

Article

Preservative Effects of Flaxseed Gum-Sodium Alginate Active Coatings Containing Carvacrol on Quality of Turbot (*Scophthalmus maximus*) during Cold Storage

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Abstract: In this article, the effect of active coatings of flaxseed gum (FG) and sodium alginate (SA) containing carvacrol (CA) on the quality of turbot (*Scophthalmus maximus*) after storage at 4 °C for 18 days was evaluated. The experimental results showed that FG/SA-CA could effectively inhibit the growth of microorganisms. At the same time, FG/SA-CA reduced the value of odorous-related compounds including thiobarbituric acid reactive substances (TBARS), total volatile base nitrogen (TVB-N), and K values. The FG/SA-CA significantly delayed the oxidation of myofibrillar protein (MP) through controlling the development of carbonyl groups and maintaining a high content of sulfhydryl groups. Thus, FG/SA-CA inhibits the growth of spoilage microorganisms, maintains the structure of the protein, and extends the refrigerated shelf life of turbot.

Keywords: *Scophthalmus maximus*; carvacrol; active coating; cold storage; quality



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1. Introduction

Turbot (*Scophthalmus maximus*) is highly favored by customers due to its abundant nutritious content and good taste [1]. Different pre-treatment methods play a crucial role in quality changes during storage of turbot. However, turbot is perishable during cold storage owing to spoilage microorganisms. It is imperative to discover methods to impede the deterioration of turbot during cold storage. Scientists have looked to plant materials for the development of food preservatives over the past few years. Essential oils are volatile secondary metabolites of plants in the form of saturated or unsaturated hydrocarbons, alcohols, esters, aldehydes, ketones, ethers, and terpenes. Carvacrol (5-isopropyl-2-methyl phenol) is a monoterpenoid phenolic compound and considered to be a natural antibacterial and antioxidant agent. Carvacrol, as a broad-spectrum fungicide, is effective in preventing microbial infestation of fish during storage. It has been found as an indispensable and major acting component in both oregano and thyme oils [2]. The antibacterial and antioxidant properties of carvacrol can benefit foods without negatively affecting their senses. Chaparro et al. [3] found that combined carvacrol and chitosan on tilapia fillets inhibited the microbial growth during the refrigeration process, but reduced the evaluation of fish texture. Alves et al. [4] prepared carvacrol microcapsules that prolonged the storage duration of frozen salmon by 4–7 days and decreased the population of thermophilic and cryophilic bacteria during the preservation process in cold storage. However, carvacrol is sensitive to temperature, oxygen, humidity, and light, and the volatility and hydrophobicity of carvacrol

prevents it from maximizing its effects [5]. Therefore, carvacrol presents difficulty for direct use in food preservation.

Edible packaging materials refer to natural substances that can be digested and absorbed by human beings as the base material, such as protein, polysaccharide, plant cellulose and fat, etc. The force between the molecules of these substances enables the generation of a with a porous network structure of packaging materials. Active packaging technology is a means of maintaining the quality of food products by changing the storage environment in food packaging, such as by adding various gas absorbers and releasers, eliminating oxygen, carbon dioxide, and other gaseous liquids inside the package, controlling temperature and humidity, or by adding bacteriostatic agents [6]. The primary goals include prolonging the food's quality assurance time, intensifying the food's scent and flavor, and enhancing the overall cleanliness and safety of the food [7]. Active packaging can be categorized as absorption, release, and coating based on the method of controlling the factors affecting preservation. Active coatings can prevent oxygen penetration, water dissipation, and inhibit microbial growth [8]. There is evidence that embedding natural preservatives into active coatings could extend the quality guarantee period of fish [9–11]. Polysaccharides are particularly excellent ingredients for making active coatings. For example, sodium alginate (SA) has been frequently used as a material for reactive or thin reactive coatings due to its excellent film-forming properties [12]. Pei et al. [13] successfully formulated gum tragacanth-SA-based active coatings containing epigallocatechin gallate and applied it to the preservation of *Larimichthys crocea*. However, the relatively poor water resistance and antioxidant activity of SA active coatings limit their application in food packaging. Flaxseed gum (FG) is a type of natural functional colloid with gel, emulsification, and thickening properties [14]. It can improve the hardness and ductility of the active coating [15]. The preservatives used on the surface of food are less effective against foodborne pathogenic microorganisms because preservatives quickly spread into food and denature with food composition [16]. Active coatings facilitate a gradual and uninterrupted long-term transfer from packaging materials to food surfaces.

Therefore, based on the study of Fang et al. [17], this research focuses on the effect of different concentrations of carvacrol FG-SA active coating on the refrigerated freshness of turbot.

2. Materials and Methods

2.1. Processing of Carvacrol Active Coatings

When making carvacrol emulsion, improvements were made on the basis of Fang et al. [18] to improve the stability of the emulsion. Carvacrol, at concentrations of 0.05%, 0.10%, and 0.20%, was mechanically mixed with 7.5 g β -cyclodextrin (β CD) and 50 g Tween 80 in a beaker to achieve a homogenous dispersion. Subsequently, 400 mL of ultra-pure water was added and agitated for a duration of 8 h at ambient temperature. The carvacrol/ β CD emulsions contained carvacrol at concentrations of 0.75, 1.50, and 3.00 μ L/mL. FG (0.5% *w/v*), glycerol (1.5% *v/w*) and SA (viscosity 200 ± 20 mPa, 1.5% *w/v*) were added to the prepared carvacrol/ β CD emulsions at a temperature of 45 °C. Afterwards, this was followed by the addition of ultrapure water to the mixture, which resulted in the final emulsion volume being equal to 1 L. Next, the emulsions were agitated for 2 h and then the mixture was made uniform by subjecting it to ultrasonic homogenization using an Ultrasonic operating at a frequency of 20 KHz and a power of 600 W for 20 min. This process resulted in the formation of coating solutions that were consistent throughout. Finally, the FG/SA solutions with carvacrol concentrations of 0.05%, 0.10%, and 0.20% were designated as FG/SA-5CA, FG/SA-10CA, and FG/SA-20CA, respectively.

2.2. Preparation of Turbot and Immersion Sample Treatment

Seventy-two turbot (500 $\pm$ 10 g) were purchased from Luchaogang Market in Shanghai, China. Afterwards, market workers carefully removed the head, internal organs, skin, and bones, leaving the dorsal muscle of the turbot. They were then quickly transported on ice

to the lab within 1 h. Subsequently, the specimens were meticulously cleansed using a 0.85% NaCl solution. The 72 turbot were randomly divided into 4 groups, with each group consisting of 18 turbot: (i) CK (uncoated); (ii) FG/SA-5CA (coated with FG/SA-5CA active coating solution); (iii) FG/SA-10CA (coated with FG/SA-10CA active coating solution) and (iv) FG/SA-20CA (coated with FG/SA-20CA active coating solution). Turbot samples from each batch were submerged in a coating solution at a ratio of 1:3 (*w/v*) for 10 min at 4 °C. A sterile biochemical incubator was used to generate the coating, and the samples were held at 4 °C for 60 min. Fish samples were removed from storage for analytical purposes on days 0, 3, 6, 9, 12, 15, and 18.

2.3. Bacteria Analysis

A representative 5 g sample of turbot dorsal muscle from each group was homogenized in 45 mL of saline solution, followed by a series of gradient dilutions. Then, 100 µL of bacterial solution (*Psychrophilic*, *Pseudomonas* spp., H₂S-producing, and lactic acid bacteria) were added to the muscle/saline medium. *Psychrophilic* bacteria were cultured on plate agar medium. *Pseudomonas* spp. were cultured on cetrimide agar medium. The H₂S-producing bacteria were cultured on iron agar medium and appear as black colonies. Lactic acid bacteria were cultured on MRS medium. *Psychrophilic* bacteria were counted after 7 days of incubation at 4 °C, and the remaining bacterial strains were cultured at 30 °C for 48 h before counting. The counts of all species of bacteria were calculated as lg CFU/g [19].

2.4. Total Volatile Basic Nitrogen (TVB-N) Analysis

Representative 5 g samples of turbot dorsal muscle from each of the four experimental groups were taken for determination of TVB-N values using a Kjeltac analyzer (FOSS 8400, Hilleroed, Denmark). The results were expressed as mg N/100 g.

2.5. K Value Analysis

A representative 5 g sample of turbot dorsal muscle from each experimental group was added to 10 mL 10% perchloric acid (PCA) and homogenized. The supernatant was collected after centrifugation at 4 °C at 8000× *g* for 10 min. The precipitate was mixed with 10 mL 5% PCA solution, and then centrifuged again at 4 °C at 8000× *g* for 10 min, repeated twice, to extract the supernatant. All supernatants were combined before adding 15 mL of ultra-pure water. The pH of the supernatant was adjusted to 6.5 with 10 mol/L KOH solution, 1 mol/L KOH solution, 5% PCA solution, and 10% PCA solution. After standing for 30 min, the supernatant was transferred to a 50 mL volumetric flask and diluted to 50 mL with ultrapure water. It was filtered with a 0.22 µm filter membrane and then injected into a sample bottle for the determination of each *K* value. Using the method of Cen et al. [20], ATP-related compounds were measured by HPLC (Waters e2695, Milford, CT, USA) and calculated as follows:

$$K \text{ value} = \frac{Hx + HxR}{ADP + AMP + Hx + HxR + ATP + IMP} \times 100\%$$

In the formula, HxR denotes inosine; Hx denotes hypoxanthine; ATP denotes adenosine triphosphate; ADP denotes adenosine diphosphate; AMP denotes adenosine 5'-monophosphate; and IMP denotes adenosine monophosphate.

2.6. Thiobarbituric Acid Reactive Substances (TBARS) Analysis

TBARS values were determined using the method devised by Chen et al. [21]. A representative 5 g sample of smashed dorsal muscle of turbot from each group was added to 97.5 mL of distilled water and 2.5 mL of 4 M HCl. Then, a 5 mL aliquot of the fraction was mixed with 5 mL of thiobarbituric reaction reagent (consisting of 0.02 M TBA and 90% glacial acetic acid) and heated in boiling water for 35 min. After cooling, the absorbance of

the pink solution was quantified using a spectrophotometer at 532 nm and 600 nm. TBARS values are expressed as mg MDA/100 g. The formula is as follows:

$$\text{TBARS values (mg MDA/100 g)} = \frac{A_{532} - A_{600}}{155} \times 726$$

In the formula, A_{532} indicates the absorbance value of the solution at 532 nm; A_{600} indicates the absorbance value of the solution at 600 nm.

2.7. Myofibrillar Protein (MP) Extraction

The MP solutions were prepared with reference to the method described by Tan et al. [22], with some modifications. A representative 2 g sample of the dorsal muscle of turbot was selected and homogenized with 20 mL of 20 mmol/L cold buffer, with a buffer concentration of 20 mmol/L and 0.05 mol/L NaCl. The precipitate was obtained by subjecting it to centrifugation at 4 °C and $10,000 \times g$ for 15 min, which was repeated twice. The collected precipitates were combined with 20 mL of a cold sodium phosphate buffer solution (20 mmol/L, comprising 0.6 mol/L NaCl) and subjected to extraction at 4 °C for 2 h. Finally, the supernatants were collected by repeated centrifugation.

2.8. Measurement of Sulfhydryl Groups (SH) and Carbonyl Groups

The index was determined according to the method of Xu et al. [23]. The SH contents measured as $\mu\text{mol/g}$ of protein were determined using the extinction coefficient of 2-nitro-5-thiobenzoate (NTB). The carbonyl content was determined by 2,4-dinitrophenylhydrazine derivatization. The results are given as $\mu\text{mol/g}$.

2.9. Measurement of Ca^{2+} -ATPase Activity

A representative 5 g sample of turbot dorsal muscle from each experimental group was added to 18 mL of 0.85% NaCl saline solution and homogenized in an ice bath. The solution underwent centrifugation at a force of $2500 \times g$ for 15 min at 4 °C. The homogenized liquid was mixed with 0.85% NaCl solution in a ratio of 1:10. The quantitative determination of Ca^{2+} -ATPase was performed using a micro assay kit provided by the Nanjing Jianjian Bioengineering Institute (Nanjing, China). The measurement of the absorption spectra was conducted at a wavelength of 636 nm. The ultimate outcome was expressed in $\mu\text{mol/mg}$ protein.

2.10. Surface Hydrophobicity of Myofibrillar Protein (MP)

Dilutions of MP solution were prepared to provide final concentrations of 0.2, 0.4, 0.8, and 1.0 mg/mL. A 4 mL sample of diluted MP solution was combined with 20 μL of ANS (8 mmol/L) and kept in darkness for 10 min. A fluorescence spectrophotometer (F-7100, Hitachi, Tokyo, Japan), with a slit width of 10 nm, an emission wavelength of 470 nm, and a detection wavelength of 390 nm was used to measure the relative fluorescence intensity (RFI) of the mixes [22].

2.11. Statistical Analysis

The experiments were carried out in triplicate. The results are shown as mean $\pm$ standard deviation. The data were analyzed by SPSS 22.0 analysis of variance (ANOVA). Graphing the data was done using Origin 2018.

3. Results and Discussion

3.1. Microbiological Analysis

The growth of five different microorganisms in turbot during cold storage is illustrated in Figure 1. The TVC of turbot was 3.3 lg CFU/g at the beginning of day 0, reflecting that the turbot was of good quality. Figure 1 shows an increase in TVC for all samples. However, the TVCs of the samples treated by other concentrations of CA were significantly higher than that of FG/SA-20CA during the whole storage process ($p < 0.05$). The microbiologically

unacceptable limit is 7 lg CFU/g [24]. The CK, FG/SA-5CA, FG/SA-10CA, and FG/SA-20CA groups exceeded this limit on the 6th, 9th, 12th and 18th day, respectively.

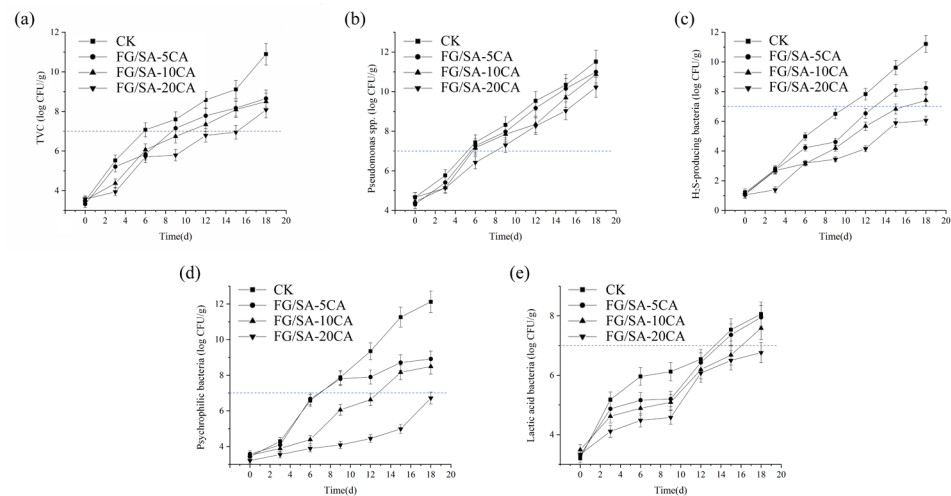


Figure 1. The growth trends of bacteria on turbot muscle with or without various concentrations of active coatings of FG/SA-CA during cold storage: total viable counts (a); *Pseudomonas* spp. counts (b); H₂S-producing bacteria counts (c); *Psychrophilic* bacteria counts (d); lactic acid bacteria counts (e).

The FG/SA-CA treatments showed a remarkable suppressive effect on bacteria ($p < 0.05$), and the inhibitory effect was more obvious at high concentrations. A lipid component of the bacterial cell wall may be penetrated by carvacrol, which then allows it to invade the bacterium. This changes the protein structure of the bacteria, causing it to die [25]. FG/SA-CA treatment showed similar inhibitory effects on the production of *Pseudomonas* spp., *Psychrophiles* bacteria, H₂S-producing bacteria, and lactic acid bacteria in turbot. The numbers of *Psychrophiles* in the FG/SA-10CA and FG/SA-CA samples were lower than that in CK, demonstrating that carvacrol effectively slowed down the growing process of *Psychrophilic* bacteria.

Pseudomonas spp. is a kind of bacteria that lives and flourishes in the presence of oxygen. It is one of the causes of spoilage of turbot during refrigerated storage [26]. The addition of carvacrol to the active coating decreased the number of *Pseudomonas* spp., suggesting that the colonization of aerobic spoilage bacteria is the primary factor in the deterioration of turbot that occurs during cold storage. The H₂S-producing bacteria are also a type of special spoilage bacteria in turbot during cold storage [27]. Bacterial counts for H₂S producing bacteria prior to the start of storage were 1.1 lg CFU/g. During refrigeration, turbot treated with carvacrol reduced the growth of H₂S-producing bacteria. Lactic acid bacteria generated organic acids in turbot during cold storage [28]. *Psychrophilic* bacteria produce metabolic chemicals such as volatile sulfides, ketones, biogenic amines and aldehydes that deteriorate the odor, texture and flavor of turbot. Following the conclusion of the storage period, the number of bacteria in the FG/SA-5CA, FG/SA-10CA and FG/SA-20CA treated groups decreased by 2.3%, 11.3%, and 13.7% compared to that of CK, respectively. Therefore, FG/SA-CA is effective in inhibiting the growth of these bacteria, and FG/SA-20CA has the strongest inhibitory effect. For this reason, FG/SA-20CA had the best effect of delaying the spoilage of turbot fillets. This is similar to the findings of Li et al. [29], who found that microencapsulated eugenol emulsion treatment of perch was effective in inhibiting microbial growth during refrigeration.

3.2. TVB-N Analysis

TVB-N indicates the content of volatile basic compounds and is one of the main indexes to determine the freshness in turbot [30]. Many basic volatile compounds are formed during the decomposition of proteinaceous and nonproteinaceous nitrogenous compounds. During cold storage, the spoilage bacteria degraded the proteins in the fish

muscle and accumulated rapidly. As shown in Figure 2a, the turbot sample had a TVB-N value of 11 mg N/100 g on day 0. This indicates that the turbot samples were in a fresh state before treatment. In the above analysis, turbot also had a low initial TVC value, both of which testified to its freshness. During cold storage, TVB-N values increased due to bacterial growth and multiplication. The allowable threshold of TVB-N is 25 mg N/100 g [26] for marine fish. The turbot in the CK sample exceeded the acceptable limits for TVB-N values after 12 days of storage. Notably, all turbot treated with FG/SA-CA had lower TVB-N values. In particular, these values in the FG/SA-10CA and FG/SA-20CA groups remained below the limit throughout the cold storage. These two active coatings could therefore lead to reduced spoilage. This indicates that FG/SA-C inhibits microbial growth and slows down protein breakdown and amine production. Additionally, the TVB-N value of turbot treated with a high concentration of carvacrol was lower. The FG/SA-5CA group enabled a longer shelf life of about 4 d, and the FG/SA-10CA and FG/SA-20CA groups extended the shelf life by about 6 d. This is analogous to the findings of Li et al. [29], who found that TVB-N values were reduced when sea bass were treated with high concentrations of eugenol.

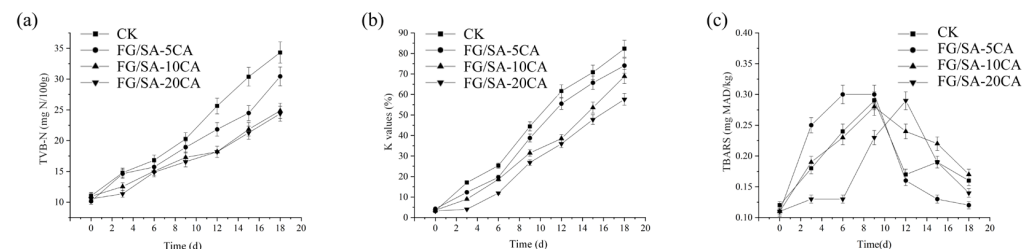


Figure 2. Changing trends in TVB-N (a), K values (b), and TBARS value (c) of turbot during cold storage.

3.3. K Values Analysis

The K values calculated from ATP catabolism are indispensable indicators for determining the spoilage of turbot [31]. The level of rejection by the K value is 60% [32]. Figure 2b illustrates the variations of ATP-related compounds during the cold storage period in turbot. As the duration of storage increases, the K values of turbot increased from 3.21% (d 0) to 80.25% (day 18) for the CK sample. By the end of cold storage, K values did not exceed 60% on day 18 in the FSG/SA-20CA group, but ranged from 60 to 80% in the FSG/SA-10CA and FSG/SA-5CA groups. The higher concentrations of FSG/SA-CA inhibited bacterial activity and nucleotide degradation, further delaying the increase in K values with storage time, which supports the conclusion of the TVB-N experiments. This result is also consistent with the colony count trends. It was demonstrated that FG/SA-CA could prevent the degradation of ATP controlled by enzymes and microorganisms, and could maintain good quality turbot during cold storage. This is analogous to the findings of Bazargani et al. [33], who investigated the effect of adding resveratrol to a sodium alginate coating on rainbow trout fillets during cold storage. They found that this treatment can effectively slow the increase of K values.

3.4. TBARS Value Analysis

TBARS represents the level of lipid oxidation. Elevated lipid oxidation levels result in the heightened buildup of lipid peroxides and the subsequent production of related secondary metabolites [34]. While turbot body fat content is low, the proportion of unsaturated fatty acids is high, and fat oxidation and acid produce odor and reduce food quality. Malondialdehyde (MDA) is one of the important oxidation products of polyunsaturated fatty acids [30]. Accumulation of MDA content reflects the increase in TBARS content during cold storage. The MDA content of the turbot sample on day 0 was 0.12 mg MDA/100 g (Figure 2c). The TBARS value exhibited an initial rise followed by a subsequent drop in all samples. On the 6th day, the FG/SA-CA groups possessed lower TBARS

values in comparison to the CK group, indicating that the FG/SA-CA inhibited fatty acid oxidation. The antioxidant effect of carvacrol caused a reduction in lipid oxidation and a decrease in the oxygen permeability of fish lipids [35]. Kostaki et al. [36] suggested that since MDA had the potential to interact with other components in fish such as furfural, alkyl aldehyde, alkenal, ketones, and carbohydrates, thus creating secondary metabolites, the MDA content in fish decreases with storage. The other reason could be attributed to the interaction between MDA and other volatile compounds [37]. However, the TBARS values were consistently low and irregular, so the results obtained did not reflect the oxidation of turbot lipids satisfactorily.

3.5. Changes of Residual Groups in Amino Acid Side-Chains

3.5.1. Total SH Counts Analysis

It is possible to determine the oxidation of proteins during fish preservation by analyzing the levels of sulfhydryl (SH) and carbon groups. The SH groups are amongst most dynamic functional groups of protein [38]. They are easily oxidized to disulfide bonds and other oxidation products as storage time increases. It is the usual practice to take into account variations from the SH groups when determining the oxidation levels of proteins found in marine commodities [39]. As depicted in Figure 3a, the total SH counts were considerably reduced across all experimental groups throughout the storage period ($p < 0.05$). The initial SH values in the CK, FG/SA-5CA, FG/SA-10CA-CA, and FG/SA-20CA groups were approximately 41.96, 41.46, 42.32, and 41.75, respectively. The initial SH counts of CK, FG/SA-5CA, FG/SA-10CA, and FG/SA-20CA groups on the 18th day decreased by 42.12%, 47.93%, 35.52%, and 33.50%, respectively. It is noteworthy that the SH values of the CK group were significantly lower than those of the FG/SA-CA-treated groups at the same storage time ($p < 0.05$), and were particularly significant for the FG/SA-20CA group ($p < 0.05$). In addition, previous research has shown that several SH groups formed as a result of increased interactions within and between proteins caused by environmental and extrinsic energy changes [40]. The results of SH content were very similar to those of Pei et al. [41], who found that epigallocatechin gallate had a protective effect on the side chain groups of myofibrillar protein.

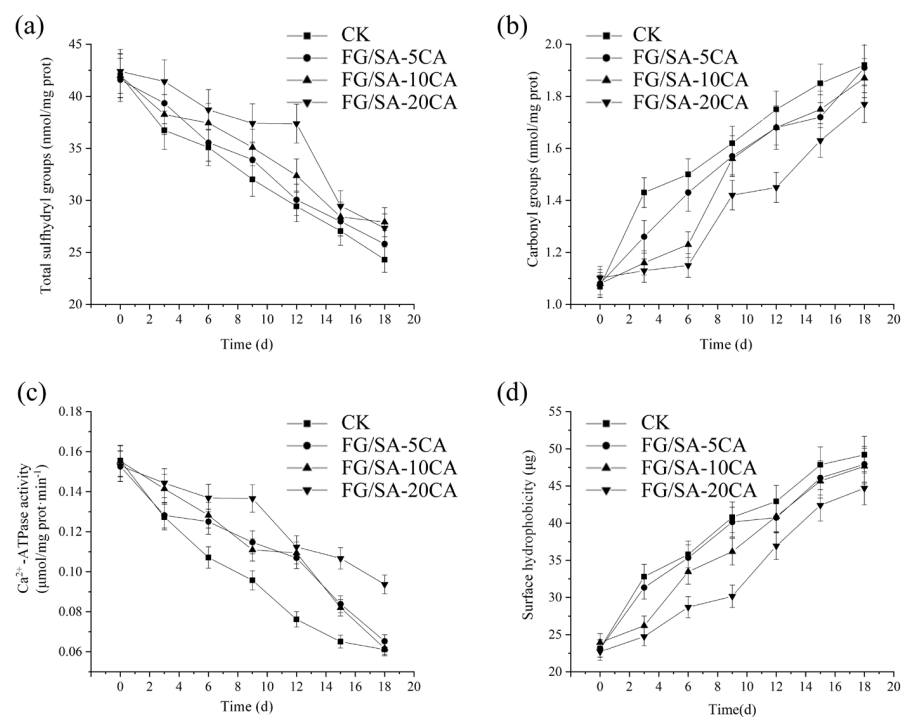


Figure 3. Changing trends in total sulfhydryl counts (a), carbonyl counts (b), Ca^{2+} -ATPase activity (c) and surface hydrophobicity (d) of turbot during cold storage.

3.5.2. Carbonyl Counts Analysis

The carbonyl groups are the primary chemical byproducts of MP oxidation. The oxidation of MPs causes backbone splitting and cross-linkage, resulting in a number of amino acid residues to turn into carbonyl groups [42]. As depicted in Figure 3b, the carbonyl content of all substances rose as the storage duration increased, suggesting that MP oxidation occurred during cold storage. The carbonyl groups in the CK group increased rapidly from 1.07 nmol/mg protein at day 0 to 1.92 nmol/mg protein at the end of storage. However, in the FG/SA-20CA group, the carbonyl groups were measured as 1.77 nmol/mg protein at the end of storage, showing that the FSG/SA-CA active coatings protected MPs from oxidation. Chamba et al. [43] previously reported an analogous outcome; discovering that there were no major differences in the carbonyl groups between FG/SA-5CA and FG/SA-10CA ($p > 0.05$). Similarly, Zhao et al. [42] found that the effect of grape seed extract on the carbonyl group was not concentration dependent. It can be inferred that concentration of the antioxidants being used has an inverse relationship with the carbonyl content of the sample.

3.6. Ca^{2+} -ATPase Activity Analysis

The activities of Ca^{2+} -ATPase could reflect the oxidative decomposition of MP [44]. Figure 3c shows the trend of Ca^{2+} -ATPase activity in each experimental group. Ca^{2+} -ATPase activity on day 0 was approximately $0.152 \mu\text{mol/mg protein} \cdot \text{min}^{-1}$ (Figure 3c). At the end of the storage period, the Ca^{2+} -ATPase activity of CK, FG/SA-5CA, FG/SA-10CA, and FG/SA-20CA groups increased by 74.54, 73.63, 69.54, and 60.45%, respectively. The present study showed that carvacrol was protective against Ca^{2+} -ATPase activities, probably due to the inhibition of oxidative denaturation of myosin by the active coatings with the addition of carvacrol, similar to the results of the carbonyl groups. Through their research, Reza et al. [45] found that even minute alterations to the structure of the MP led to a reduction in the activity of Ca^{2+} -ATPase. It is possible that the function of Ca^{2+} -ATPase decreases as a result of the aggregation of myosin and the oxidation of the head SH bond. The inhibition of Ca^{2+} -ATPase function was associated with the modification of protein structure resulting from the creation and interconnection of disulfide bonds within or between polypeptides [46]. He et al. [47] found that eugenol had a certain inhibitory effect on proteases, which could slow down alteration in Ca^{2+} -ATPase activity.

3.7. Surface Hydrophobicity Analysis

The degree of protein denaturation could also be measured by surface hydrophobicity. Exposing hydrophobic groups results in an elevation of protein hydrophobicity, hence increasing the extent of MP denaturation [48]. The protein surface hydrophobicity increased with storage time (Figure 3d). This may be due to the slow dissociation rate of MP molecules during storage, resulting in the exposure of hydrophobic amino acid residues and the change of hydrophilic and hydrophobic groups [49]. On day 0, there was no significant difference in surface hydrophobicity between coated and uncoated proteins ($p > 0.05$). The surface hydrophobicity of MP in each group increased significantly with the prolongation of the storage term ($p < 0.05$). In contrast, the growth was slower in the carvacrol-treated group, probably due to the strong antioxidant properties of carvacrol that inhibited protein degradation [18]. This is analogous to the findings of Hu et al. [50], who treated beef with tea polyphenols, and found that the trend of increasing surface hydrophobicity was slowed down during cold storage. Therefore, turbot that has been treated with carvacrol has the potential to inhibit the exposure of some hydrophobic groups.

4. Conclusions

The results of the study showed that the FG/SA-CA active coating inhibits bacterial growth and reduces TVB-N, TBARS, and K levels, thus maintaining the high quality of turbot during cold storage and extending the quality guarantee period of food. The application of FG/SA-CA treatment can successfully suppress the oxidation of fish proteins

and the formation of alkaline compounds, such as amines, in the fish. The shelf life of turbot is greatly extended as a result of this treatment, which also helps to maintain the fish's distinctive freshness. The preservation effect was augmented as the carvacrol level in the film increased. In comparison with the CK group, the FG/SA-CA groups exhibited obvious protective functions against protein oxidation, preventing the addition of carbonyl counts and the reduction in SH counts and Ca^{2+} -ATPase activity. Furthermore, the composite coating treatment suppressed the enhancement of surface hydrophobicity and stabilized the protein structure. Overall, FG/SA-20CA indicated optimal preservation performance and prolonged the shelf life for turbot during cold storage.

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Conflicts of Interest: The authors declare no conflicts of interest.

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