



OPEN Production of *Oncorhynchus mykiss* biosensor based on polyvinyl alcohol/chitosan nanocomposite using phycocyanin during refrigerated storage

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Smart packaging, also known as intelligent packaging, is responsive to external stimuli, moisture, light, oxygen, heat, pH, and bacterial growth. In this study, polyvinyl alcohol/nanochitosan/phycocyanin nanocomposite (PVA/NCH/PC-NC) for fish fillets of *Oncorhynchus mykiss* rainbow trout coating was prepared. Five treatments were prepared over a period of 14 days (0, 1, 7 and 14 days) under treatments of T₁: fish coated with PVA/NCH-NC without PC; T₂, T₃, T₄ and T₅ fish coated with PVA/NCH/PC-NC (0.5, 1, 1.5 and 2% PC respectively). Moreover, the results showed that higher concentrations of PC in PVA/NCH polymer matrix resulted in a net-like morphology on the film's surface. Also, after 21 days of storage, the T₄ treatment had the lowest levels of mesophilic, psychrophilic, and Enterobacteriaceae bacteria (8.17 ± 0.02 , 7.90 ± 0.04 , and 60.67 ± 0.02 log cfu/g, respectively). Additionally, it was seen that PVA/NCH/PC-NC improved the Sensory evaluation of fish fillet samples during 14 days of storage ($p < 0.05$). Overall, the results showed that the prepared PVA/NCH/PC-NC (2% PC) film function as an intelligent packaging solution in food preservation and freshness monitoring applications of *Oncorhynchus mykiss* fillet in terms of mechanical, microbial and sensorial evaluation.

Keywords Phycocyanin, *Spirulina* sp., Fish fillet, Biofilms, Polyvinyl alcohol/chitosan nanocomposite

The global demand for high-quality food products is increasing, with the third generation of packaging being crucial for preserving food quality. This generation uses smart materials to monitor spoilage metabolism and functions like protection, communication, containment, and convenience. The second generation focuses on minimizing environmental impact, while the third-generation smart packaging monitors fish quality and alerts to potential issues. The goal is to promote safer, longer-lasting foods with sanitation, detectability, and quality¹.

Fish and fishery products (FFP) are a significant source of essential amino acids, accounting for 20% of per capita animal protein consumption. They also contain n-3 polyunsaturated fatty acids, which reduce heart disease risk, and various macronutrients that improve health outcomes².

The Salmonidae family includes Pacific salmon (*Oncorhynchus* sp.), Atlantic salmon, trout (*Salmo* sp.), char (*Salvelinus* sp.), graylings (*Thymallus* sp.), whitefish (*Coregonus* sp.), and various other genera. Rainbow trout is among approximately 70 species of salmonids found in natural ecosystems. Iran, Chile, and Turkey are the leading producers of rainbow trout³. Furthermore, rainbow trout farming is conducted in various countries, such as the United States, Norway, Spain, Italy, and France^{4,5}. Rainbow trout (*Oncorhynchus mykiss*) has increasingly established itself in the market as a species of economic significance. This species is classified as a fatty fish and ranks among the most commonly farmed fish. In comparison to wild (Pacific species) and cultured (Atlantic species) salmon, it exhibits lower prices and significant differences in flesh color, and intramuscular fat layer⁶. This fish contains a significant quantity of unsaturated fatty acids, which offer numerous health benefits and serve as an important and appropriate protein source for humans.

Microbial spoilage and oxidation are primary factors contributing to the deterioration of fish, leading to its disposal by consumers. FFP's elevated moisture content, which facilitates microbial growth and participates

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in various hydrolytic processes, classifies it as a highly perishable product⁷. Unsaturated fats exhibit a higher susceptibility to oxidation, resulting in diminished nutritional value and the development of undesirable odor and flavor. Proteolytic enzymes and intestinal microflora significantly degrade proteins, producing ammonia and nitrogen compounds that influence their immunological and sensory properties⁸. Artificial colors in food packaging may contain toxic and mutagenic compounds, which can inadvertently contaminate the packaged product, rendering it unsafe for consumption and contributing to increased waste generation⁹. Furthermore, the rising cost of petroleum resources and growing concerns about environmental degradation represent additional negative consequences of artificial colors^{10,11}. Utilizing advanced packaging methods can extend the shelf-life of related products^{12,13}.

Currently, there is ongoing research into the possible use of biopolymers as an alternative to synthetic polymers in food packaging. Chitosan, derived from marine crustaceans or insects, is a non-toxic, biodegradable material with excellent film-forming properties^{14–16}. The degree of deacetylation and concentration of chitosan influences its antibacterial activity. Higher deacetylation and concentration increase its effectiveness¹⁷. Studies have shown that chitosan can prolong the shelf-life of carp slices and cherry slices^{18,19}. However, single chitosan films are brittle and have a narrow antibacterial spectrum, limiting their use in food packaging²⁰. Researchers have identified Polyvinyl alcohol (PVA) as a promising option to enhance the mechanical properties of chitosan films. This makes chitosan a versatile material for various industries, including medicine, food packaging, and the chemical industry^{21,22}.

Researchers have addressed the challenges related to food packaging films by integrating natural active agents into chitosan/polyvinyl alcohol films, including anthocyanin, clove essential oil, ginger essential oil, curcumin, and others^{23–29}. The addition of these natural compounds improves the physical, antimicrobial, and other properties of the chitosan/polyvinyl alcohol film while also serving as a freshness indicator and extending the shelf life of food. Therefore, selecting a suitable natural active agent is crucial in the development of intelligent active films³⁰.

Natural dye-based packages have a high sensitivity to changes in product quality, such as pH changes, ammonia gas emissions, and exposure to inappropriate storage temperatures and UV light. Functional colorimetric indicators with natural colors can monitor food quality by interacting with changes in pH, gases, pathogens, temperature and humidity³¹. These indicators are related to the quality and appropriateness of packaged food consumption, according to consumers' points of view³².

Phycocyanin (PC) is a phycobiliprotein with antioxidant, anti-inflammatory, liver, and nerve protective properties. Experimental studies suggest it can treat Alzheimer's and Parkinson's disease, prevent skin-mucosal cancers, and prevent chronic myeloid leukemia in humans^{33–39}. However, studies have shown that the incorporation of PC into biodegradable polymers can create effective pH indicators that monitor food quality. It makes the quality of food possible⁴⁰.

Given the growing global awareness among consumers regarding the value of food, its safety, and its components, there is a clear preference for the use of natural preservatives. Research has indicated that the utilization of biodegradable films as carriers of probiotic bacteria can have beneficial effects on fresh meat products. These effects include antioxidant and antimicrobial properties, making them a viable option for a cost-effective and accessible natural preservative⁴¹.

In summary, this study aimed to prepare PVA/NCH/PC-NC using PC inclusion complexes. Previous studies in the literature have not examined the utilization of PC extracted from *Spirulina* sp. with PVA/NCH/NC over refrigerated storage. The objective of this study was to assess the impact of a film containing a natural cyanobacterial pigment on the preservation of fresh *Oncorhynchus mykiss* rainbow trout fillets. The evaluation focused on antimicrobial, chemical, physical, mechanical, and sensory effects. The fillets were stored at a temperature of 4 ± 1 °C for a duration of 14 days.

Material and methods

Culture conditions of the *Spirulina* sp.

Cyanobacterial strains *Spirulina* sp. isolated from Cyanobacteria culture collection (CCC) of herbarium ALBORZ at the Science and Research Branch, Islamic Azad University, Tehran, were grown in modified Z8 and illuminated ($300 \text{ m}^{-2} \text{ s}^{-1}$) growth rooms at 28 ± 2 °C for 10–15 days, respectively^{42,43}.

Extraction and purification of analytical grade of PC

The extraction and purification of PC was carried out as described by Nowruzi et al.⁴⁴. The PC was extracted from 500 mL of homogenized log-phase (14-day-old) culture after being centrifuged at 4,000 rpm to obtain a pellet. The pellet was suspended in 100 mL of 20 mM acetate buffer (pH 5.1). Extraction was carried out by repeated freezing (-20 °C) and thawing (room temperature) methods for 4 days until cell biomass became dark purple. Cell debris was removed by centrifugation at 5,000 rpm for 10 min, and a crude extract was obtained. Purification was carried out according to Afreen and Fatma⁴⁵. Briefly, solid ammonium sulfate was slowly added to the crude extract to achieve 65% saturation by continuous stirring. The resulting solution was allowed to stand for 12 h in a cold room and centrifuged at $4,500 \times g$ for 10 min. The pellets were resuspended in a small volume of 50 mM acetic acid-sodium acetate buffer (pH 7.1) and dialyzed overnight. The extracts were recovered from the dialysis membrane and filtered through a $0.45 \mu\text{m}$ filter (Fig. 1).

Antioxidant activity of extracted PC

Diphenyl-2-picrylhydrazyl (DPPH)

The antioxidant activity was measured by adding 100 μL of sample extract to 500 μL of DPPH (0.2 mM) solution. The absorbance decreased for 15 min, indicating higher radical-scavenging activity (517 nm). Positive controls

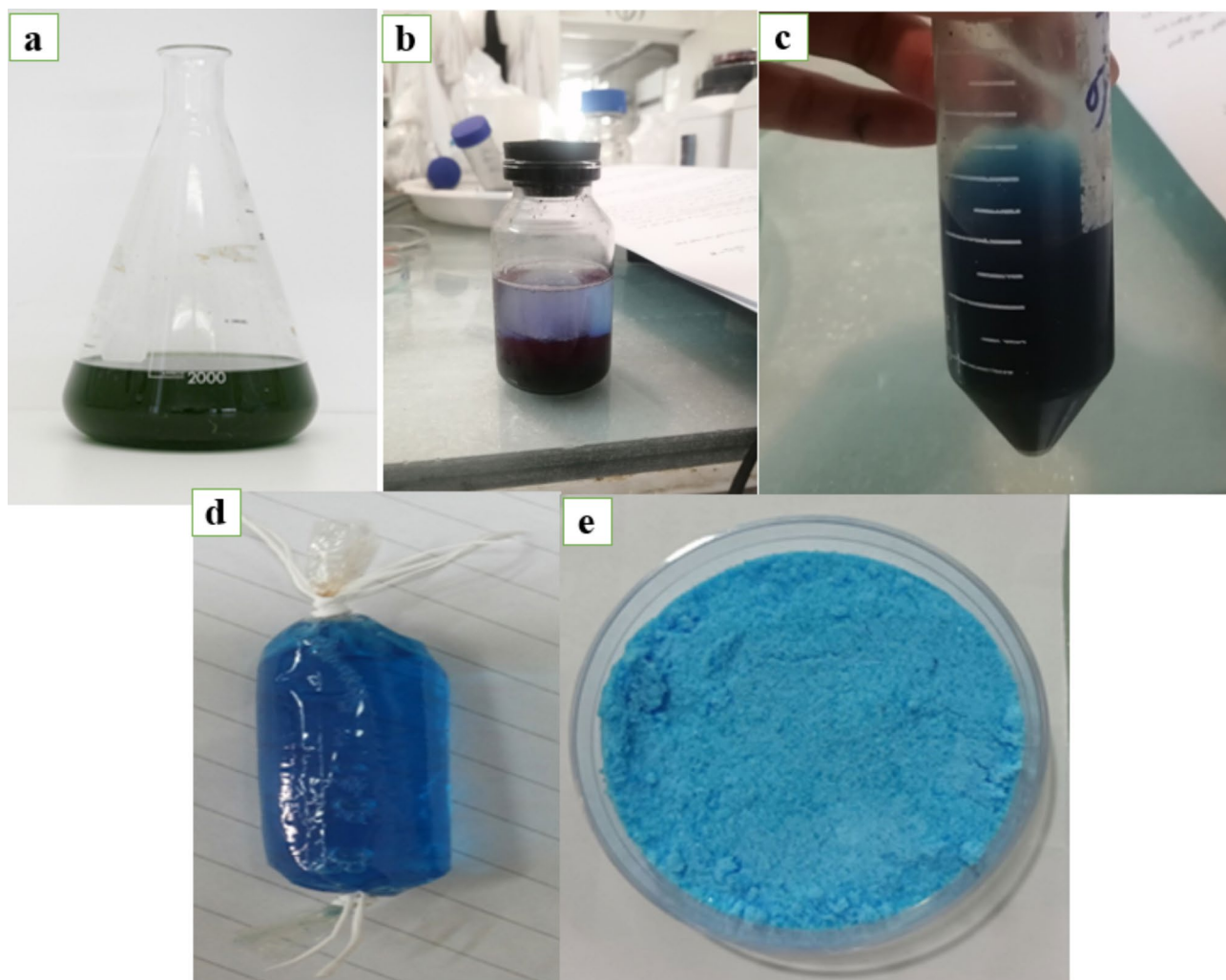


Fig. 1. The process of extraction and purification of PC. (a) Culture, (b) extraction, (b and c) purification, (d) dialysis, and (e) purified PC.

included ascorbic acid, BHA, and Trolox. Radical scavenging activity was calculated as a percentage of DPPH and discoloration using the equation:^{46,47}

$$\text{DPPH \%} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

FRAP assay

A mixture of extract and FeCl_3 in 0.05 M HCl was incubated for 30 min in a water bath at 37 °C. Then, 200 μL of TPTZ in 0.05 molar hydrochloric acid were introduced. The absorbance was measured at 620 nm, and the findings were classified as gallic acid or Trolox. The blank used was 50 μL of HCl 0.05 M⁴⁶.

Preparation of PVA/NCH/PC-NC

In order to encapsulate the PC-NC, 10 g of PVA was added to 90 ml of 80 °C water and continuously stirred for 4 h. Also, 4 g (w/v) of nanochitosan was dissolved in 100 ml of water (60 °C). Different concentrations of PC extract (0, 0.5, 1, 1.5, and 2%) were added to the solution and stirred at room temperature for 60 min to ensure uniformity. Afterwards, 15% (w/w) glycerol was added as a plasticizer, and the obtained solution was poured into a Petri dish and kept at room temperature for 48 h for evaporation and film formation. The biofilms were dried in an oven at 50 °C for 5 h after being separated from the platters⁴⁸ (Fig. 2).

Physical properties of biofilms

FTIR

The FTIR spectrum of the films was measured using weak total reflection (ATR) in the smart FTTR method with a resolution of 14 cm. The films (2 mg) were directly placed in the ZnSe ATR cell, and the spectra were recorded at a wave number of 500–4000 cm^{-1} ⁴⁹.

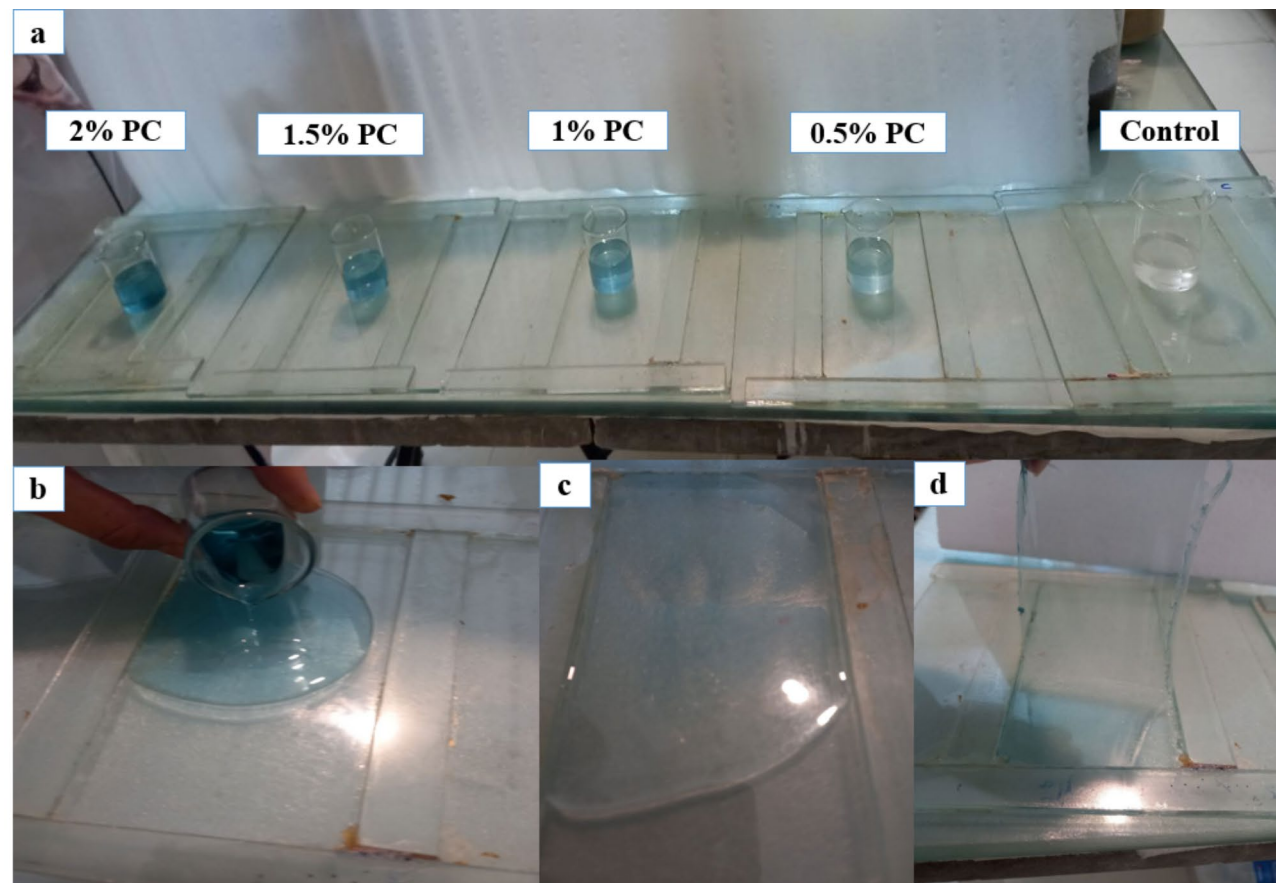


Fig. 2. The preparation process of polyvinyl alcohol/nano chitosan/phycoerythrin nanocomposite. (a) preparation of biofilms with different PC concentration of (control, 0.5%, 1%, 1.5%, and 2% PC), (b) pouring the biofilms on glass surface, (c) formation of biofilms, and (d) separating biofilms from the glass surface.

SEM analysis

The packaging biofilms were cut into 2×2 cm dimensions and then dissolved in a mixture of glutaraldehyde and acetonitrile solvent. They were carefully adhered to metal bases using glue after the solvent evaporated in the spa tucker device. The gold coating was applied using argon gas, as gold possesses exceptional electrical conductivity that is further enhanced by argon gas. Subsequently, the samples from the spa tucker stage were transferred to the electron microscope chamber for electron bombardment, yielding the desired signals. The image was used to assess the size and distribution of particles within the coating⁵⁰.

The thickness of the biofilms

The biofilm thickness was determined at three locations using a high-resolution digital micrometer with a precision of 0.001 mm. The average results were then computed⁵¹.

The moisture content of the biofilms

The biofilm pieces were trimmed to dimensions of 4×4 cm and measured in terms of weight. The initial weight was determined based on the measured value. The sample pieces were subsequently placed in an oven set at a temperature range of 50–60 °C for a period of 24 h until they reached their final dry weight. At this point, the weight and quantity of the samples were deemed to be the final dry weight⁵².

Mechanical properties of the biofilms

The biofilms' mechanical properties were measured using a histometer. The biofilms were divided into 1×7 cm sections and subjected to a controlled environment with a relative humidity of 50% and a temperature of 25 °C. The Instron device had a fixed distance of 50 mm between its two jaws. The upper jaw moved at a speed of 50 mm/min, while the lower jaw remained stationary. The amount of resistance to stretch ability and elongation was calculated from the following equation⁴⁸:

$$\text{Tensile strength (megapascal)} = \frac{\text{Maximum power}}{\text{Primary level}}$$

$$\text{Stretch ability} = \frac{\text{Elongation at the moment of rupture}}{\text{Initial length}} \times 100$$

Film color characteristics

The color content was determined using Hunter Lab parameters in terms of L^* (brightness-darkness), a^* (red-green), and b^* (yellow-blue) by a colorimeter device (Hunter Lab, color Flex, Virginia, USA).

Determination of color change in biofilms

The biofilms underwent color changes when buffer solutions with a pH range of 1 to 13 were used. In order to achieve this objective, the films were prepared with dimensions of 2 × 2 cm and submerged in buffer solutions. The overall color difference (ΔE^*) at various pH levels was then determined using the provided equation⁵³:

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

Packaging of *Oncorhynchus mykiss*

The fish samples of *Oncorhynchus mykiss* were promptly acquired from the local market in Karaj and promptly transported to the laboratory on crushed ice within a span of 6 h. The fillets were cut into pieces measuring 25 × 10 cm² and weighing 55 g. Following a thorough washing with sterile distilled water and subsequent drying in sterile conditions, the fillets were then packed with samples of the specified biofilms. These fillets were carefully placed in sterile stomacher bags, while control samples were also prepared and placed in their respective sterile stomacher bags. The samples were stored at a temperature of 4 °C and were subsequently analyzed for chemical, microbial, and organoleptic changes over a period of 14 days (0, 1, 7, and 14 days). Totally, five codes were prepared under treatments of T₁: fish coated with PVA/NCH-NC without PC; T₂: fish coated with PVA/NCH/PC-NC (0.5% PC); T₃: fish coated with PVA/NCH/PC-NC (1% PC); T₄: fish coated with PVA/NCH/PC-NC (1.5% PC) and code 5: fish coated with PVA/NCH/PC-NC (2% PC) (Fig. 3).

Evaluation Tests of *Oncorhynchus mykiss*

pH

The samples were homogenized by an electric stirrer in a 250-ml beaker, using 5 g of each sample and 45 ml of distilled water. The pH of the samples was measured using a pH meter on days 0, 1, 7, and 14⁵⁴.

Thiobarbituric acid reactive substance (TBARS)

This experiment measured TBAR using a colorimetric method. A 25 mL volumetric flask was filled with 1 g of minced fish meat, filled with 1-butanol, and a test tube was filled with 5 mL of the solution. TBA reagent was added, and the tubes were placed in an oven at 95 °C for 2 h. The absorbance of the solution was measured at 530 nm using a spectrophotometer with distilled water as a blank. The TBARS amount was calculated using the given equation on days 0, 1, 7, and 14. The equation takes into account the sample absorption (A_s) and blank absorption (A_c)⁵⁵.

$$\text{TBARS} = \frac{(V A_s - A_c) \times 50}{200} \quad (1)$$

Total volatile basic nitrogen (TVB-N)

The measurement of total volatile base-nitrogen (TVB-N) was conducted according to the method outlined by Aubourg et al. (1997) on days 0, 1, 7, and 14⁵⁶. Initially, 10 g of fish sample were dissolved by means of distilled water. The combination was produced for immediate use following full agitation and subsequent 30 min incubation. The solution was filtered and then evaluated using the semi-micro kjeldahl nitrogen determination device. The findings were expressed as milligrams of TVB-N per 100 g of muscle, using the equation V (volume of sulfuric acid) and N (normality of sulfuric acid)⁵⁷.

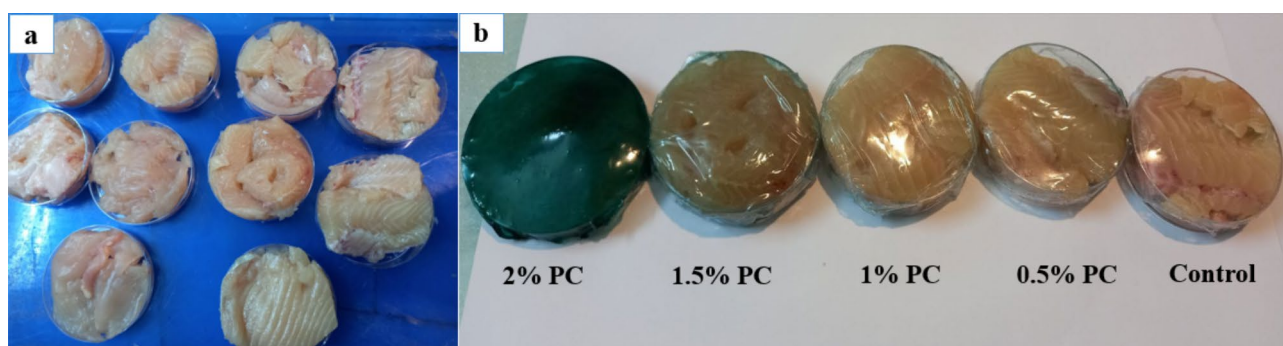


Fig. 3. The application of produced biofilms in fish fillet packaging. **(a)** Fish fillets and **(b)** packaging of fish fillet with different concentration of produced biofilms (control, 0.5%, 1%, 1.5%, and 2% PC).

$$\text{TVB} - \text{N} = (\text{V} \times \text{C} \times 14 \times 100) / 10\%$$

Microbiological analysis

Total psychrophilic bacteria count

Total viable psychrophilic bacteria counts were carried out according to Raeisi et al. (2016) at days of 0, 1, 7, and 14⁵⁸.

Total mesophilic bacteria count

Samples (1 ml) of serial dilutions of sample homogenates were plated on PCA and incubated at 37 °C for 2 days for determination of total mesophilic bacteria counts at days of 0, 1, 7, and 14⁵⁹.

Enumeration of total E. coli

The most probable number (MPN) method is used for the quantitative estimation of total coliforms at days of 0, 1, 7, and 14^{60,61}.

Sensory evaluation

The sensory characteristics such as color, odor, appearance, flavor, texture, and overall acceptability were scored using a 5-point hedonic scale at days of 0, 1, 7, and 14, according to Altug and Elmacı (2011)⁶². Samples were heated in the oven using an oven bag at 180 °C for 10 min immediately before serving.

Statistical analysis

The results of each experiment were analyzed using ANOVA with the statistical software package SPSS version 24. A significance level of 95% was used to determine statistical significance. The Tukey test was used to assess the significance of differences in mean values following a significant variation ($p \leq 0.05$) detected by the ANOVA test. Three replicated measurements were conducted for each treatment, and the mean values $\pm$ standard error of mean were determined⁶³.

Results

Antioxidant activity of PC

According to the results, the amounts of antioxidant activity by the DPPH and FRAP methods were 2.40 and 0.503 mg/g, respectively.

The results of biofilms tests

FTIR

FT-IR test was used to check the chemical bonds in the examined samples. In FT-IR spectroscopy, the infrared ray energies coincide with the vibrational energies of molecules, and this adaptation causes the sample to absorb electromagnetic radiation energy. Therefore, by changing the frequency of the radiation in a specific range (infrared), a spectrum is obtained whose transmission value is reduced in some wavelengths, or, in other words, it is absorbed by the molecules of matter. In Fig. 4, the FT-IR spectrum of PVA/NCH containing 0.5, 1, 1.5, and 2% v/w PC and control treatment is shown (Table 1). The FTIR spectrum of the samples in this study revealed a peak at 3280 cm⁻¹ wave number, which corresponds to the stretching vibrations of O-H and N-H bonds in the hydroxyl and amide structures of PVA/NCH. The peak at 2870 cm⁻¹ wave number also corresponds to the stretching vibrations of C-H bonds in the methyl and methylene structures of these two structures. The bending vibrations of O-H and N-H bonds, as well as the C-H bending vibrations, are also associated with the peaks located at wave numbers of 1632 cm⁻¹ and 1551 cm⁻¹. It falls within the wave numbers of cm⁻¹ 1456 and cm⁻¹ 1346. Additionally, the stretching vibrations of C-N and C-O bonds are associated with multiple peaks appearing in the range of 1081 cm⁻¹ wave number. In chitosan, the dancing vibration of the C-H bond and the bending vibration of the aromatic rings also showed absorption peaks at 840 cm⁻¹ and 563 cm⁻¹ wavenumbers, respectively. The results showed that by adding the weight percentage of PC, the intensity of this peak increased, and its position shifted to lower wave numbers. The presence of O-H and N-H bonds in the PC structure may have intensified the peak associated with these bonds, but also shifted its position by forming hydrogen bonds with the polar groups in PVA and chitosan. Also, the presence of C-H bonds in the structure of PC has an effect on the bending vibration of these bonds. Higher percentages of PC in these nanocomposites have intensified the peak associated with the C-H bending vibration, owing to the presence of this bond in the PC structure. The presence of higher PC percentages clearly shifts the minimum peak point to higher wave numbers, indicating the interaction between the methyl and methylene groups in this nanocomposite. Additionally, the presence of C-O/C-N bonds and aromatic rings in PC has made the peaks at 1080 cm⁻¹ and 560 cm⁻¹ wavenumbers stronger. The FTIR test results confirm the existence of chemical bonds in PVA and chitosan compounds, and they also show that the presence of O-H, N-H, C-H, C-O, and C-N bonds and aromatic rings in the PC chemical structure enhances the intensity of the corresponding peaks. We investigated these links by increasing the weight percentage of this structure in the nanocomposite.

Electron microscope (SEM) test of NC

The morphology of the indicator films in the Co sample revealed a smooth, uniform, and continuous morphology, with cross-linking occurring without any tears, creases, or cracks.

Adding 0.5% of PC, uniform spherical structure and gaps between different spheres were observed on the surface of the biofilms. The addition of PC (1, 1.5, and 2%) resulted in a web-like shape on the film's surface (Fig. 5).

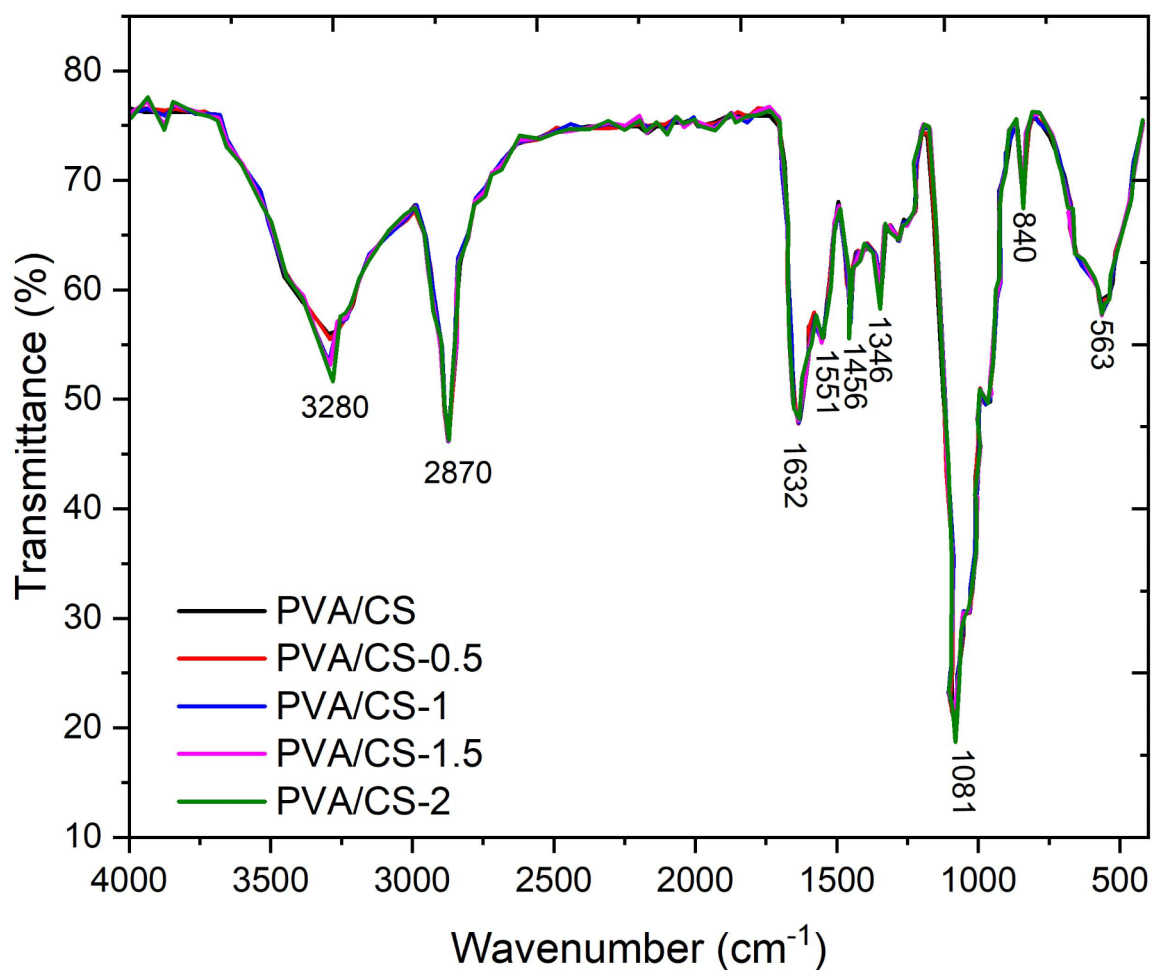


Fig. 4. FT-IR spectra of indicator biofilms. (black legend: control sample; red legend: film containing 0.5% PC; blue legend: film containing 1% PC; pink legend: carpet containing 1.5% PC; green legend: film containing 2% PC).

Functional group	T ₁	T ₂	T ₃	T ₄	T ₅	Changes
Stretching vibrations of O-H bonds	55.96	55.51	53.39293	53.13034	51.60894	Decrease
Stretching vibrations of N-H bonds	55.96	55.51	53.39293	53.13034	51.60894	Decrease
Stretching vibrations of C-H bonds	46.15	46.21	46.15738	46.19944	46.27547	Increase
Bending vibrations of O-H bonds	48.11	47.75	47.85358	48.00687	48.18935	Increase
Bending vibrations of N-H bonds	55.64	55.47	58.81899	60.41261	60.29337	Increase
Bending vibrations of C-H bonds	59.24	59.10	61.91734	63.48924	62.70562	Increase
Bending vibrations of C-H bonds	58.58	58.99	65.40272	65.76016	65.30374	Increase
Stretching vibrations of C-N bonds	20.62	20.55	25.00531	28.36906	28.77245	Increase
Stretching vibrations of C-O bonds	20.62	20.55	25.00531	28.36906	28.77245	Increase
Bending vibrations of C-H bonds	68.73	68.04	75.26303	74.16848	71.64724	Increase
Bending vibration of aromatic rings	59.56	59.25	67.88514	70.87242	75.5092	Increase

Table 1. Peaks corresponding to functional groups and changes in indicator biofilms. T₁: fish coated with PVA/NCH-NC without PC; T₂: fish coated with PVA/NCH/PC-NC (0.5% PC); T₃: fish coated with PVA/NCH/PC-NC (1% PC); T₄: fish coated with PVA/NCH/PC-NC (1.5% PC) and code T₅: fish coated with PVA/NCH/PC-NC (2% PC).

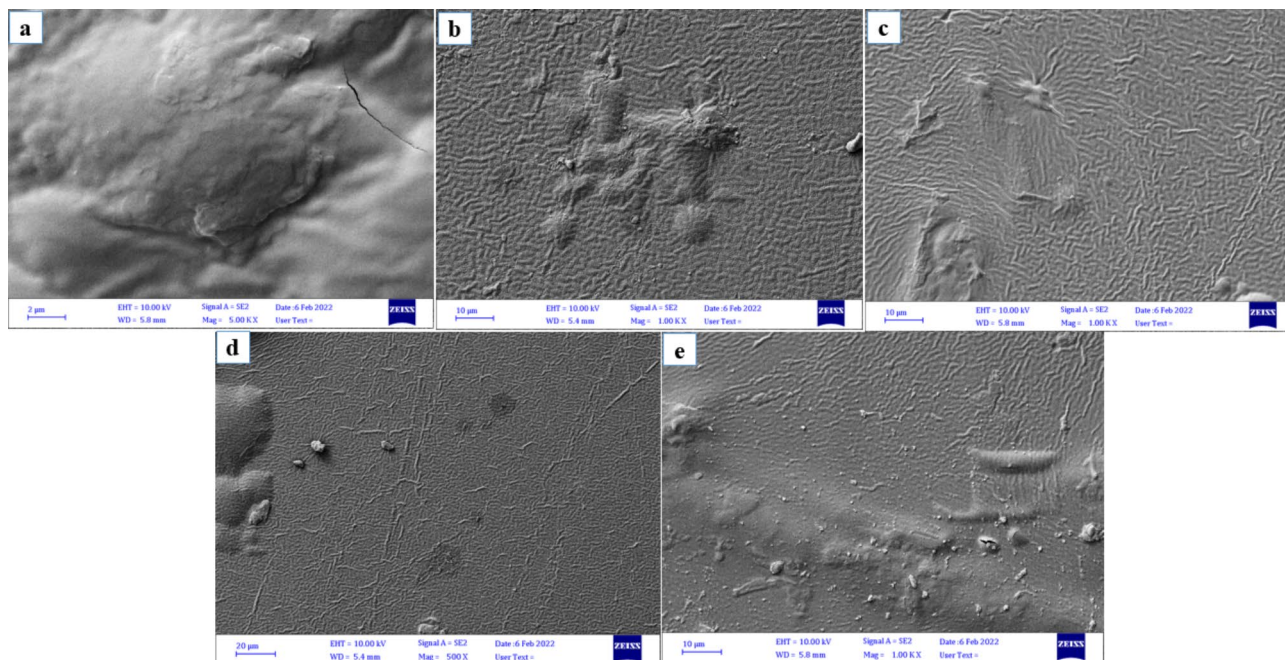


Fig. 5. The results of the morphology of the indicator films. (a) Co: control sample; (b) T_1 : indicator film containing 0.5% PC; (c) T_2 : indicator film containing 1% PC; (d) T_3 : indicator film containing 1.5% PC, (e) T_4 : indicator film containing 2% PC.

Thickness test

The results of the thickness of the films indicated that the different treatments did not have a statistically significant difference in terms of thickness (0.01 ± 0.37 mm) ($P > 0.05$).

Moisture test

The results showed that in different treatments, there was a statistically significant difference in terms of humidity ($P < 0.05$). The highest moisture content of the control sample was $7.85 \pm 0.00\%$, and treatments T_1 , T_2 , T_3 , and T_4 had 7.74 ± 0.14 , 7.62 ± 0.02 , 7.26 ± 0.20 , and $6.92 \pm 0.26\%$ moisture, respectively ($P < 0.05$). In general, adding PC significantly reduced the moisture content of the biofilm by 0.88 times compared to the control ($P < 0.05$).

The results of testing the mechanical properties of nanocomposites

Tensile strength of NC

The results showed that different treatments had statistically significant differences in terms of tensile strength ($P < 0.05$). The control sample had the lowest amount of elasticity at $323.81 \pm 8.25\%$, and treatments T_1 , T_2 , T_3 , and T_4 had 357.14 ± 14.29 , 404.76 ± 8.25 , 419.05 ± 8.25 , and $423.81 \pm 16.50\%$ elasticity, respectively. In general, the addition of PC significantly increased the ability to stretch the biofilms by 1.30 times compared to the control ($P < 0.05$). However, no statistically significant difference was observed in different biofilms containing PC ($P > 0.05$).

Stretch ability of NC

The results showed that different treatments showed a statistically significant difference in terms of tensile strength ($P < 0.05$). The control sample had the highest tensile strength of 0.00 ± 0.97 N/cm², and treatments T_1 , T_2 , T_3 , and T_4 had tensile strengths of 0.93 ± 0.00 , 0.93 ± 0.00 , 0.94 ± 0.00 , and 0.93 ± 0.00 N/cm², respectively ($P < 0.05$). The addition of PC significantly reduced the biofilms' tensile strength to 0.95 ($P < 0.05$). However, different biofilms containing PC showed no statistically significant difference ($P > 0.05$).

The results of the color index test of NC

The results of the changes in brightness (L^*), redness (a^*), and yellowness (b^*) of the indicator biofilms during 14 days of storage are shown in Table 2. According to the results, different treatments showed statistically significant differences in terms of L^* , a^* , and b^* index in the indicator biofilms during 14 days of storage ($P < 0.05$). The highest level of color indicators was related to the control sample ($P < 0.05$). The addition of PC in the formulation of the biofilms caused a significant decrease in the L^* , a^* , and b^* index of the indicator biofilms to 0.23/0.88 and 0.89, respectively compared to the control. ($P < 0.05$).

The color change (ΔE)

The results of the total color changes of the indicator biofilms during 14 days of storage are shown in Table 3. According to the results, different treatments showed statistically significant differences in terms of overall color

Brightness index (L^*)					
Time	T ₁	T ₂	T ₃	T ₄	T ₅
Day 0	77.03 ± 0.11 ^{Aa}	54.05 ± 0.46 ^{Ab}	52.61 ± 0.46 ^{Ac}	50.79 ± 0.53 ^{Ad}	50.35 ± 0.39 ^{Ad}
Day 1	77.00 ± 0.06 ^{Ba}	52.78 ± 0.61 ^{Bb}	51.69 ± 0.25 ^{Bc}	49.30 ± 0.22 ^{Bd}	48.72 ± 0.35 ^{Bcd}
Day 7	76.37 ± 0.02 ^{Ca}	50.98 ± 0.71 ^{Cb}	49.78 ± 0.22 ^{Cc}	47.97 ± 0.15 ^{Cd}	47.45 ± 0.48 ^{Cd}
Day 14	76.08 ± 0.21 ^{Da}	48.73 ± 0.22 ^{Db}	47.42 ± 0.71 ^{Dc}	46.98 ± 0.39 ^{Dd}	44.72 ± 0.24 ^{De}
Redness (a^*)					
Time	T ₁	T ₂	T ₃	T ₄	T ₅
Day 0	1.48 ± 0.19 ^{Ca}	13.27 ± 0.17 ^{De}	12.76 ± 0.25 ^{Cd}	11.21 ± 0.54 ^{Dc}	9.27 ± 0.54 ^{Db}
Day 1	1.71 ± 0.21 ^{Ba}	12.05 ± 0.21 ^{Ce}	11.69 ± 0.79 ^{Bd}	7.68 ± 0.50 ^{Cc}	6.05 ± 0.26 ^{Cb}
Day 7	1.70 ± 0.25 ^{Ba}	7.60 ± 0.22 ^{Bd}	6.76 ± 0.47 ^{Ac}	6.83 ± 0.14 ^{Bc}	5.60 ± 0.10 ^{Bb}
Day 14	1.98 ± 0.15 ^{Aa}	6.31 ± 0.25 ^{Ad}	6.03 ± 0.60 ^{Ac}	2.62 ± 0.18 ^{Ab}	2.15 ± 0.25 ^{Ab}
Yellowness (b^*)					
Time	T ₁	T ₂	T ₃	T ₄	T ₅
Day 0	3.57 ± 0.09 ^{Ca}	37.85 ± 0.76 ^{Cc}	39.46 ± 0.19 ^{Cb}	40.02 ± 0.37 ^{Dd}	42.69 ± 0.15 ^{Ce}
Day 1	3.75 ± 0.21 ^{BCa}	36.34 ± 0.76 ^{Bbc}	37.02 ± 0.76 ^{Bb}	39.45 ± 0.35 ^{Cd}	42.89 ± 0.30 ^{Ce}
Day 7	3.89 ± 0.29 ^{Ba}	34.17 ± 0.40 ^{Ac}	35.23 ± 0.12 ^{Ab}	38.64 ± 0.40 ^{ABd}	40.08 ± 0.17 ^{Be}
Day 14	3.91 ± 0.06 ^{Aa}	34.00 ± 0.12 ^{Ac}	35.15 ± 0.40 ^{Ab}	38.09 ± 0.17 ^{Ad}	38.30 ± 0.87 ^{Ad}

Table 2. The results of the average brightness index (L^* , a^* and b^*) of the biofilms during 14 days of storage. T₁: fish coated with PVA/NCH-NC without PC; T₂: fish coated with PVA/NCH/PC-NC (0.5% PC); T₃: fish coated with PVA/NCH/PC-NC (1% PC); T₄: fish coated with PVA/NCH/PC-NC (1.5% PC) and code T₅: fish coated with PVA/NCH/PC-NC (2% PC). Different small and capital letters indicate a significant difference in the column and row respectively ($p < 0.05$).

Time	T ₁	T ₂	T ₃	T ₄	T ₅
Day 0	0.00 ± 0.00 ^{Ca}	0.00 ± 0.00 ^{Ca}	0.00 ± 0.00 ^{Ca}	0.00 ± 0.00 ^{Ca}	0.00 ± 0.00 ^{Ca}
Day 1	0.42 ± 0.01 ^{Bc}	1.80 ± 0.01 ^{Bb}	2.41 ± 0.01 ^{Bb}	4.90 ± 0.01 ^{Ba}	4.86 ± 0.01 ^{Ba}
Day 7	1.04 ± 0.22 ^{Ac}	8.17 ± 3.76 ^{Aab}	8.94 ± 0.27 ^{Ab}	9.75 ± 0.58 ^{Aa}	10.39 ± 0.57 ^{ABa}
Day 14	1.00 ± 0.35 ^{Ac}	8.06 ± 1.26 ^{Ab}	8.78 ± 0.03 ^{Ab}	9.21 ± 0.22 ^{Aa}	9.47 ± 0.20 ^{Aa}

Table 3. The results of the average overall color changes (ΔE) of the biofilms during 14 days of storage. T₁: fish coated with PVA/NCH-NC without PC; T₂: fish coated with PVA/NCH/PC-NC (0.5% PC); T₃: fish coated with PVA/NCH/PC-NC (1% PC); T₄: fish coated with PVA/NCH/PC-NC (1.5% PC) and code T₅: fish coated with PVA/NCH/PC-NC (2% PC). Different small and capital letters indicate a significant difference in the column and row respectively ($p < 0.05$).

changes of the indicator biofilms during 14 days of storage ($P < 0.05$). The inclusion of PC in the film formulation caused a significant increase in the overall color changes of the indicator biofilms ($P < 0.05$). An increase in color changes was observed in biofilms containing a higher amount of PC ($P < 0.05$). However, no significant difference in the amount of changes (ΔE) was observed in all treatments on days 7 and 14.

The results of determining color changes (ΔE) of indicator biofilms at different pHs

The results of the general color changes of the indicator biofilms at different pHs are shown in Table 4 and Fig. 6. According to the results, different treatments showed statistically significant differences in terms of overall color changes at different pHs ($P < 0.05$). The control sample, as expected, showed no color changes ($P > 0.05$). The inclusion of PC in the film formulation caused a significant increase in the color changes of the indicator biofilms ($P < 0.05$). The most color changes were observed at neutral pH ($P < 0.05$).

Results of *Oncorhynchus mykiss* tests

pH

The results of pH changes of fish samples covered with indicator biofilm showed that different treatments and duration of storage had a significant effect on the pH of fish fillets ($P < 0.05$). On the first day, no statistically significant difference was observed between different treatments ($P > 0.05$). From the 7th day of the experiment, a statistically significant difference was reported in the study groups ($P < 0.05$). A significant increase in the pH of fish samples during 21 days of storage was reported in all investigated groups ($P < 0.05$). However, adding PC slowed down the increase in pH of fish samples ($P < 0.05$). This decreasing trend was significant with an increasing percentage of PC on day 21 (6.39 ± 0.01 Ae01) ($P < 0.05$) (Table 5).

pH range	T ₁	T ₂	T ₃	T ₄	T ₅
pH ₁	0.00 ± 0.00 ^d	43.98 ± 0.96 ^c	44.15 ± 0.97 ^{bc}	45.38 ± 0.53 ^{ab}	46.22 ± 0.53 ^a
pH ₂	0.00 ± 0.00 ^d	48.75 ± 0.58 ^c	49.03 ± 0.58 ^{bc}	49.50 ± 0.26 ^b	50.56 ± 0.15 ^a
pH ₃	0.00 ± 0.00 ^d	36.55 ± 0.22 ^c	37.70 ± 0.20 ^c	41.19 ± 0.92 ^b	46.14 ± 0.71 ^a
pH ₄	0.00 ± 0.00 ^e	40.07 ± 0.51 ^d	41.29 ± 0.49 ^c	44.56 ± 0.65 ^b	49.63 ± 0.85 ^a
pH ₅	0.00 ± 0.00 ^d	42.29 ± 0.53 ^c	42.95 ± 0.51 ^c	47.84 ± 0.64 ^b	50.46 ± 0.23 ^a
pH ₆	0.00 ± 0.00 ^e	45.94 ± 0.05 ^d	47.24 ± 0.48 ^c	51.11 ± 0.49 ^b	53.04 ± 0.00 ^a
pH ₇	0.00 ± 0.00 ^e	49.47 ± 0.91 ^b	50.83 ± 0.91 ^b	53.32 ± 0.75 ^a	54.47 ± 0.47 ^a
pH ₈	0.00 ± 0.00 ^e	45.42 ± 0.22 ^d	47.11 ± 0.24 ^c	49.89 ± 0.92 ^b	51.58 ± 0.10 ^a
pH ₉	0.00 ± 0.00 ^c	45.19 ± 0.67 ^b	46.33 ± 0.67 ^b	51.58 ± 0.68 ^a	52.36 ± 0.87 ^a
pH ₁₀	0.00 ± 0.00 ^d	52.04 ± 0.57 ^c	55.84 ± 0.83 ^b	56.57 ± 0.81 ^b	58.06 ± 0.82 ^a
pH ₁₁	0.00 ± 0.00 ^e	50.96 ± 0.28 ^d	57.13 ± 0.68 ^c	60.74 ± 0.76 ^b	62.66 ± 0.76 ^a
pH ₁₂	0.00 ± 0.00 ^e	35.48 ± 0.31 ^d	41.10 ± 0.32 ^c	46.02 ± 0.18 ^b	47.85 ± 0.16 ^a
pH ₁₃	0.00 ± 0.00 ^d	26.07 ± 0.03 ^c	26.81 ± 0.29 ^c	32.98 ± 0.90 ^b	34.81 ± 0.88 ^a

Table 4. The average results of determining the color changes of indicator biofilms at different pH. T₁: fish coated with PVA/NCH-NC without PC; T₂: fish coated with PVA/NCH/PC-NC (0.5% PC); T₃: fish coated with PVA/NCH/PC-NC (1% PC); T₄: fish coated with PVA/NCH/PC-NC (1.5% PC) and code T₅: fish coated with PVA/NCH/PC-NC (2% PC). Different small and capital letters indicate a significant difference in the column and row respectively ($p < 0.05$).

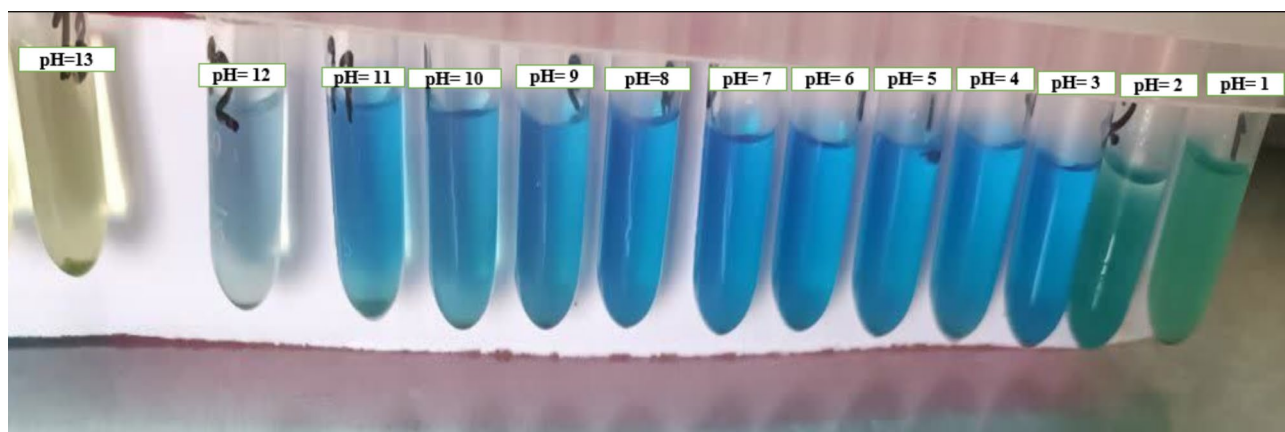


Fig. 6. The test to determine the color change of films at different pH.

Treatment	Day 0	Day 1	Day 7	Day 14
T ₁	6.02 ± 0.01 ^{Da}	6.25 ± 0.02 ^{Ca}	6.64 ± 0.03 ^{Ba}	7.02 ± 0.02 ^{Aa}
T ₂	6.02 ± 0.01 ^{Da}	6.21 ± 0.02 ^{Cb}	6.38 ± 0.03 ^{Bb}	6.67 ± 0.01 ^{Ab}
T ₃	6.02 ± 0.01 ^{Da}	6.14 ± 0.02 ^{Cc}	6.30 ± 0.03 ^{Bc}	6.55 ± 0.01 ^{Ac}
T ₄	6.01 ± 0.01 ^{Da}	6.10 ± 0.01 ^{Cd}	6.35 ± 0.02 ^{Bb}	6.45 ± 0.02 ^{Ad}
T ₅	6.01 ± 0.01 ^{Da}	6.13 ± 0.01 ^{Cd}	6.27 ± 0.01 ^{Bc}	6.39 ± 0.01 ^{Ae}

Table 5. Average pH results of fish fillets covered with indicator biofilms during 14 days of storage. T₁: fish coated with PVA/NCH-NC without PC; T₂: fish coated with PVA/NCH/PC-NC (0.5% PC); T₃: fish coated with PVA/NCH/PC-NC (1% PC); T₄: fish coated with PVA/NCH/PC-NC (1.5% PC) and code T₅: fish coated with PVA/NCH/PC-NC (2% PC). Different small and capital letters indicate a significant difference in the column and row respectively ($p < 0.05$).

According to the Iranian National Standard No. 13314 ISIRI, the appropriate pH of fish should be in the range of 6.5–6. Therefore, the results of the T_2 , T_3 , and T_4 treatments up to day 21 showed that they were able to last 14 days longer than the control fish samples within the maintained range of the standard.

TBARS

The results of changes in thiobarbituric acid in fish samples covered with indicator biofilm showed that different treatments and storage duration had a significant effect on the amount of TBARS in fish fillets ($P < 0.05$). All investigated groups reported a significant increase in TBARS in fish samples during 21 days of storage ($P < 0.05$); however, the increase in fish coated with high concentrations of PC was reported at a slower rate. Therefore, on day 21, T_4 treatment had the lowest amount of TBARS (1.23 ± 0.04 ppm) with a significant difference (Fig. 7).

According to the Iranian national standard number 11242ISIRI, for raw fish, the amount of TBARS should be less than 1 ppm or 1 mg (mg MDA/kg) per kilogram of fish meat. Therefore, the approval of all treatments extends only to the 14th day.

TVB-N

The changes in TVB-N compounds in fish samples covered with indicator biofilm showed that the amount of these compounds in fish fillets changed significantly depending on the treatment and storage time ($P < 0.05$). In all investigated groups, there was a significant increase in the formation of TVB-N compounds in fish samples during 21 days of storage ($P < 0.05$). However, the increasing trend in fish coated with high concentrations of PC was reported at a slower rate, so that on the 21st day, T_4 treatment had the lowest amount of TVB-N compounds (19.53 ± 0.21 mg/100 g), with a significant difference (Fig. 8).

According to the Iranian national standard number 13866ISIRI for raw fish, the amount of TVB-N should usually be less than 20 to 30 mg of nitrogen per 100 g (mg/100g). Therefore, based on the obtained results, the national standard approves all treatments until the 21st day.

Microbial analysis results

The results of total mesophilic bacteria, psychrophilic bacteria, and Enterobacteriaceae

The total counting of mesophilic, psychrophilic, and enterobacteriaceae in fish samples covered with indicator biofilm revealed that different treatments and storage durations had a significant effect on bacterial analysis in fish fillets. ($P < 0.05$). A significant increase in the number of bacteria in fish samples during 21 days of storage was reported in all investigated groups ($P < 0.05$). However, the increase in the fish coated with a high concentration of PC was reported at a slower rate, so that on the 21st day, the T_4 treatment had the lowest amount of mesophilic, psychrophilic, and Enterobacteriaceae bacteria with a significant difference of 8.17 ± 0.02 , 7.90 ± 0.04 , and 60.67 ± 0.02 log cfu/g, respectively (Fig. 9).

According to the Iranian national standard number 2-5272, 1382 ISIRI for raw fish, the maximum number of numerical mesophilic bacteria is less than 7 (log107 CFU/g). According to the obtained results, treatments T_1 ,

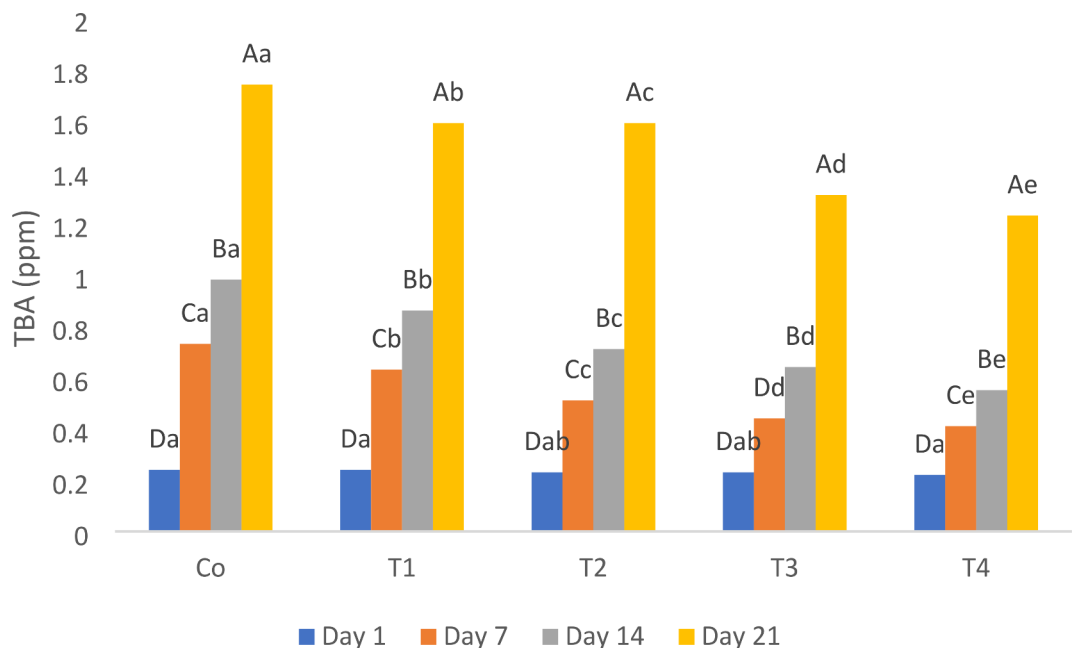


Fig. 7. The average results of thiobarbituric acid (ppm) of fish fillets covered with indicator film during 21 days of storage. Co: control sample/ T_1 : indicator film containing 0.5% phycocyanin; T_2 : indicator film containing 1% phycocyanin; T_3 : indicator film containing 1.5% phycocyanin; T_4 : indicator film containing 2% phycocyanin.

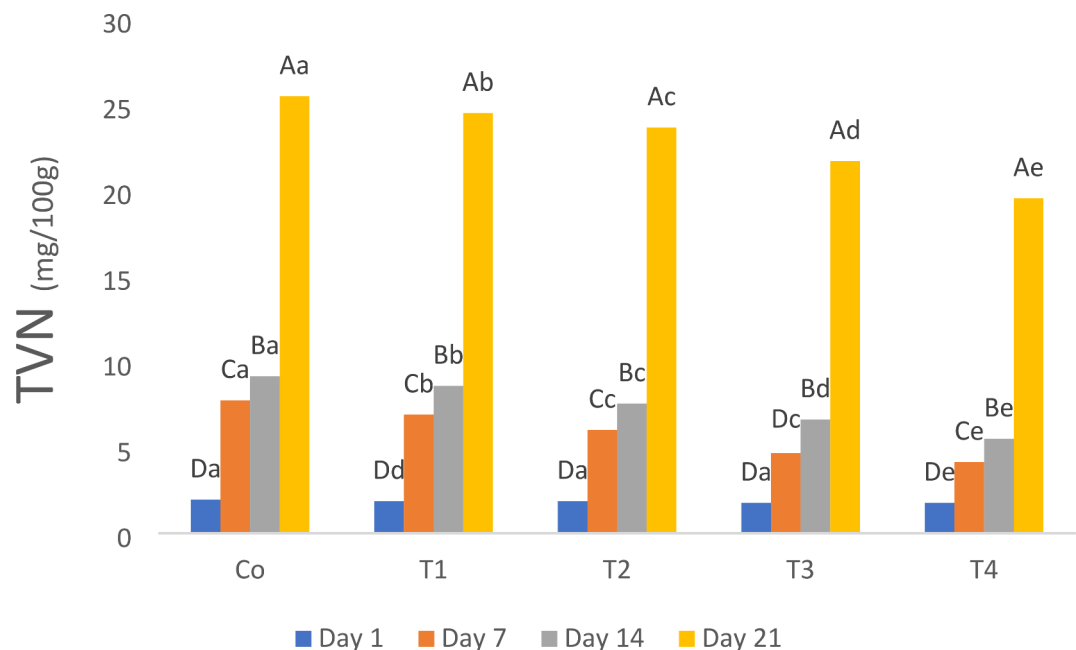


Fig. 8. The average results of total volatile nitrogen (mg/100g) of fish fillets covered with indicator film during 21 days of storage. Co: control sample/T₁: indicator film containing 0.5% phycocyanin; T₂: indicator film containing 1% phycocyanin; T₃: indicator film containing 1.5% phycocyanin; T₄: indicator film containing 2% phycocyanin.

T₂, T₃, and T₄ are approved until the 7th days and they were able to keep the fish samples in the standard range for 6 days more than the control.

According to the Iranian National Standard No. 1382-5624 for fresh fish and marine products, the limit of the number of psychrophilic bacteria is usually less than 6 (log₁₀ CFU/g). According to the obtained results, T₂, T₃, and T₄ treatments are approved until the 7th days and they were able to keep the fish samples within the standard range for 6 days more than the control.

According to the Iranian national standard number ISIRI 14224, 1382, for raw fish, the number of Enterobacteriaceae bacteria should usually be less than 3 (log₁₀ cfu/g). According to the obtained results, only the T₄ sample complies with the national standard until the seventh day and was able to maintain the standard range for 6 days more than the control fish samples.

Evaluation results of organoleptic properties

Aroma and odor

The results of sensory evaluation of the aroma and odor of fish samples covered with indicator biofilm showed that different treatments and duration of storage had a significant effect on the sensory evaluation of the fish fillets ($P < 0.05$). On the first day, no statistically significant difference was observed in the study groups ($P > 0.05$). However, from the 7th day, a statistically significant difference was observed in the studied groups ($P > 0.05$). A significant decreasing trend was reported in the sensory evaluation of the aroma and odor of fish samples during 21 days of storage in all the studied groups ($P < 0.05$). However, the decreasing trend in fish coated with high concentrations of PC was reported at a slower rate, so that on day 21, the T₄ treatment had the lowest amount (3.33 ± 0.58) with a significant difference (Fig. 10).

The sensory results of aroma and odor showed that until the seventh day, all the samples are approved. (Up to the number of 3 criteria is acceptable in the measurement, the results of the sense of smell show that until day 21, only the T₄ sample is approved). The rate of sensory acceptance of aroma and odor in the T₄ treatment was higher than the control for 14 days.

Color

The results of sensory color evaluation of fish samples covered with indicator biofilm showed that different treatments and duration of storage had a significant effect on the sensory color evaluation of fish fillets ($P < 0.05$). A significant decreasing trend was reported in the color sensory evaluation of fish samples during 21 days of storage in all investigated groups ($P < 0.05$). So, on the 21st day, T₄ treatment had the lowest amount (3.00 ± 0.00) with a significant difference (Fig. 11).

The color sensory results indicated that all samples received approval up until the seventh day. However, the T₄ treatment received approval until the 21st day, resulting in a 14-day increase in the acceptability rate compared to the control.

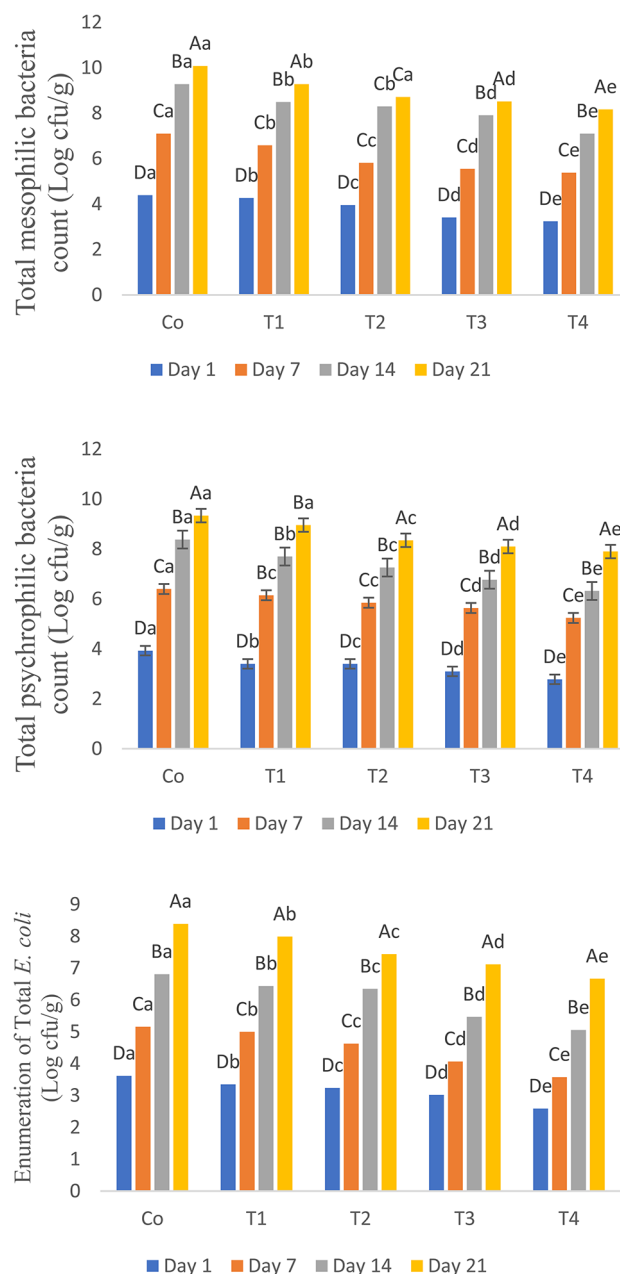


Fig. 9. Average results of psychrophilic mesophilic bacteria and Enterobacteriaceae (in Log cfu/g) of fish fillets covered with indicator film during 21 days of storage. Co: control sample/T₁: indicator film containing 0.5% phycocyanin; T₂: indicator film containing 1% phycocyanin; T₃: indicator film containing 1.5% phycocyanin, T₄: indicator film containing 2% phycocyanin.

Texture

The results of sensory evaluation of the texture of fish samples covered with indicator biofilm showed that different treatments and duration of storage had a significant effect on the sensory evaluation of the texture of fish fillets ($P < 0.05$). A significant decreasing trend was reported in the sensory evaluation of fish samples during 21 days of storage in all investigated groups ($P < 0.05$). However, the decreasing trend was reported in fish coated with high concentrations of PC at a slower rate, so that on day 21, T₄ treatment had the lowest amount (3.00 ± 0.00) with a significant difference (Fig. 12).

The texture results revealed that all the samples received approval up until the seventh day. However, the T₄ treatment received approval until the 21st day, resulting in a 14-day increase in the acceptability rate compared to the control.

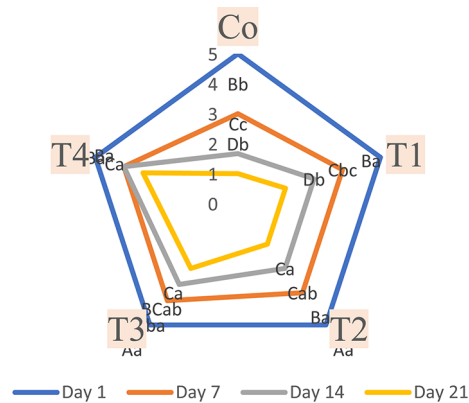


Fig. 10. The results of the average sensory evaluation of the aroma and odor of fish fillets covered with an indicator film during 21 days of storage. Co: control sample/T₁: indicator film containing 0.5% phycocyanin; T₂: indicator film containing 1% phycocyanin; T₃: indicator film containing 1.5% phycocyanin, T₄: indicator film containing 2% phycocyanin.

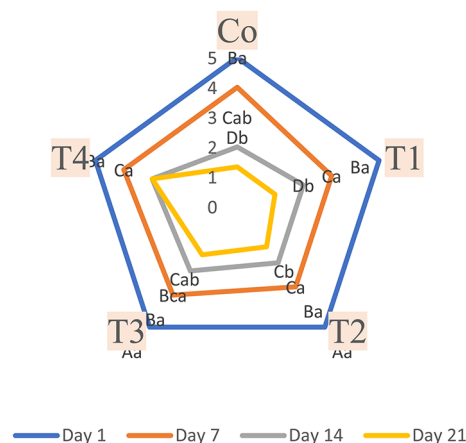


Fig. 11. The results of the average color sensory evaluation of fish fillets covered with indicator film during 21 days of storage. Co: control sample/T₁: indicator film containing 0.5% phycocyanin; T₂: indicator film containing 1% phycocyanin; T₃: indicator film containing 1.5% phycocyanin, T₄: indicator film containing 2% phycocyanin.

Overall acceptability

The results of sensory evaluation of overall acceptance of fish samples covered with indicator biofilm showed that different treatments and duration of storage had a significant effect on the amount of sensory evaluation of overall acceptance of fish fillets ($P < 0.05$). A significant decreasing trend was reported in the sensory evaluation of the overall acceptance of fish samples during 21 days of storage in all investigated groups ($P < 0.05$). However, the decreasing trend was reported in fish coated with high concentrations of PC at a slower rate, so that on day 21, T₄ treatment had the lowest with a significant difference (3.33 ± 0.58) (Fig. 13).

The overall acceptance sensory results indicated that all samples received approval until the seventh day. However, the T₄ treatment received approval until the 21st day, resulting in a 14-day increase in the acceptability rate compared to the control.

Discussion

Food packaging is crucial for food safety, quality, and durability; protecting food from environmental pollution, mechanical forces, and breakage; enhancing shelf life; and reducing waste, with recent advancements making it more efficient⁶⁴. Fish decomposition is linked to microbiological, chemical, and physical changes that occur during storage. Bacteria associated with fish decomposition proliferate, leading to protein breakdown and the subsequent formation of ammonia, indole, methanethiol, putrescine, trimethylamine, and other odorless chemicals. It is critical to effectively preserve fish using appropriate treatments⁶⁵. Researchers have found that combining microalgae extracts in seafood products can reduce microbial growth, increase oxidative stability, and protect sensory characteristics, thereby increasing shelf life. Cyanobacteria's colored compounds, such as polyphenols, phycobiliproteins, and vitamins, are highly effective water-soluble antioxidants. Antioxidant

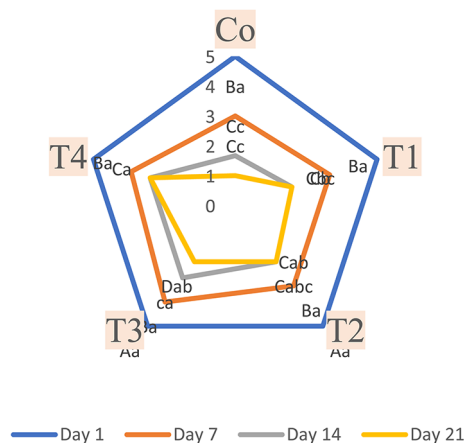


Fig. 12. The average results of sensory evaluation of the texture of fish fillets covered with indicator film during 21 days of storage. Co: control sample/T₁: indicator film containing 0.5% phycocyanin; T₂: indicator film containing 1% phycocyanin; T₃: indicator film containing 1.5% phycocyanin, T₄: indicator film containing 2% phycocyanin.

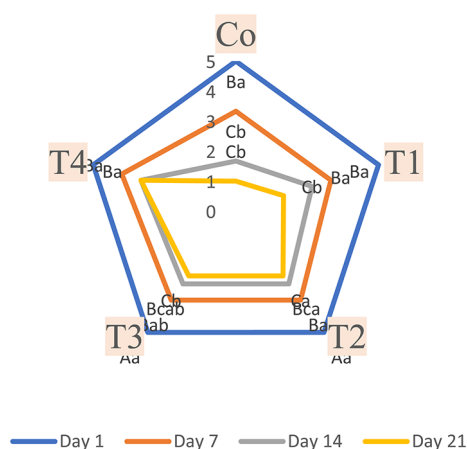


Fig. 13. Results of average sensory evaluation of overall acceptance of fish fillets covered with indicator film during 21 days of storage. Co: control sample/T₁: indicator film containing 0.5% phycocyanin; T₂: indicator film containing 1% phycocyanin; T₃: indicator film containing 1.5% phycocyanin, T₄: indicator film containing 2% phycocyanin.

compounds delay lipid oxidation by inhibiting free radical formation and propagation through various mechanisms, including species generation, chelation of metal ions, quenching of oxygen, breaking self-oxidative chain reactions, and decreasing local oxygen concentration⁶⁶. As a result, the purpose of this research is to develop an industrial *Oncorhynchus mykiss* biosensor based on PVA, NC, and PC derived from the cyanobacterium *Spirulina* sp. during refrigerator storage.

The antioxidant activity results indicated that the extracted PC from *Spirulina* sp. was 2.40 mg/g and 0.503 mg/g as determined by the DPPH and FRAP methods, respectively. Similarly, Gabr et al. (2020) demonstrated that the aqueous and ethanolic extracts of PC from *Spirulina* sp. exhibited antioxidant activities of 73.58% and 96.33%, respectively⁶⁷. Agrawal et al. (2021) demonstrated that the antioxidant activity, as measured by the DPPH and FRAP methods, was 158.3 µg/ml and 152.7 µg/ml, respectively⁶⁸.

The results of FTIR spectroscopy indicated that PC enhanced the intensity of the peaks in the PVA/NCH-NC polymer matrix. According to the results, all functional group stretching vibrations of C-H, C-O, and C-N bonds, as well as bending vibrations of O-H, N-H, C-H, and aromatic rings, have increased, with the exception of the stretching vibrations of O-H and N-H. This information is supported by studies conducted by Shahmoradi et al. (2021), Aworinde et al. (2020), and Das et al. (2017)^{69–71}. The micrographs of the control sample exhibited a smooth, uniform, and continuous morphology, devoid of tears, folds, or cracks. The results indicated that the control sample (PV/NCH) demonstrated a smooth surface, a homogeneous matrix with minimal pores and cracks, and maintained good structural integrity. The uniform dispersion of CH within the PVA matrix results in a homogeneous composition characterized by effective interfacial adhesion⁷².

The addition of 0.5% PC to the film-forming solution resulted in a uniform spherical structure on the film surfaces, with observable gaps between the spheres attributed to the PC inclusion (0.5%). The film surfaces displayed wrinkles that resembled a network structure, effectively facilitating the film's compression. The incorporation of PC at higher concentrations (1, 1.5, and 2%) resulted in a net-like morphology on the film's surface, suggesting a favorable compatibility of PC within the PV/NCH polymer matrix. This cross-linking interaction between PC and the polymer matrix may enhance the film's properties. The observed phenomenon is likely due to the arrangement of PV/NCH chains during film formation, which becomes more organized with the addition of PC, thereby serving as an effective biofiller for the polymer matrix. The increase in PC concentration from 0.5% to 2% resulted in a more distinct web-like structure on the film's surface⁴⁸.

The film thickness has a significant impact on various physical and mechanical properties, particularly mechanical characteristics and water vapor permeability⁷³. The study found no significant differences in the thickness of indicator films across different treatments, with all showing 0.37 mm. The discrepancy may be due to the low amount of PC used in the films⁵⁰. Similarly, Moghaddas Kia et al. (2018) demonstrated that the inclusion of PC derived from the microalga *Spirulina platensis* did not significantly influence the thickness of the degummed/sodium caseinate film, possibly due to its minimal concentration in the film formulation⁷⁴. However, Chenti et al. (2019) showed that adding PC to gelatin-based films, especially at concentrations of 6.25% and 12.5%, made the films much thicker than the control film, which was different from what this study found⁴⁸.

Understanding the moisture barrier performance of packaging films is critical, as they must sustain a specific moisture level in packaged products. If the packaging material is highly susceptible to moisture, exposure to a high relative humidity environment may alter its practical properties due to moisture absorption, potentially leading to qualitative changes and a reduction in the shelf life of the packaged product. Consequently, it is essential to ascertain the total dry matter or moisture content of films intended for food applications⁷⁵.

The moisture content analysis of indicator films revealed that the control sample had the highest moisture content. PC significantly reduced the film's moisture content, and the reduction in humidity was more pronounced with a higher percentage of PC. This resulted in rigid sections, stiffened matrix structure, and reduced moisture content across layers⁷⁶. Sun et al. (2023) investigated the effects of curcumin, PC, or modified lycopene dyes and showed a decrease in moisture content of whey protein-cellulose nanocrystal packing films⁷⁷.

The mechanical properties of packaging materials are crucial for protecting food from environmental factors. Understanding indicators like tensile strength and elongation percentage at rupture is essential for designing biodegradable films. These parameters dictate the quality of polymer layers suitable for food packaging. The film's ability to withstand various stresses during preparation, handling, and storage while preserving its integrity is crucial. The bonds and solubility of compounds, along with intermolecular interactions among polymer chains, influence the mechanical properties of nanocomposite films. The presence of integral compounds can cause structural changes in the film matrix, facilitating enhanced interactions between components⁷⁸.

The study found that the stretchability of edible film increases with tensile stress, indicating its flexibility. The addition of PC also significantly enhanced the maximum increase in film length from initial to breaking point. The study also revealed that the addition of PC significantly reduced the tensile strength of indicator films, with no significant difference observed across different films. The control sample exhibited the highest tensile strength, suggesting that PC functions as a plasticizer, enhancing chain entanglements and enhancing the stretchability of the indicator film⁷⁹. PC, similar to a plasticizer, can form hydrogen bonds, thereby interacting with polymers by disrupting polymer-polymer bonds and increasing the distance between polymer chains⁸⁰. Chenti et al. (2019) found that PC inclusion in an indicator film reduced its tensile strength and elasticity due to the decreased distance between chains. The decrease in free volume, molecular mobility, and flexibility of gelatin chains resulted in decreased tensile strength, which correlates with elasticity⁴⁸.

Color and opacity serve as significant indicators for assessing the physicochemical properties of bioactive films. The color characteristics of bioactive films serve as significant indicators of packaging materials, encompassing aspects such as appearance, functionality, and consumer acceptance. The color index L^* quantifies the extent of darkness and brightness, ranging from 0 to 100. The color index a^* quantifies the redness in samples, ranging from +120 (absolute red) to -120 (absolute green). A positive score signifies the presence of redness in the sample, whereas a negative score denotes a greenish hue. The positive values of the color index b^* reflect the extent of yellowness in the sample, while negative values denote the degree of blueness. This can be expressed as the absolute yellowness minus the absolute blueness, with a range from +120 to -120.

The study found that the addition of PC to indicator films significantly decreased the color indicators of brightness, redness, and yellowness. This decrease was more severe in higher amounts of PC. After 14 days of storage, the films showed a significant increase in overall color changes (ΔE). The results showed that adding PC to the films significantly reduced these indicators, with the control sample showing the highest level of change ($P < 0.05$). Compared to our research, Tie et al. (2024) discovered that adding PC to a polymer matrix of carboxymethyl chitosan and pectin made films less bright. This was true for both red/green and yellow/blue values. The film turned reddish brown and blue, with the highest value of a^* observed at 20 g/100 g of PC nanoemulsion, indicating a tendency towards green color instead of red⁸¹. Nami et al. (2024) also discovered that adding PC nanoliposomes to a soy protein film matrix made the film less bright over time, with 20 g/100 g being the highest value seen⁷⁶.

The analysis of color changes in the indicator films across various pH levels revealed that at pH 1 and 2, the samples exhibited a green color. In contrast, at pH levels exceeding 3, the films transitioned to blue, with the most pronounced color changes occurring within the neutral pH range. Altering the pH from the neutral range to pH = 13 resulted in a diminished intensity of color changes in the film. At pH levels 13, the biofilms exhibited a color change to pale yellow. The results of this study align with the findings of Li et al.'s (2021) research. Their

study demonstrated that incubating PC under acidic conditions (pH 4–8) diminished PC adsorption and led to a rapid degradation of the pigment exceeding 50% at approximately 620 nm⁸².

Changes in pH serve as a quality indicator for fish spoilage. The pH of live fish muscle typically ranges from 6.7 to 7. Following death, it can vary between 6 and 7, influenced by factors such as season and species⁸³. The pH levels of fish fillets covered with indicator film were measured over the course of 21 days. The control sample had the highest pH level, while adding PC lowered the pH levels of the fish samples. With an increased percentage of PC, the decreasing trend was more pronounced.

Spoilage bacteria, which produce compounds like trimethylamine, dimethylamine, and ammonia, as well as protein breakdown and volatile nitrogenous bases, are responsible for the gradual increase in pH. In fish meat, edible coatings containing bioactive compounds can prevent enzyme activity, microbial growth, protein decomposition, and alkaline compound accumulation⁸⁴. The presence of bioactive compounds also changes the permeability to carbon dioxide gas, with higher concentrations of PC increasing the concentration in the coating. This prevents the release of carbon dioxide gas, leading to the formation of carbonic acid and a decrease in the product's pH. After death, the pH of fish fillets ranges from 6 to 7, and the antibacterial and antioxidant properties of phycobiliproteins, which inhibit microorganism activity and oxidation, allow for their consumption within 21 days⁸⁵. Tavakoli et al. (2023) developed films incorporating anthocyanin and phycobilin derived from cyanobacteria to inhibit spoilage in carp fish. Their findings indicated a gradual increase in pH over time, with the inclusion of anthocyanin and phycobilin pigments significantly enhancing this trend⁸⁶. Stejskal et al. (2020) demonstrated that PC extract from *Spirulina platensis* incorporated into the gelatin film matrix significantly inhibited the rise in pH of the samples. The increase in fish muscle pH signifies the accumulation of alkaline compounds, including ammonia and volatile amines such as trimethylamine, primarily resulting from microbial activity⁸⁷.

The TBARS is a method for detecting lipid oxidation, primarily involving malondialdehyde (MDA) and hydroperoxides, which are the primary reaction products of polyunsaturated fatty acids with oxygen. Fish and fish products are rich sources of long-chain polyunsaturated fats, especially omega-3 fatty acids, which are highly susceptible to oxidative degradation. Lipid oxidation is a destructive reaction that limits the acceptability and quality of seafood food products. It is carried out through a free radical chain mechanism called self-oxidation, which involves three stages: initiation, propagation, and termination. This process can be influenced by natural enzyme systems, metal catalysis, solar or thermal energy, and non-radical and radical oxygen species⁸⁸. The study discovered that the control sample had the most thiobarbituric acid. Adding PC at 1.5% and 2% levels greatly decreased the amount of TBARS. The lower levels of TBARS in samples that contained PC are due to the presence of phenolic and terpene compounds in the sample⁸⁹. The ability of biofilms containing biological compounds to act as a barrier to oxygen diffusion is critical in mitigating lipid oxidation. During 21 days of storage, fish samples showed a significant increase in TBARS, possibly due to increased free iron and peroxides in fish muscle. Fat oxidation produces unstable hydroperoxides, which can cause unpleasant taste and smell. Oxygen attacks on unsaturated fatty acids can initiate free radical chain reactions⁹⁰. The amount of TBA in freshly caught fish is between 3 and 5 mg MAI/kg, but the acceptable limit of TBARS is 5 to 8 mg MAI/kg in fish stored in ice⁸⁹. As a result, all of the examined fillets met the TBA limit within 21 days of storage. Balti et al. (2020) investigated edible coatings made from active exo-polysaccharides enriched with red seaweed extract (*Gracilaria gracilis*) to enhance shrimp storage in refrigeration. The study found that these coatings also reduced the increase in thiobarbituric acid levels in shrimp⁹¹.

Volatile total nitrogen serves as a critical indicator of seafood quality, as it correlates directly with microbial growth and the subsequent production of metabolic byproducts, including ammonia, trimethylamine, diethylamine, and methylamine. Volatile nitrogen, primarily composed of trimethylamine, dimethylamine, and ammonia, results from the decomposition of proteins and non-protein nitrogenous compounds by the natural enzymes of proteolytic bacteria⁹². The study found that the control sample had the highest amount of volatile nitrogen compounds, while PC added significantly decreased their formation, particularly with an increasing PC percentage. However, all investigated groups reported a significant increase in volatile nitrogen compound formation during 21 days of storage. The increase in the amount of total volatile nitrogen in the present study in all samples during the storage period can be related to the activity of spoilage bacteria. The high activity level of bacteria breaks compounds such as trimethylamine oxide, peptides and amino acids into volatile bases⁹³. The control sample had excellent quality within 14 days, while other samples had 21 days of shelf life. The lower TVN-B values of samples with PC may be due to a faster decrease in bacterial population or decreased capacity for oxidative deamination of non-protein nitrogen compounds. However, the antioxidant and antibacterial properties of chitosan in the film matrix should not be overlooked, as it inhibits bacteria activity and reduces TVN-B compound formation in fish samples⁹⁴. Özogul et al. (2021) showed that coating sardine fish fillets in a coating containing PC extracted from microalgae reduced the formation of TVN-B compounds compared to the control sample⁸⁹.

Meat's high perishability poses a significant challenge to its health and quality. To prevent spoilage, various storage methods like low temperatures, freezing, drying, salting, heating, canning, and preservatives have been employed. Researchers are now exploring edible coatings and films containing natural antimicrobial compounds to improve and maintain the quality of fish and its products, thereby increasing the shelf life of this valuable product⁹⁵. Antimicrobial food coatings reduce the surface contamination of food products through the gradual release of antimicrobial compounds and increase the shelf life of products⁹⁶.

Microalgae, a rich source of biopreservatives, play a crucial role in the seafood industry by increasing the shelf life of commercial products. These bioactive compounds can disrupt the integrity of bacterial cell membranes, leading to extensive leaching of vital ions and potentially cell death. The introduction of hydrophobic compounds from microalgae extracts with bacterial cell wall phospholipids causes this disruption⁹⁷. The cationic property of chitosan lets the NH₃ group on the glucosamine monomer interact with the microbial cell membrane

electrostatically. This causes intracellular component leakage, which may be what gives it its antimicrobial properties. Additionally, its selective permeability reduces oxygen transfer to meat and meat products, increasing stability and durability⁹⁸.

The results indicated that the incorporation of PC into fish samples coated with indicator film led to a significant decrease in the counts of mesophilic, psychophilic, and enterobacteriaceae. This reduction was more pronounced with higher percentages of PC in the film formulation. The International Committee for Determining the Microbiological characteristics of foods has established a limit for total bacterial load in raw fish at 7 log cfu/g⁹⁹. Consequently, all samples containing PC can be stored in the refrigerator for a duration of 7 days. The results obtained are consistent with the research of Devi et al. (2016), which showed the antimicrobial effects of *S. platensis* protein extract against bacterial pathogens of fish under in vitro conditions. The results of these researchers showed that bioactive compounds extracted by methanol/ethanol/acetone mixture (1:1:1) prevented the growth of *Proteus mirabilis*, *Bacillus pumilus* and *Staphylococcus aureus* in infected fish¹⁰⁰.

The consumption of fish and fish products is primarily influenced by sensory characteristics, with color, smell, taste, and texture identified as the key determinants of quality. Phycobiliproteins are extensively utilized as natural protein food colors owing to their bright coloration, availability, non-toxic protein characteristics, potential free radical scavenging ability, and water solubility¹⁰¹. The sensory evaluation of fish encompasses four factors: smell, color, texture, and overall acceptance. Each quality feature is scored on a scale from 1 to 5, where a higher score indicates a greater level of preference. The sensory evaluation of fish fillet samples revealed that sensory scores were highest on the first day of storage. However, during storage, aroma, color, texture, and general acceptability scores decreased significantly. The addition of PC to the polymer matrix maintained the samples' organoleptic quality, with sample T₄ (containing 2% PC) having the highest sensory evaluation score. In line with the obtained results, Nowruzi et al. (2024) also showed that coating rainbow salmon fillets with phycoerythrin (PE) increased the organoleptic properties of the samples¹⁰¹.

Conclusion

Fish is the main source of unsaturated fatty acids and high-quality proteins for humans. However, it is highly vulnerable to oxidation and microbiological deterioration. As a result, this study aimed to produce an industrial fish freshness biosensor based on PVA/NCH/PC-NC during storage in the refrigerator. Spectroscopy (FTIR) results showed that phycocyanin (PC) made the peaks of the PVA/NCH-NC polymer matrix more intense. Based on the results, except stretching vibrations of O-H and N-H, all the functional group of stretching vibrations of C-H/C-O/C-N bonds, bending vibrations of O-H/N-H/C-H, and bending vibration of aromatic rings has increased. Moreover, the results showed that higher concentrations of PC in PVA/NCH polymer matrix resulted in a net-like morphology on the film's surface, suggesting compatibility and potential enhancement of the film's properties due to cross-linking interactions. According to the results, the addition of PC reduced the moisture content and stretch ability of nanocomposites in by 0.88 and 0.95, respectively. However, the tensile strength of nanocomposites increased by 1.30 times in T₄ compared to the control during 14 days of storage. Moreover, the results showed that the addition of 2% PC on the PVA/NCH-NC film reduced the increase rate of pH, thiobarbituric acid, and total volatile nitrogen factors, displaying great potential in indicating the shrimp freshness and delaying spoilage. The results of color index test showed that brightness (*L**), redness (*a**), and yellowness (*b**) reduced by addition of PC in T₄ by 0.23, 0.88, and 0.89 during 14 days of storage compared to the control. The color change (ΔE) also increased with the addition of PC during 14 days of storage. The results of measuring pH, thiobarbituric acid (TBARS), and total volatile nitrogen (TVB-N) showed that according to the standard, the treatment of PVA/NCH/PC-NC (2% PC) increased the shelf life of fish fillets up to 14, 14, and 21 days, respectively. The results of mesophilic, psychophilic, and Enterobacteriaceae bacteria showed that the addition of 2% PC on the PVA/NCH-NC film could increase the bacterial level for additional 6 days compared to the control condition. Summarizing the results, we found that the T₄ treatment (PVA/NCH/PC-NC (2% PC)) was the most effective treatment in this research, improving the quality of coated fish fillet in terms of mechanical, microbial, and sensorial evaluation.

Data availability

All data generated or analysed during this study are included in this published article.

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The authors declare no competing interests.

Ethics and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. Authors did not do any experiments on humans and/or the use of human tissue samples.

Consent for publication

Not applicable.

Live vertebrates and/or higher invertebrates

This study is not on live vertebrates and/or higher invertebrates.

Images

All the images are original and not copy from other papers.

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