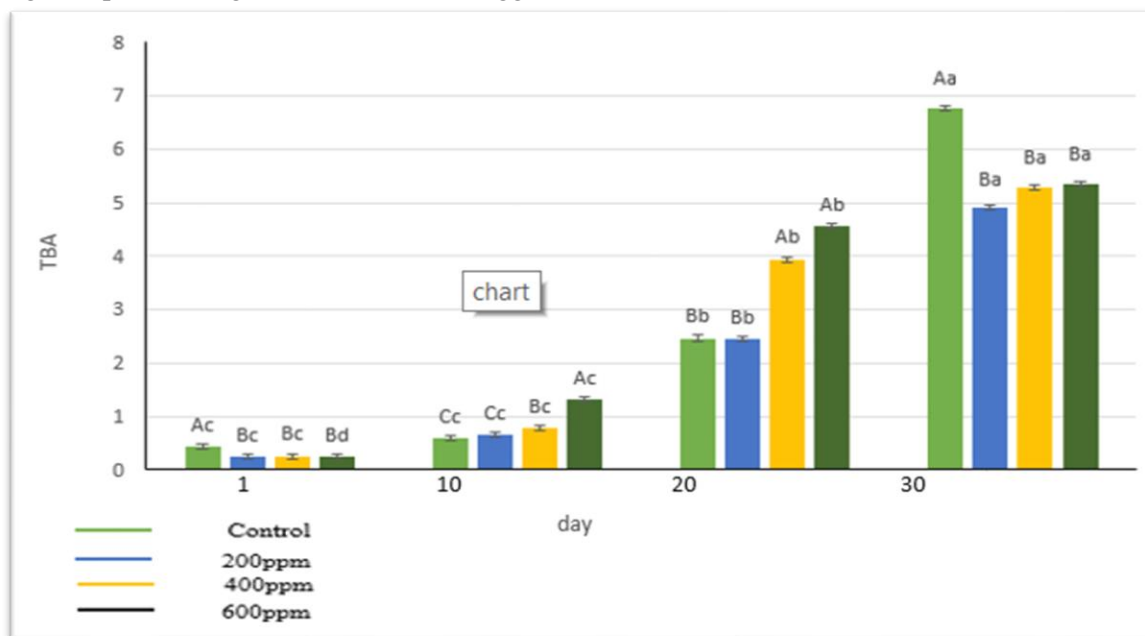


Figure 8. Comparison of changes in thiobarbituric acid of fish coated with a film containing basil stem color marker based on rice bran fiber

### 3-12-FTIR spectroscopy

The FTIR spectrum of coated fish containing anthocyanin indicator from the film containing basil stem color indicator based on rice bran fiber in the range of 400-4000  $\text{cm}^{-1}$  is presented in the graph. OH stretching vibrations show inter or intramolecular hydrogen bonds. 2800-3000  $\text{cm}^{-1}$  peaks are related to NCH- and CH<sub>2</sub>-bending and stretching vibrations of alkanes and alkenes. Typical bands assigned to cellulose can be seen in the region of 1630-900  $\text{cm}^{-1}$ . The absorption band in the range of 897  $\text{cm}^{-1}$  is related to ether bonds of glucose units in cellulose. The bandwidth at 3000-3500  $\text{cm}^{-1}$  is related to the vibrational stretching of hydroxyl groups, which indicates water retention and the ability of hydrogen bonding in polysaccharides and prevents the increase of fish spoilage during the period (1 to 30). The peaks related to the out-of-plane bending of H-C bonds in benzene rings at 722  $\text{cm}^{-1}$  with out-of-plane vibrations of H-N indicate the nanoemulsion of

rice bran fiber compounds from basil stem anthocyanin (Ghiasi et al., 2020; Łupina et al., 2019). In addition, it has been reported that the 1700-1600  $\text{cm}^{-1}$  range is assigned to the amide spectral region as well as to the secondary structure of proteins, which is mainly the result of the stretching of bonds at C=O of peptide structures. Other peaks at 2932, 1238, 1020  $\text{cm}^{-1}$  may be attributed to carbon-hydrogen and carbon-oxygen single bonds. The peak in the range of 1301  $\text{cm}^{-1}$  is also attributed to the C-O-C stretching vibration bond (Khezerlou et al., 2023). According to the graphs of the functional groups in the fish coated with the indicator from the film containing the basil stem color indicator based on rice bran fiber, it shows that on day zero, the removal of the carbonyl in the anthocyanin of the basil stem and the broadening of the OH hydroxyl band made the environment slightly more acidic. but in the lower areas of the system, it has become more acidic. In addition, the antioxidant properties of basil stem anthocyanin extract and phenolic

compounds such as carvacrol, thymol and methyl ether derivatives prevent the initiation of chain reactions of free radicals and thus

reduce them (Tavakoli et al. , 2023; Zeng et al., 2023).

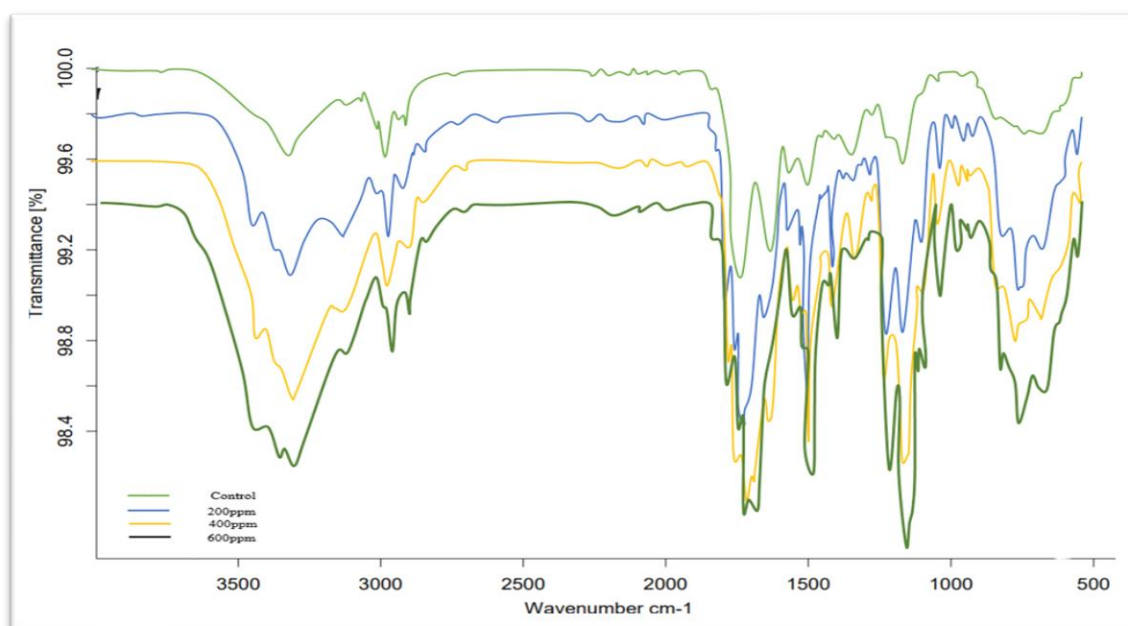


Figure 9. Spectrometry FTIR of coated fish containing anthocyanin indicator from the film containing basil stem color indicator based on rice bran fiber

#### 4-Conclusion

The anthocyanin extract extracted from the basil stem has appropriate amounts of phenolic, flavonoid and anthocyanin compounds, and for this reason, it has antioxidant properties. In addition, these phenolic compounds can also show antimicrobial properties. The use of this extract improved the shelf life of lion fish fillet. As it is known, different indicators show different shelf life for fish. This shows that relying on a qualitative index to diagnose the health and quality of fish cannot be very reassuring. The simultaneous measurement of chemical index (especially TBARS and N-TVB) along with the estimation of microbial quality can provide an almost adequate view of the quality and health of fish. In general, although the treatment containing nanoemulsion extract of anthocyanin extract brought the longest shelf life to lionfish fillets, this increase in shelf life in the period of less than ten days was not significantly different from the control sample. Edible film based on

wheat bran fiber with anthocyanin from basil stem was prepared in terms of transparent color and an acceptable amount of tensile strength, high solubility and moisture content. Therefore, it can cover food and increase its shelf life. The antioxidant activity of the film with basil stem anthocyanin composition increased significantly compared to the control. Biodegradable films based on anthocyanin composition of basil stem can be used as a safe packaging material with the ability to inhibit free radicals in food. The use of smart packaging that includes color markers based on natural extracts (such as anthocyanins from the basil stem) can be an effective way to monitor the quality and spoilage of lionfish. These indicators accurately and quickly show changes in quality and can help consumers and distributors to know the freshness status of the product. Color indicators can change color quickly and indicate spoilage or reduced quality of fish. This helps consumers to avoid buying low-quality products. The use of natural extracts in markers ensures the safety and

health of consumers and helps to reduce the use of harmful chemicals. By using this type of packaging, you can extend the life It increased the usefulness of marine products and reduced food waste.

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مقاله علمی-پژوهشی

### بررسی عملکرد فیلم حاوی نشانگر رنگی ساقه ریحان بر پایه فیبر سبوس برنج به منظور پایش فساد

ماهی شیر (*Siganus commersonnii*)

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#### اطلاعات مقاله

#### چکیده

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۵/۱۳

تاریخ پذیرش: ۱۴۰۳/۱۰/۲

کلمات کلیدی:

پایش فاسدشدن،

فیلم خوراکی،

نشانگر رنگی

تولید فیلم های هوشمند شامل استفاده از شاخص های رنگ پاسخگو به pH است که از منابع طبیعی به دست می آیند. در راستای این روند، هدف در این پژوهش طراحی یک حسگر حاوی نشانگر رنگی ساقه ریحان بر پایه فیبر سبوس برنج به منظور تعیین کیفیت ماهی شیر (*Siganus commersonnii*) نگهداری شده به مدت ۱ تا ۳۰ روز در دمای یخچال است. در این مطالعه از یک طرح فاکتوریل برای ارزیابی اثرات تیمارهای کنترل، ۲۰۰ پی پی ام، ۴۰۰ پی پی ام و ۶۰۰ پی پی ام استفاده شد. نتایج میکروسکوپ الکترونی روبشی نشان داد که عصاره آنتوسیانین ساقه ریحان باعث تغییرات در زنجیره های پلیمری و کاهش تخلخل ماتریس فیلم می شود. آنتوسیانین با بسیاری از گروه های هیدروکسی به عنوان پلاستی سایزر، حجم آزاد و تحرک ماکرومولکولی بین پلیمری به دلیل کاهش نیروهای بین مولکولی و چگالی کمتر افزایش داد و در نتیجه قابلیت کشش و انعطاف پذیری لایه ها را بهبود بخشید. علاوه بر این، خواص شیمیایی (pH، تیوباریتوریک اسید و ترکیبات نیتروژن) برای همه نمونه ها با افزایش زمان نگهداری تا روز ۳۰ روند افزایشی داشت. در طیف سنجی FTIR، مشاهده شد که در روز صفر حذف کربنیل در عصاره آنتوسیانین ساقه ریحان باعث بزرگ شدن باند هیدروکسیل و اسیدی شدن محیط شده است. خواص آنتی اکسیدانی عصاره آنتوسیانین ساقه ریحان در حضور ترکیبات فنلی از شروع واکنش های زنجیره ای رادیکال های آزاد جلوگیری کرده است و باعث کاهش آن شد. فیلم حاوی نشانگر رنگی عصاره آنتوسیانین ساقه ریحان بر پایه فیبر سبوس برنج، پتانسیل افزایش کیفیت و ماندگاری فیلدهای ماهی شیر را دارد. عصاره آنتوسیانین ساقه ریحان و فیبر سبوس برنج می تواند به عنوان یک شاخص مناسب برای بررسی تغییرات فساد در محصولات غذایی بسته بندی شده باشد.

DOI:10.22034/FSCT.22.159.169.

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