

# Effect of Sodium Alginate Coating Incorporated with Epigallocatechin Gallate and Peanut Oil on Refrigerated Hilsa (*Tenualosa ilisha*) Chunks

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## Abstract

Hilsa, the national fish of Bangladesh, is highly perishable despite its high nutritional value. Low-temperature preservation, salting, and synthetic preservatives are used to extend the shelf life of hilsa. Nowadays, researchers are searching for safer alternatives. Edible coatings are a safe and effective method for extending the shelf life of fish. This research aims to assess the efficacy of a sodium alginate-based edible coating enriched with the antioxidant and antimicrobial agent epigallocatechin gallate (EGCG) and peanut oil for preserving the quality and extending the shelf life of refrigerated hilsa fish chunks (HFC). In the experiment, HFC samples were divided into four treatment groups: T1 (sodium alginate, 2%), T2 (sodium alginate 2% with peanut oil 1%), T3 (sodium alginate 2%, peanut oil 1%, and EGCG 0.2%), and a control group (CON) treated with distilled water. Samples were stored at  $4\pm 1$  °C and evaluated over 12 days for biochemical parameters, including pH, total volatile base nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), and microbial counts on tryptic soy agar (TSA) and violet-red bile agar (VRB). Initial pH values ( $5.73\pm 0.03$  to  $5.74\pm 0.04$ ) were not significantly different ( $p>0.05$ ), but T3 exhibited a significant increase by day 12 ( $6.54\pm 0.04$ ,  $p<0.05$ ). TVB-N levels showed that T3 remained within acceptable limits until day 9, while CON and T1 exceeded spoilage limits by day 6. TBARS values surpassed the oxidative rancidity threshold of 2 mg MDA/kg by day 6 in CON and day 9 in T1 and T2, while T3 remained acceptable until day 9. Sensory evaluation indicated that T3 maintained its quality longer than the other treatments. These findings suggest that sodium alginate coatings with EGCG and peanut oil effectively extend the shelf life of refrigerated HFC, offering a sustainable alternative to synthetic preservatives.

Keywords: edible coating, epigallocatechin gallate, hilsa, peanut oil, sodium alginate.

## Introduction

Hilsa fish (*Tenualosa ilisha*) is a culturally and nutritionally significant species in South Asia, particularly in Bangladesh, where it plays a vital role in the diet and economy. In the year of 2023-2024 its production was around 529,000 tonnes in Bangladesh, which account 75% of the global catch. Hilsa is well known for its high nutritional composition (moisture 63.83%, protein 19.85%, lipid 13.85%, ash 2.19%) (Majumdar et al., 2024). Biochemicals suggested that this fish species contains a long chain of  $\omega$ -3 (avg. 177.89 mg/100 g) and  $\omega$ -6 (avg. 391.96 mg/100 g) polyunsaturated fatty acids (PUFA) while myristic acid (C14:0) is the predominant saturated fatty acid (avg. 684.67 mg/100 g) (Acharya et al., 2022). PUFA provides numerous health benefits, including reduced inflammation and improved cardiovascular health (Pal et al., 2011). Despite its high nutritional value, hilsa is highly perishable due to the combined effects of

microbial growth, enzymatic activity, and post-harvest chemical reactions (Alam et al., 2012). The development of undesirable odor, flavor, and texture found in spoiled hilsa decreases market value, which demands processing and preservation to minimize this loss. Hilsa is commonly preserved by icing, freezing, salting, or salt fermentation to a large extent, and reports on vacuum packaging and canning at a laboratory scale (Khatun et al., 2021; Habib et al., 2025). Recently, synthetic preservatives have been preferred to minimize post-mortem loss in fish. Nowadays, shelf-life extension of food by the application of edible coatings made from natural polysaccharides or proteins is attracting researchers' attention.

Edible coatings made from biopolymers such as sodium alginate, chitosan, and others are effective in extending the shelf life of fish, shrimp, and other seafood (Dehghani et al., 2018). Edible coatings provide a natural barrier against moisture loss, microbial growth, and oxidation (Gull et al., 2023). These coatings

enhance shelf life and quality by forming a protective layer around the fish. Sodium alginate, a marine alga-derived polysaccharide, is used in food coating due to its biocompatibility, biodegradability, environmental friendliness, renewability, non-toxicity, and low cost (Chen et al., 2021). It exhibits liquid-gel behavior and gel-forming properties when exposed to divalent ions, such as calcium (Ahmad et al., 2023). Incorporating antimicrobial agents into sodium alginate films has shown effectiveness against both Gram-positive and Gram-negative bacteria (Yan et al., 2024). The use of natural preservatives, such as chitosan and essential oils, in conjunction with sodium alginate coatings offers a sustainable alternative to synthetic preservatives (Dehghani et al., 2018). Additionally, epigallocatechin gallate (EGCG), a polyphenolic compound found in green tea, exhibits potent antioxidant and antimicrobial properties. Its ability to scavenge free radicals and inhibit microbial growth makes it a valuable candidate for food preservation (Chen et al., 2018). In tilapia preservation, EGCG can delay oxidation and enhance shelf life when incorporated into edible coatings (Cao et al., 2020). Similarly, peanut oil, rich in monounsaturated fat (oleic acid) and antioxidants, serves as an effective medium for marination and preservation, extending the shelf life of fish (Mei et al., 2019).

So far, there is no report on the hilsa shelf-life extension by a sodium alginate-based coating. Therefore, this study aims to investigate the efficacy of a sodium alginate-based edible coating enriched with epigallocatechin gallate (EGCG) and peanut oil for preserving the quality and extending the shelf life of refrigerated hilsa fish chunks (HFC). These natural coatings not only enhance shelf life and maintain quality but also align with consumer preferences for clean-label products. By employing such an edible coating, stakeholders can effectively reduce post-harvest losses, ensure food safety, and promote sustainable preservation practices for hilsa.

## Materials and Methods

### Chemicals and Reagents

The following analytical grade chemicals and reagents were used in the experiment. Sodium alginate powder, where viscosity: 15,000-20,000 cps, density: 1.261 g/cm<sup>3</sup>, and medium molecular weight: 80,000-120,000 Da (Sigma-Aldrich, China), glycerol (Sigma-Aldrich, Malaysia), calcium chloride (Duksan Pure Chemicals, S. Korea), 2% boric acid (Fujifilm Wako Pure Chemical Corporation, Japan), magnesium oxide (Fujifilm Wako Pure Chemical Corporation, Japan), sulfuric acid (Emsure, Germany), hydrochloric acid (Emsure, Germany), sodium chloride (Emsure, Germany), TBA

reagent (Sigma-Aldrich, China), antifoam (Sigma-Aldrich, USA), tryptone soya agar and violet red bile glucose agar (Hi-media Laboratories Pvt. Ltd, India).

### Collection of samples and preparation of HFC

The hilsa fish, approximately 10 kg, was purchased from Bahadur Bazar, Dinajpur. The fish were carefully transported to the laboratory within 45 mins in insulated boxes packed with sufficient ice (fish: ice ratio 1:2). Upon arrival at the lab, the fish were descaled by scraping, the head was removed by cutting just behind the gills, and the fish were rinsed thoroughly with running water. The cleaned beheaded fish was cut transversely into uniform hilsa fish chunks (HFC) using a sharp knife, and washed with chilled water to remove any residual blood, scales, or entrails as required. Each chunk measured 4 inches in length, 2.5 inches in width, and weighed an average of 45 g. The prepared chunks were inspected for any remaining bones and set aside for further experimental use.

### Coating of HFC

The coating solution was prepared according to the method of Cai et al. (2015) with slight modifications. The alginate solution was prepared by dissolving sodium alginate (2% w/v) in distilled water and stirring the mixture at 70°C until it became clear. To enhance the strength, flexibility, and oxygen permeability of coatings, 10% (w/w) glycerol was added as a plasticizer to a polysaccharide-based edible coating and mixed thoroughly (Treatment 1). Subsequently, 1% (v/v) peanut oil was added to the alginate solution, followed by 2.5% (v/v) polysorbate 80 with proper mixing to form the alginate/oil solution (Treatment 2). Lastly, EGCG (0.2%, w/v) was added to the alginate/oil solution and homogenized (Treatment 3). Hilsa chunks were divided into four treatment groups. The CON group fish chunks were immersed in distilled water for 1 minute at room temperature. In other treatment groups, fish chunks were dipped in the above coating solutions for 1 minute at room temperature. A consistent fish-to-solution ratio of 1:2 was maintained across all treatment groups. A 2% (w/v) calcium chloride solution was prepared and used as a cross-linking agent. This solution facilitates the formation of a stable gel matrix, which is essential for the desired structural and functional properties. After dipping, the chunks were allowed to drain for 1 minute on a sterile stainless-steel wire mesh screen at room temperature to remove excess solution. Subsequently, the chunks were immersed in calcium chloride solution for 1 minute. The samples were then individually packed in airtight polyethylene pouches and stored at 4 ± 1 °C. Each treatment group included three replicates, which were analyzed at 3-day intervals.

## Chemical analysis

### pH value determination

Ten (10) g of hilsa flesh was taken and homogenized with 100 mL of distilled water (Cai et al., 2015). Then, the pH of the homogenate was measured with a pH meter (HI11301-Edge, Hanna Inst, Romania).

### Estimation of total volatile-base nitrogen (TVB-N)

The total volatile base nitrogen (TVB-N) value was determined as described by Akhtar et al., (2025). Initially, 2 g of magnesium oxide (MgO) and 2-3 drops of antifoam were added to 10 g of homogenized fish samples, which were subsequently mixed with 200 mL of distilled water. The resulting mixture underwent distillation, yielding approximately 150 mL of distillate, which was collected in a flask containing a 2% boric acid solution with 3-5 drops of methyl red as a pH indicator. The distillate was titrated with 0.1 N sulfuric acid until a light pink endpoint was achieved. The TVB-N value, expressed as milligrams of nitrogen per 100 grams of sample, was calculated based on the following equation:

$$\text{TVB-N (mg N/100 g of fish)} = (\text{final mL of titrant solution} - \text{starting point of mL titrant solution}) \times 14$$

### Estimation of thiobarbituric acid reactive substance (TBARS)

The measurement of thiobarbituric acid-reactive substances (TBARS) was performed using a method adapted from Akhtar et al. (2025). Initially, 10 g of the homogenized sample was combined with 97.5 mL of distilled water and 2.5 mL of 4N hydrochloric acid (HCl). This mixture was subsequently heated in a Kjeldahl distillation unit to collect 50 mL of distillate. Next, 5 mL of the distillate was mixed with 5 mL of a thiobarbituric acid (TBA) reagent, prepared by dissolving 0.0283 g of TBA in 90% glacial acetic acid. The resulting mixture was incubated in boiling water for 35 minutes. Following incubation, the solution was allowed to cool, and the absorbance of the pink solution was measured at 538 nm using a spectrophotometer (PG Instrument, UK, model T80+). The TBARS value was calculated using a constant of 7.8, and the results were expressed as milligrams of malonaldehyde equivalents per kilogram of sample. To calculate the TBARS value following equation was used as follows:

### Microbiological analysis

Hilsa fish samples were prepared for microbial analysis in a sterile environment (Akhtar et al., 2025). On the day of sampling, the fish flesh was aseptically cut into small pieces using a sterile knife and stored at 4°C. A 10 g

portion of the sample was transferred to a sterile stomacher bag, mixed with 90 mL of physiological saline solution (PS), and homogenized for 30 seconds using a stomacher blender (BK-SHG05, China), resulting in a 1:10 dilution. Serial dilutions were performed by transferring 1 mL of the homogenized sample into a glass bottle containing 9 mL of PS. This mixture was thoroughly shaken using a vortex mixer to ensure uniformity. The prepared dilutions were then utilized for colony counting using the pour plate method on Tryptone Soya Agar (TSA) and Violet Red Bile (VRB) media. Each medium was incubated at 37°C for 48 hours to facilitate microbial growth.

### Sensory analysis

The sensory evaluation of hilsa fish samples (HFC) was conducted following the sensory assessment technique established by Ebadi et al. (2019). A panel consisting of ten trained members evaluated the fish samples based on four quality attributes: color, odor, firmness, and overall acceptance. Panelists used a scoring system ranging from 1 to 5, with 5 indicating excellent quality and 1 indicating unacceptable samples. This structured approach ensured a comprehensive assessment of the sensory characteristics of the fish.

### Statistical analysis

Each sample was analyzed in triplicate to ensure the reliability of the results. The data are presented as average values accompanied by their standard deviations (mean  $\pm$  SD). Differences among the treatment groups were examined using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify specific differences. Statistical analyses were accomplished using Prism software (Version 10). A significance level of  $p < 0.05$  was established for all comparisons.

## Results and Discussion

### pH value of HFC

pH is a key indicator of microbial spoilage in fish, as certain psychrophilic bacteria break down carbohydrates, thereby increasing the acidity of the medium. It is widely used to determine fish freshness, with fresh fish typically having a near-neutral pH (Majumdar et al., 2024). After death, the breakdown of nitrogenous compounds could raise pH (Cai et al., 2015). According to the Argentine food code, fish with a pH higher than 7.5 is considered unfit for consumption (Goncalves, 2017). During refrigerated storage, significant pH changes were observed in the HFC. In this study, the pH of HFC increased progressively across all treatments throughout the 12-day refrigerated storage (Figure 1). The pH values ranged from  $5.74 \pm 0.04$  to  $7.21 \pm 0.02$  in the CON group, indicating fluctuations over time. This rise is attributed to

the production of basic nitrogenous compounds, such as ammonia and amines, resulting from microbial enzyme-mediated degradation of proteins and amino acids. In the CON group, the pH increase was the most rapid and significant ( $p < 0.05$ ), reflecting intense microbial activity and spoilage. Conversely, T3 maintained a significantly lower pH compared to other groups ( $p < 0.05$ ), indicating better preservation of fish muscle integrity. The effectiveness of T3 is due to EGCG's antimicrobial properties, which suppress the growth of spoilage bacteria and limit the production of alkaline compounds. Sodium alginate forms a semipermeable barrier, reducing oxygen exchange and bacterial proliferation, while peanut oil contributes antioxidative effects, slowing enzymatic reactions. These factors collectively stabilize pH in T3-treated samples. Similar results were found in the study by Ruan et al. (2019). For the three other treatment groups, the initial pH values were  $5.74 \pm 0.01$  for T1,  $5.73 \pm 0.03$  for T2, and  $5.73 \pm 0.03$  for T3. By the end of storage, the pH values had increased to  $6.84 \pm 0.04$ ,  $6.64 \pm 0.03$ , and  $6.54 \pm 0.04$ , respectively. Based on these results, it can be concluded that sodium alginate coating incorporating EGCG and peanut oil had a slight limiting effect on the pH changes of HFC. It slowed the increment of pH values. Similar findings were reported in the study by Pei et al. (2022).

### Total volatile-base nitrogen (TVB-N) value

Total volatile base nitrogen (TVB-N) in fish meat consists of compounds such as trimethylamine,

dimethylamine, ammonia, and other volatile nitrogenous substances (Akhtar et al., 2025). TVB-N is commonly used as an indicator of fish quality, as its increase is directly linked to the activity of spoilage bacteria and the breakdown of proteins by endogenous enzymes (Özoğul et al., 2004; Zakhariya et al., 2015). During storage, significant differences ( $p < 0.05$ ) in TVB-N content were observed among the HFC samples. TVB-N indicates fish muscle protein decomposition, which is driven by increased values significantly over time ( $p < 0.05$ ) in all groups. This is due to microbial degradation of proteins into volatile amines, such as ammonia, trimethylamine, and dimethylamine. At the start of storage, the TVB-N values were  $23.03 \pm 0.73$  mg of N/100 g sample in the CON group, whereas  $22.89 \pm 0.56$  mg of N/100 g,  $21.5 \pm 0.68$  mg of N/100 g sample, and  $22.43 \pm 0.13$  mg of N/100 g sample were observed in T1, T2 and T3, respectively (Figure 2). However, as storage time progressed, TVB-N levels continued to increase among samples, surpassing the acceptable limit by the 6th day in the CON group ( $42.99 \pm 0.48$  mg of N/100 g sample), T1 by the 9th day ( $50.82 \pm 0.32$  mg of N/100 g sample) and T3 by the 12th day ( $55.12 \pm 0.66$  mg of N/100 g sample), respectively ( $p < 0.05$ ). Figure 2 shows that the increase in TVB-N was slower in T3 than in the other groups, indicating that T3 was more effective at maintaining fish quality over time. The slower increase in T3 is due to EGCG's antimicrobial effects, which limit bacterial enzymatic activity responsible for deamination. Sodium alginate coating restricts moisture loss and microbial

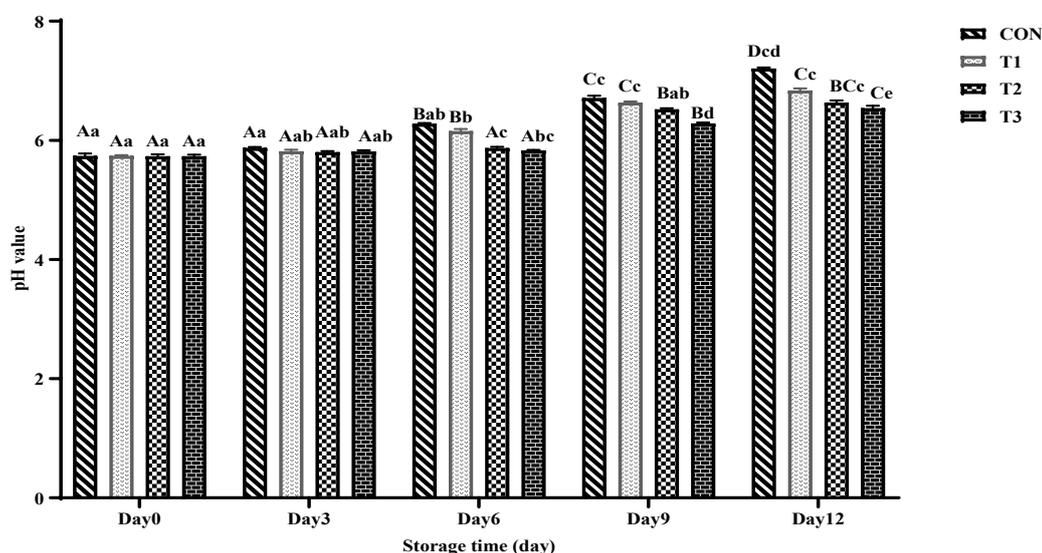


Figure 1. Changes in pH value of HFC stored at refrigerated temperature ( $4^{\circ}\text{C}$ ). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

infiltration, while peanut oil acts as a physical and oxidative barrier. According to Akhater et al. (2025), the acceptable TVB-N limit for fish freshness is 35-40 mg N/100 g sample, and in this study, the threshold was set at 40 mg N/100 g. Cai et al. (2015) reported that Japanese sea bass treated with an  $\epsilon$ -polylysine and alginate coating maintained TVB-N levels below 30 mg N/100 g for 12 days at 4°C. In contrast, untreated samples exceeded 40 mg N/100 g by day 8, supporting the efficacy of bioactive coatings in delaying protein degradation. Similarly, Dai et al. (2022) observed that bighead carp fillets coated with chitosan and EGCG exhibited TVB-N values below 30 mg N/100 g sample for 10 days at chilled storage, further corroborating the antimicrobial and preservative effects of EGCG-enriched coatings. These findings suggest that T3 was the most effective treatment for reducing TVB-N levels, helping to extend the freshness of the HFC during refrigerated storage up to 9<sup>th</sup> days within an acceptable range.

### Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) are widely used indicators of the extent of secondary lipid oxidation in food products (Akhtar et al., 2025). The TBARS test primarily measures malondialdehyde (MDA), a compound that represents aldehydes formed during lipid oxidation. This oxidation process can

negatively impact food quality by reducing its nutritional value and altering its texture and color (Lie, 2001). In this study, significant changes were observed in the TBARS values of HFC coated with different ratios of sodium alginate alone or combined with peanut oil and epigallocatechin gallate, which were then stored under refrigeration (Figure 3). The results showed a statistically significant difference ( $p < 0.05$ ) among the groups. At the beginning of storage (day 0), the TBARS values were  $1.29 \pm 0.02$  mg MDA/kg sample in the CON,  $1.20 \pm 0.02$  mg MDA/kg sample in T1,  $1.19 \pm 0.02$  mg MDA/kg sample in T2, and  $1.23 \pm 0.06$  mg MDA/kg sample in T3, respectively. As storage progressed, the TBARS values increased across all groups. By the end of the storage period, the final values were  $4.68 \pm 0.06$  mg MDA/kg sample for the CON,  $3.42 \pm 0.02$  mg MDA/kg sample for T1,  $2.74 \pm 0.04$  mg MDA/kg sample for T2, and  $2.31 \pm 0.04$  mg MDA/kg sample for T3. The CON group exhibited the highest TBARS levels, indicating rapid rancidity. T3 showed the lowest TBARS values, suggesting effective retardation of lipid peroxidation.

The acceptable limit for TBARS is 2 mg MDA/kg (Akhtar et al., 2025). Among the treatment groups, T3 consistently showed the lowest TBARS value throughout storage. This suggests that T3 had better oxidation stability compared to the other treatments. In contrast, T2 and T3 showed positive effects from the addition of peanut oil and epigallocatechin gallate. The antioxidative

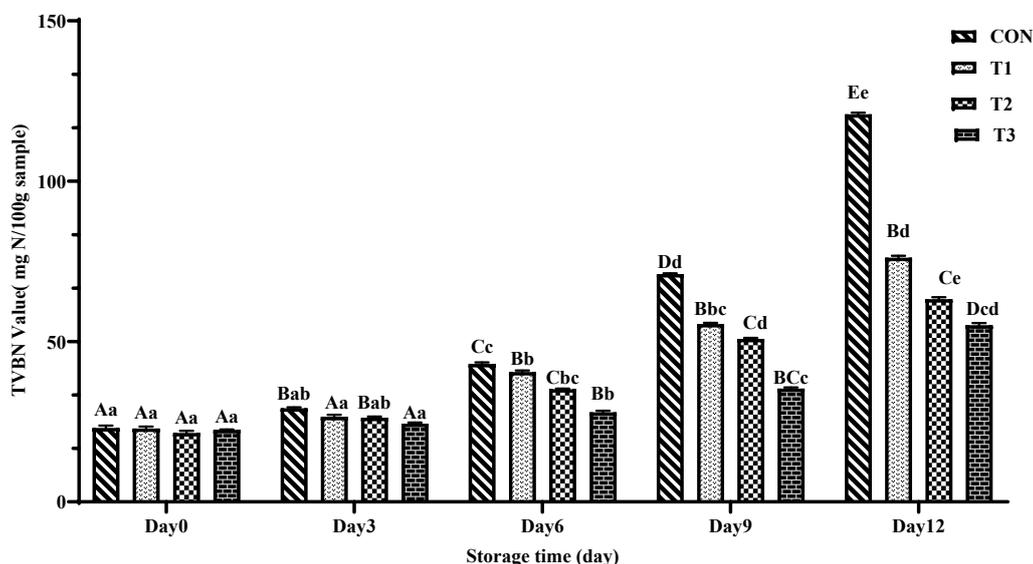


Figure 2. Changes in TVB-N value of HFC stored at refrigerated temperature (4°C). Different uppercase letters (A-E) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

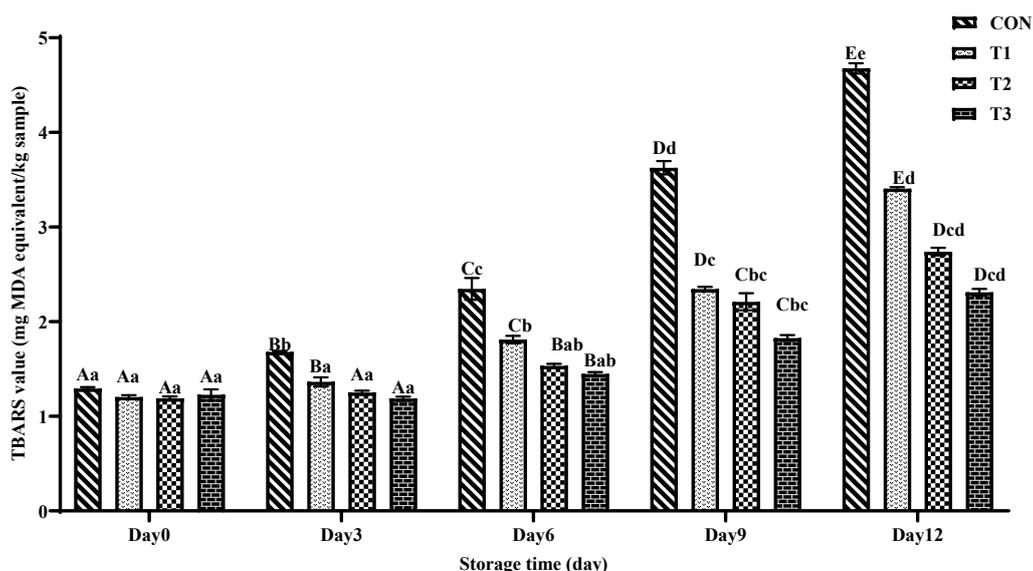


Figure 3. Changes in TBARS value of HFC stored at refrigerated temperature (4°C). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

properties of EGCG and vitamin E in peanut oil played critical roles in reducing TBARS formation (Pei et al., 2022). Sodium alginate created a moisture and oxygen barrier, minimizing lipid exposure to oxidative elements. These mechanisms delayed the breakdown of fatty acids into malondialdehyde (MDA), the main TBARS compound. Based on these findings, T3 appears to be the most effective coating for preserving the quality of fish Chunks, as it resulted in the lowest lipid oxidation levels. Meanwhile, T1 and T2 showed no significant benefits. This suggests that T3 is the most suitable treatment for maintaining the freshness and stability of HFC during refrigerated storage.

### Microbial load count of HFC

#### Total viable count in TSA media

Microbial growth was monitored using total viable count (TVC) in TSA media to evaluate bacterial contamination in HFC during storage (Figure 4). The results showed a steady increase in microbial counts across all groups, with the CON group experiencing the fastest bacterial growth. On day 0, CON, T1, T2 and T3 microbial loads were  $4.28 \pm 0.06$  log CFU/g sample,  $4.27 \pm 0.12$  log CFU/g sample,  $4.25 \pm 0.04$  log CFU/g sample and  $4.33 \pm 0.12$  log CFU/g sample, respectively. Over time, microbial counts exceeded the threshold

limit of 7 log CFU/g, reaching  $7.56 \pm 0.08$  log CFU/g in the CON group by day 12, while T1 and T2 recorded  $7.14 \pm 0.03$  log CFU/g and  $6.71 \pm 0.04$  log CFU/g, respectively. T3 exhibited the slowest microbial growth, with  $5.89 \pm 0.08$  log CFU/g sample on day 12, indicating better preservation. This highlights the efficacy of T3 in microbial control. Protein and amino acid breakdown by bacteria leads to the formation of volatile compounds and spoilage. EGCG in T3 inhibits bacterial enzymes, while sodium alginate and peanut oil restrict moisture and nutrient availability. Despite refrigeration, psychrophilic bacteria such as *Pseudomonas* spp. can proliferate; however, EGCG suppresses their growth. Previous studies have reported similar microbial growth patterns in refrigerated fish fillets. Kim et al. (2018) found that microbial proliferation is a major factor in fish spoilage, with bacterial counts exceeding 7 log CFU/g indicating the onset of spoilage. Similarly, Sáez et al. (2020) observed that a sodium alginate coating enriched with tannins reduced microbial counts and remained within acceptable limits in refrigerated rainbow trout fillets until day 12, aligning with the findings of this study, where the CON group exceeded acceptable microbial levels by day 12. The effectiveness of treatments in slowing microbial growth has also been documented. Charoenphun et al. (2023) found that an alginate-based coating containing a plant extract can delay microbial spoilage, a trend similar to that observed in this study, where T3 exhibited

the slowest bacterial growth. Additionally, Rayeni et al. (2016) emphasized that antimicrobial coatings can significantly extend the shelf life of fish products, further supporting the results of this study. These findings align with previous research, confirming that antimicrobial treatments, such as coatings, can effectively slow bacterial growth, thereby extending the shelf life of fish fillets during refrigerated storage.

Total viable count was analyzed using violet red bile (VRB) agar, which specifically targets enteric bacteria.

The results showed a steady increase in bacterial counts across all groups, with the CON group experiencing the most rapid growth. On day 0, CON, T1, T2, and T3 microbial loads were  $3.25 \pm 0.05$  log CFU/g sample,  $3.22 \pm 0.01$  log CFU/g sample,  $3.28 \pm 0.11$  log CFU/g sample and  $3.17 \pm 0.09$  log CFU/g sample, respectively (Figure 5). As storage progressed, bacterial counts rose, reaching  $7.19 \pm 0.06$  log CFU/g in the CON by day 12, whereas T1 and T2 had  $6.51 \pm 0.04$  log CFU/g and  $6.28 \pm 0.09$  log CFU/g, respectively. T3 exhibited

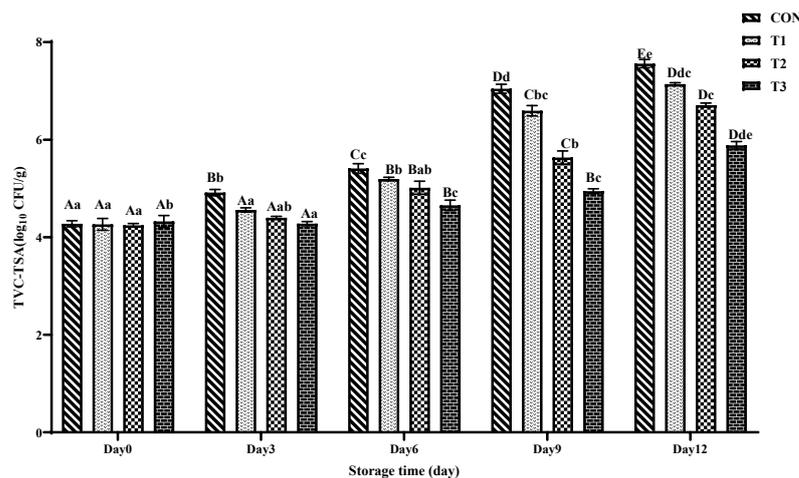


Figure 4. Changes in TVC value in TSA media of HFC stored at refrigerated temperature (4°C). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

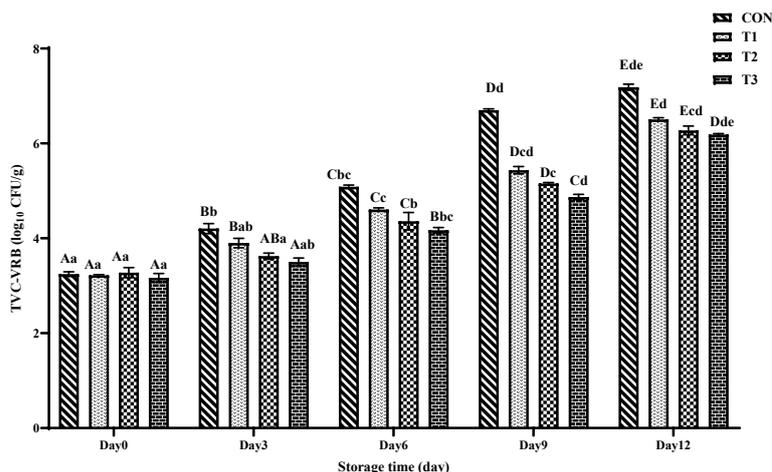


Figure 5. Changes in TVC value using VRB media of HFC stored at refrigerated temperature (4°C). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

the slowest growth, with  $6.19 \pm 0.02$  log CFU/g sample on Day 12, highlighting the antimicrobial efficacy of EGCG, particularly against Gram-negative bacteria. Microbial spoilage in fish is facilitated by high water activity and nutrient content. Sodium alginate coatings reduce surface contamination, while peanut oil creates hydrophobic barriers. The polyphenolic structure of EGCG damages bacterial membranes, reducing coliform growth. Similar trends have been reported in previous studies investigating bacterial growth in fish fillets during storage. Research by Özoğul et al. (2014) found that bacterial counts increased significantly in untreated fish fillets, exceeding acceptable levels within 10-12 days of refrigerated storage. Studies by Pezeshk et al. (2011) and Akhtar et al. (2025) demonstrated that antimicrobial coatings effectively reduced microbial proliferation. The slower bacterial growth in T3 in this study supports these findings, indicating that certain treatments can extend the shelf life of fish fillets by limiting bacterial contamination. These results reinforce previous research, showing that appropriate treatments, such as natural coatings or antimicrobial compounds, can significantly delay bacterial growth, improving fish fillet quality and safety during refrigerated storage.

### Sensory attributes

The development of off-odors, caused by microbial and chemical changes, was evaluated in CON, T1, T2, and T3 samples over a 12-day storage period. Initially, there were no noticeable differences in appearance, color, odor, or texture between the CON and treated samples before storage ( $p > 0.05$ ). However, storage time clearly

affected the sensory quality of all samples. Both the CON and treated groups showed a gradual decrease in sensory scores for odor, texture, appearance, and color over time (Figures 6-9). Similarly, Pei et al. (2022) found that an alginate coating incorporating EGCG and lysozyme retained better color in large yellow croaker than alginate alone. In the current study, the initial sensory scores for all groups were 5.00. By the 12th day, the scores had dropped to  $3.15 \pm 0.04$  for the CON group. By the 12th day, the scores were  $3.15 \pm 0.05$  in T1,  $3.23 \pm 0.04$  in T2, and  $4.23 \pm 0.03$  in T3. According to Figure 6-9, a sensory score of 5 indicates the best quality, a score of 3 is considered safe for human consumption, and a score below 3 indicates poor quality and spoilage (Akhtar et al., 2025). However, on the 12<sup>th</sup> day, T3 had the highest score, indicating better quality than the other treatments. Fish freshness deteriorates due to protein denaturation, lipid oxidation, and bacterial spoilage. EGCG's antioxidant properties help preserve color and texture, while antimicrobial action reduces odor-causing bacteria. Peanut oil enhances gloss and reduces oxidative discoloration. Sodium alginate preserves moisture, firmness, and glossiness. Akhtar et al. (2025) reported that a sodium acetate-enriched sodium alginate coating maintains better color, appearance, texture, and odor in refrigerated hilsa fish chunks than untreated hilsa fish chunks. Similarly, EGCG incorporated into sodium alginate and carboxymethyl cellulose maintains sensory properties for an additional 1-3 days compared to uncoated pork (Ruan et al., 2019). Xu et al. (2024) also found similar results, stating that an EGCG-enriched sodium alginate coating resists changes in color, odor, softness, and overall acceptability compared to the uncoated sample in super-chilled large

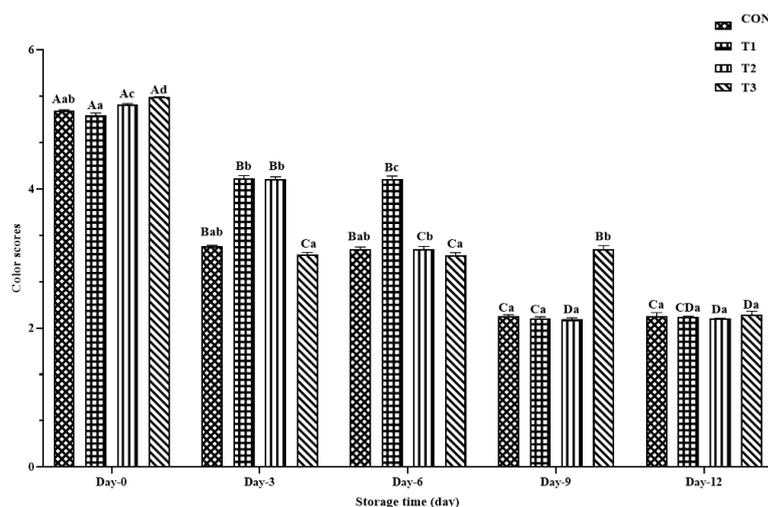


Figure 6: Changes in color of HFC stored at refrigerated temperature ( $4^{\circ}\text{C}$ ). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

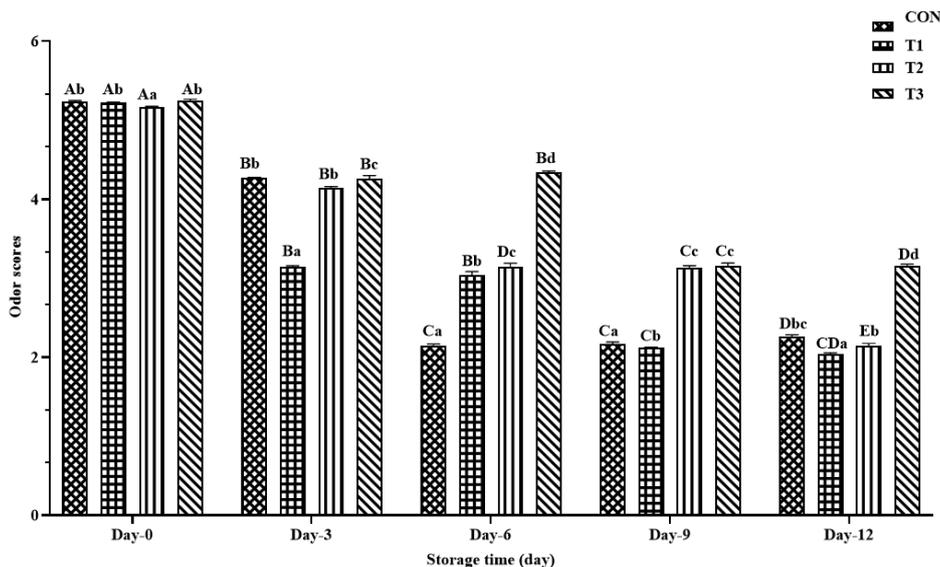


Figure 7. Changes in odor of HFC stored at refrigerated temperature (4°C). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

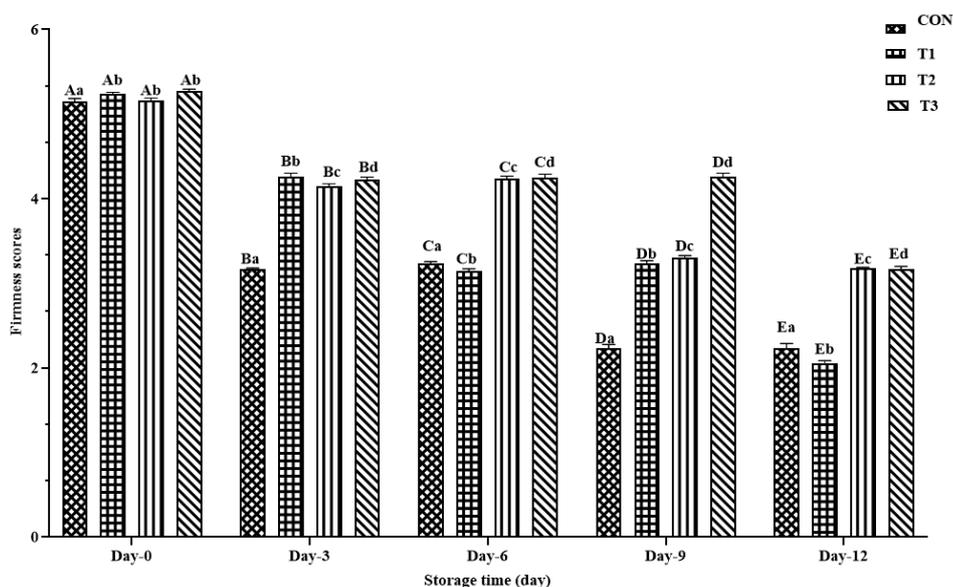


Figure 8. Changes in firmness of HFC stored at refrigerated temperature (4°C). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

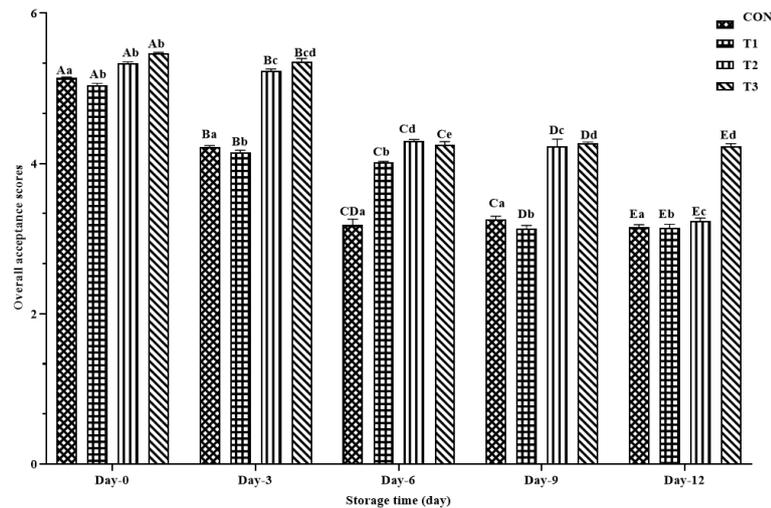


Figure 9. Changes in overall acceptance of HFC stored at refrigerated temperature (4°C). Different uppercase letters (A-D) on the bars within the same storage time indicate significant differences ( $p < 0.05$ ). Different lowercase letters (a-e) on the bars within the same treatments indicate significant differences ( $p < 0.05$ ). Bars represent the standard deviation ( $n=3$ ). The sample codes are denoted as CON: control sample dipped into distilled water, T1: treatment with 2% sodium alginate, T2: treatment with 2% sodium alginate and 1% peanut oil and T3: treatment with 2% sodium alginate, 1% peanut oil and 0.2% epigallocatechin gallate (EGCG).

yellow croaker. Based on these findings, T3 is the most effective chemical treatment for prolonging the shelf life of refrigerated HFC, maintaining good sensory quality throughout the entire storage period. Overall, the sensory ranking based on the results was  $T3 > T2 > T1 > CON$ , with T3 performing the best by extending the shelf life and maintaining freshness better than the other treated samples.

## Conclusion

The study investigated the synergistic effects of sodium alginate coating with peanut oil and epigallocatechin gallate on the quality of refrigerated hilsa fish chunks. Chunks treated with sodium alginate containing peanut oil and epigallocatechin gallate (T3) exhibited significantly lower pH, TVB-N, TBARS, TVC, and hold sensory attribute levels than those treated with sodium alginate alone, sodium alginate with peanut oil, or left untreated. Additionally, T3 was more effective in inhibiting microbial spoilage than the formulation containing sodium alginate and peanut oil alone. The antioxidant and antibacterial properties of the coating were most evident when peanut oil was used at a 1% concentration. These coatings present a promising natural alternative to synthetic preservatives, potentially enhancing food quality and prolonging freshness. The results concluded that T3 was the most effective, while T1 and T2 showed no significant impact. Hilsa can be preserved for up to 9 days when incorporated with 2% sodium alginate, 1% peanut oil, and epigallocatechin

gallate, provided the conditions are acceptable. This edible coating extends shelf life, offering a natural alternative to synthetic preservatives such as formalin and other harmful chemicals.

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