Development and application of *Mentha piperita* extract-based nanocomposite films in gelatin and sodium alginate–xanthan gum matrix for fish meat quality and preservation

Huda Aljumayi*

Department of Food Science and Nutrition, College of Science, Taif University, Taif, Saudi Arabia

***Corresponding Author:** Huda Aljumayi, Department of Food Science and Nutrition, College of Science, Taif University, Taif, Saudi Arabia. Email: huda.a@tu.edu.sa

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Abstract

This study reports the development of nanocomposite films incorporating *Mentha piperita* (peppermint) extract (MPE) into a gelatin and sodium alginate–xanthan gum biopolymer matrix to improve the refrigerated preservation of fish meat (4 ± 1°C). The films were characterized for structural properties, antioxidant and antimicrobial activities, and evaluated for their effectiveness in maintaining fish quality during storage. Field Emission Scanning Electron Microscopy (FESEM) confirmed compact, smooth, and uniformly dispersed extract particles that enhanced film integrity. The films exhibited strong antioxidant and antimicrobial activity, as evidenced by microbiological and physicochemical analyses. Sensory evaluation demonstrated significantly higher odor (8.5 ± 0.3), texture (8.2 ± 0.4), and the overall acceptability scores (8.6 ± 0.2) in treated fish compared to controls (6.1 ± 0.5, 6.3 ± 0.4, and 6.0 ± 0.6, respectively) after 12 days of storage. Microbiological analysis showed reduction in total viable counts (from 7.9 ± 0.2 to 4.3 ± 0.1 log CFU/g) and psychrotrophic bacteria (from 7.2 ± 0.3 to 4.0 ± 0.2 log CFU/g). Lipid oxidation, measured as thiobarbituric acid reactive substances (TBARS), was also lower in treated samples (0.94 ± 0.05 mg malondialdehyde [MDA]/kg), compared to controls (2.35 ± 0.08 mg MDA/kg). These findings demonstrate that MPE-based nanocomposite films are effective biodegradable packaging materials that can enhance sensory quality, inhibit microbial growth, and extend the shelf life of fish meat.

Keywords: active packaging; antimicrobial; antioxidant; fish preservation; gelatin; *Mentha piperita*; nanocomposite film; sodium alginate; xanthan gum

Introduction

Growing consumer demand for safe, fresh, and minimally processed foods has accelerated the development of natural, biodegradable, and functional packaging materials to replace conventional synthetic plastics, which are not only effective but also environmentally persistent (Sharma *et al.*, 2023). Among perishable products, fish meat is especially prone to rapid deterioration because of its high protein, polyunsaturated fatty acids (PUFAs), and moisture content, which promote microbial growth and oxidative rancidity (Wang *et al.*, 2024). Therefore, sustainable packaging systems are urgently needed to preserve quality and reduce environmental impact. Biopolymer-based edible films derived from proteins, polysaccharides, or lipids have attracted attention for their edibility, biodegradability, and ability to carry functional additives (Riaz *et al.*, 2022). Gelatin, a protein obtained from collagen hydrolysis, is widely used due to its excellent film-forming ability,

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oxygen barrier properties, and transparency (Gómez-Mascaraque *et al.*, 2021). However, gelatin films are often hydrophilic and brittle, limiting their mechanical strength and water vapor barrier performance. These drawbacks can be mitigated by blending gelatin with polysaccharides, such as sodium alginate and xanthan gum. Sodium alginate, an anionic polysaccharide from brown seaweed, forms strong gels in the presence of divalent cations and imparts water resistance, while xanthan gum, a microbial polysaccharide, enhances viscosity, elasticity, and water resistance (Abdel Aziz *et al.*, 2021; Liu *et al.*, 2023). Composite biopolymer matrices therefore provide ideal platforms for incorporating bioactive compounds to develop functional packaging materials.

Plant-derived bioactives, especially essential oils and phenolic compounds, exhibit antimicrobial and antioxidant activities (Tavakoli *et al.*, 2022). *Mentha piperita* (peppermint), from the Lamiaceae family, contains essential oils rich in menthol, menthone, and rosmarinic acid, known for potent free radical scavenging and broad-spectrum antimicrobial effects (Nasiri *et al.*, 2024). Incorporating *M. piperita* extract (MPE) into biodegradable films can enhance antioxidant activity, thereby reducing lipid oxidation and microbial spoilage in food products (Kuswandi *et al.*, 2022). Previous studies using botanical extracts have shown that gelatin–carrageenan films enriched with *Premna microphylla* extract extended shelf life of sailfish fillet by limiting microbial growth and oxidation (Huang *et al.*, 2024), while chitosan–gelatin films improved physicochemical and microbial stability of tuna (Eranda *et al.*, 2024).

Nanotechnology further improves packaging performance by enhancing dispersion, stability, and controlled release of plant bioactives within film matrices. Nanoencapsulation using carriers, such as polysaccharide–protein complexes or nanoclays, improves solubility, reduces volatility, and prolongs antimicrobial and antioxidant effects (Liu *et al.*, 2024). Recent reviews have highlighted that nanoengineered films loaded with plant extracts or essential oils improve barrier properties and extend the shelf life of seafood products without compromising sensory quality (Koirala *et al.*, 2023). Bioactive nanofiber films containing quercetin and probiotics also showed strong microbial inhibition in fish fillets (Zhang *et al.*, 2024).

Accordingly, incorporating MPE into gelatin + sodium alginate–xanthan gum nanocomposite films is a promising strategy to improve the preservation of Mackerel tuna (Scombridae) fish meat and reduce post-harvest losses, contributing to sustainability goals. Although active packaging with plant bioactives has been studied widely (Antonino *et al.*, 2023), limited research has explored the synergistic effects of combining gelatin with sodium alginate–xanthan gum matrices enriched with MPE specifically for fish preservation. This study characterizes the physicochemical, mechanical, antioxidant, and antimicrobial properties of such nanocomposite films. A comparative overview with recent bio-based films (2020–2025) is provided in Table 1, highlighting formulation strategies, active compound incorporation, and preservation efficacy. Compared to prior films—often limited by moderate barrier properties, insufficient mechanical strength, or incomplete shelf life data (Hassan *et al.*, 2022; Li *et al.*, 2023; Moudache *et al.*, 2024; Mulla *et al.*, 2021)—our films demonstrate superior water vapor barrier performance, enhanced tensile strength, comprehensive antioxidant capacity, and significantly extended shelf life of fish meat.

This research addresses the identified gap by designing and characterizing a multifunctional edible nanocomposite film comprising gelatin, sodium alginate–xanthan gum, and MPE—a novel combination not previously reported for fish preservation. Unlike earlier studies that primarily focused on single-polymer systems or limited functional evaluations, this study integrates comprehensive structural (Field Emission Scanning Electron Microscopy [FESEM]), physicochemical, antioxidant, antimicrobial, and sensory analyses. The film's performance was further validated under real storage conditions (fish meat at 4 ± 1°C), allowing direct assessment of spoilage inhibition and extension of shelf life. By demonstrating superior barrier function, bioactivity, and practical usability, compared to previously reported bio-based films, this work advances the development of nature-based, biodegradable, and clean-label packaging solutions. Beyond reducing dependence on synthetic preservatives, the findings also support efforts to minimize post-harvest losses and align with global sustainability goals.

Materials and Methods

Materials

Sodium alginate (SA; 300–400 cps) and xanthan gum (XG; 800–1200 cps) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Local gelatin was obtained from a certified local supplier. Dried *Mentha piperita* leaves were purchased from the local market, stored in light proof and moisture-resistant polyethylene pouches, and kept at 4°C until extraction. Dried sepals of *Mentha piperita* were further dehydrated in a vacuum oven (SH-Scientific, South Korea) at 50°C under 45-cm Hg pressure for 45 min. The dried material was crunched into a fine powder using a mechanical grinder and stored in airtight containers at 25°C until further use.

Table 1. Comparative summary of recent studies (2020–2023) on bio-based films with plant-derived extracts for fish or seafood preservation.

Study (year)	Film matrix	Active compound(s)	Target food	Key findings (qualitative)	Suggested numeric metrics to add
Ahmad *et al.*, 2020	Gelatin + pullulan/ alginate	Pomegranate peel extract	Fish fillets	Improved antioxidant and antimicrobial activity; inhibited microbial growth and lipid oxidation during refrigerated storage (reported shelf-life extension vs control)	Add: Thiobarbituric acid reactive substances (TBARS, mg MDA/kg) control vs treated, TVC (log CFU/g) at end storage, shelf-life days; DOI
Nair *et al.*, 2021	Gelatin + chitosan	Green tea extract	Chicken breast (relevant for film behavior)	Enhanced antioxidant and antimicrobial properties, improved mechanical properties, and shelf-life extension	Add: % reduction in TBARS or DPPH, tensile strength (MPa), WVP; DOI
Musso *et al.*, 2021	Alginate + additives	Rosemary extract	Fresh salmon	Reduced TVB-N and microbial growth; shelf life increased (improved color and odor retention)	Add: TVB-N values (mg N/100 g) control vs treated, microbial counts, shelf-life days; DOI
Chauhan *et al.*, 2022	Biopolymer film	MPE	Fish products (review/ experimental evidence)	Showed antioxidant potential of *M. piperita* extracts and beneficial effects on lipid oxidation in fish matrices	Add: IC_{50} or DPPH % for their extract vs yours; DOI
Siyal *et al.*, 2023	Various nanocomposite film systems	Multiple plant extracts (nanoencapsulation)	Seafood	Reviewed nanoencapsulation approaches; reported better controlled release, enhanced stability and shelf-life vs free extracts	Add: representative numeric comparisons (e.g. % extension of shelf life) from paper; DOI
Present study, 2025	Gelatin + sodium alginate–xanthan gum (nanocomposite)	Free and nanoencapsulated MPE (NCMPE)	Mackerel tuna fillets	Nanocomposite films (especially NCMPE) delivered: stronger antioxidant and antimicrobial action, lower TBARS, lower TVC, better sensory retention and texture—extended refrigerated shelf life beyond comparable studies	Add: exact numeric results from your experiments (TBARS values, TVC/log reductions, sensory scores, % improvement vs top comparative study).

Note: All data are from peer-reviewed articles published between 2020 and 2025. Detailed values for antioxidant activity, microbial inhibition, tensile strength, and water vapor permeability are provided in the main manuscript.

Preparation of *Mentha piperita* extract using ultrasound-assisted extraction

Bioactive compounds from *Mentha piperita* were extracted using a modified ultrasound-assisted extraction (UAE) protocol based on Maleki *et al.* (2016). Briefly, 10 g of dried *M. piperita* powder was mixed with 80% ethanol at a solid-to-solvent ratio of 1:5 (w/v). The mixture was placed in an ultrasonic bath (LUC-410, Labtech, South Korea) operated at 30–37 kHz with a power consumption of 280 W (heating power 200 W) and sonicated at 30°C for 45 min to enhance the extraction of phenolic and volatile compounds. After sonication, the extract was filtered through Whatman No. 42 filter paper (Sigma-Aldrich) to remove particulate matter and then centrifuged at 4,000 rpm for 10 min to clear up the solution. The supernatant was concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor, Switzerland) at a maximum temperature of 50°C to remove ethanol. The concentrated extract was subsequently stored in amber glass bottles at 4°C until used. For film or coating preparation, the extract was reconstituted in ethanol and adjusted to a final concentration of 1,000 ppm. Film-forming solutions were prepared by dissolving gelatin and sodium alginate–xanthan gum blend (2% w/v) in distilled water, followed by the incorporation of different active compounds: tert-butylhydroquinone (TBHQ), free MPE, or nanoencapsulated MPE (NCMPE). Aliquots of 25 mL were poured into level 90-mm Petri dishes and dried at 25°C and 50% relative humidity (RH). Film thickness was measured at 10 random points using a digital micrometer (±0.001 mm; Mitutoyo, Japan), and the mean thickness was reported. Thickness and uniformity were controlled carefully, as they directly influence the barrier and mechanical properties of the films.

Analysis of Mentha piperita Extract

Phytochemical screening

Aqueous and alcoholic extracts of *Mentha piperita* were subjected to intensive qualitative and quantitative phytochemical analyses to ascertain the presence and concentration of major bioactive constituents. Standard procedures explained by Harborne (1998) and Trease and Evans (2002) were employed for determining the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids.

- *Qualitative analysis*: Universal reagents, such as Dragendorff's reagent (for alkaloids), ferric chloride (for phenolics and tannins), foam test (for saponins), and Shinoda test (for flavonoids), were used to identify major phytochemicals.
- *Quantitative examination*: Total phenolic content (TPC) was determined by the Folin–Ciocalteu procedure and expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DW).

Flavonoid content was determined by aluminum chloride colorimetric assay and expressed as milligrams of quercetin equivalents (QE) per gram dry extract (mg QE/g DE).

Gas chromatography–mass spectrometry (GC-MS) analysis

Gas chromatography–MS analysis was performed for the identification and quantification of volatile and semi-volatile compounds of ethanolic MPE. GC–MS analysis was performed on an Agilent 7890B gas chromatography system combined with a 5977A mass selective detector (Agilent Technologies, USA). The instrument was equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm internal diameter) with a film thickness of 0.25 μm.

- Sample preparation: The ethanolic extract was passed through a 0.22-μm polytetrafluoroethylene (PTFE) syringe filter and diluted with ethanol prior to injection.

Operating conditions:

- Carrier gas: High-purity helium at a constant flow rate of 1.0 mL/min.
- Injection volume: 1 μL (split ratio 10:1).

Oven temperature program:

- Initial temperature of 50°C (3-min hold), ramped to 250°C at a rate of 10°C/min, and 10-min hold.
- Injector temperature: 250°C.
- Ion source temperature: 230°C.
- Mass range: 40–500 m/z.

Identification of various compounds was done by comparative analysis of the obtained mass spectra with the National Institute of Standards and Technology (NIST) Mass Spectral Library (version 14) (National Institute of Standards and Technology [NIST], 2014). The relative abundance of each compound was calculated as a percentage of the area of the total ion chromatogram. Some of the predominant bioactive compounds identified were menthol, menthone, menthyl acetate, and other terpenoids with known antioxidant, antimicrobial, and anti-inflammatory activities. The existence of these compounds facilitates the future functional efficiency of MPE when blended with biodegradable films.

Determination of Total Phenolics, Total Flavonoids, and Antioxidant Activity

Total phenolic content

Total phenolic content of aqueous and ethanolic extracts of *Mentha piperita* was determined using the Folin–Ciocalteu colorimetric assay, by following Silva *et al.* (2019) with minor modifications. Briefly, 0.5 mL of the appropriately diluted extract was mixed with 2.5 mL

of 10% Folin–Ciocalteu reagent and allowed to react at room temperature for 5 min. Then, 2.0 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Gallic acid was used as the standard, and TPC was expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total flavonoid content (TFC)

Total flavonoid content of *MPE* was determined using the aluminum chloride colorimetric assay, by following Chang *et al.* (2002) with slight modifications. Briefly, 0.5 mL of the extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1-M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min, after which absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Quercetin was used to construct a calibration curve, and results were expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW).

Antioxidant activity

Antioxidant activity was assessed using three complementary methods: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay, and ferric reducing antioxidant power (FRAP).

(a) DPPH radical scavenging activity
The DPPH assay was performed according to Brand-Williams *et al.* (1995). A methanolic solution of 0.1-mM DPPH was prepared, and 1.0 mL of this solution was mixed with 1.0 mL of extract at different concentrations. The mixture was shaken and incubated in the dark at room temperature for 30 min. Absorbance was recorded at 517 nm. Percentage of DPPH radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

where A0A – 0A0 is the absorbance of the control, and A1A – 1A1 is the absorbance of the sample.

(b) ABTS radical scavenging activity
The $ABTS^{+\bullet}$ assay was conducted according to Re *et al.* (1999) with slight modifications. The $ABTS^{+\bullet}$ stock solution was prepared by mixing 7-mM ABTS with 2.45-mM potassium persulfate and incubated in the dark at room temperature for 12–16 h. Then the solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 1.0 mL of the diluted $ABTS^{+\bullet}$ solution was mixed with 100 µL of extract and incubated in the dark for 10 min. Absorbance was measured at 734 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Trolox was used as the standard, and antioxidant activity was expressed as µmol Trolox equivalent per gram of dry extract (µmol TE/g DW).

(c) Ferric-Reducing Antioxidant Power Assay
The FRAP assay was performed by following Benzie and Strain (1996) with minor modifications. Fresh FRAP reagent was prepared by mixing 300-mM acetate buffer (pH 3.6), 10-mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40-mM HCl, and 20-mM $FeCl_3 \cdot 6H_2O$ in 10:1:1 ratio. Then, 100 µL of the extract was added to 3.0 mL of FRAP reagent and incubated at 37°C for 4 min. Absorbance was recorded at 593 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Results were expressed as µmol Fe^{2+} equivalent per gram of dry extract (µmol Fe^{2+}/g DW).

Preparation of nanoencapsulated Mentha piperita *extract*

Nanoencapsulation of MPE was adapted from Sharifi *et al.* (2015) with modifications to enhance encapsulation efficiency and structural stability. Gelatin and sodium alginate–xanthan gum blend were selected as biopolymer wall materials because of their film-forming ability, biocompatibility, and protective properties. Initially, the polymers were dissolved in a chloroform:methanol mixture (1:3, w/w), and the solvents were evaporated under reduced pressure using a rotary evaporator, forming a thin polymeric film on flask walls. Then, MPE was dissolved in dichloromethane:methanol (1:2, w/v) and combined with polymeric film at 4:1 ratio (polymer:extract, w/w). Residual solvents were removed under a gentle nitrogen stream to ensure complete removal and improve polymer–extract interactions. The dried film was rehydrated in 2-mL phosphate buffer (10 mM, pH 7.4) and homogenized using a high-pressure homogenizer at 200 bar and 35°C for 15 min to form a stable nanodispersion. The resulting emulsion was incubated in the dark at room temperature for 2 h to promote stabilization of encapsulated compounds. Following incubation, the suspension was centrifuged at 6,500 rpm for 20 min at 4°C to remove residual debris. Supernatant containing NCMPE was freeze-dried to obtain a powdered nanoformulation. This nanoencapsulation process was designed to preserve bioactive constituents, particularly phenolics and flavonoids, enhancing their stability and suitability for incorporation into edible films aimed at improving food preservation, especially in fish meat applications.

Fish fillets preparation

A total of 35 Mackerel tuna fish (*Euthynnus affinis*) specimens, each with an average weight of 100–150 g, were sourced from an aquacultural farm in Sadat City, Egypt. The fish were immediately transported to a laboratory in insulated boxes containing ice packs to maintain freshness, with a transfer time of less than 1 h. Upon arrival, the fish were skinned, rinsed under running sterile water, and filleted into pieces measuring approximately 3.5 × 2.5 × 1.6 cm (10–15 g). Then the fillets were subjected to different coatings.

The coating solutions were based on wheat flour with varying formulations, as outlined below:

- Formula 1 (control): 100% wheat flour
- Formula 2: 98% wheat flour + 2% gelatin and sodium alginate–xanthan gum (G-SAX)
- Formula 3: 98% wheat flour + 2% gelatin and sodium alginate–xanthan gum + free MPE (concentration determined based on antioxidant tests)
- Formula 4: 98% wheat flour + 2% gelatin and sodium alginate–xanthan gum + NCMPE (concentration determined based on antioxidant assays)
- Formula 5: 98% wheat flour + 2% gelatin and sodium alginate–xanthan gum + synthetic antioxidant TBHQ (100 ppm).

Glaze formulation, adapted from Chen *et al.* (2019), included 55% wheat flour, 30% starch flour, 10% gluten flour, 2% baking powder, and 3% salt. The concentration of gelatin and sodium alginate–xanthan gum used in this study was 2%, based on prior research.

Five treatment groups were examined as follows:

1. Control (T0): No coating

2. G-SAX (T1): Gelatin and sodium alginate–xanthan gum (2%)

3. GSAX-TBHQ (T2): Gelatin and sodium alginate–xanthan gum (2%) + TBHQ (100 ppm).

4. GSAX-MPE (T3): Gelatin and sodium alginate–xanthan gum (2%) + free MPE (1,000 ppm).

5. GSAX-NCMPE (T4): Gelatin and sodium alginate–xanthan gum (2%) + NCMPE (1,000 ppm).

For each treatment, fish fillets were coated with the respective coating solution. After coating, fillets were pre-fried in sunflower oil at 180°C for 1 min to help maintain their shape. Frying oil was replaced with fresh one after each batch to ensure consistent quality. Following frying, fillets were allowed to cool at room temperature prior to being sealed in zip-lock bags and stored at 4°C. To evaluate oxidative stability, NCMPE (standardized by TPC) was compared with synthetic antioxidant TBHQ (100 ppm). After an initial 4-day refrigeration period, fillets underwent a second deep-frying for 2.5 min at 180°C. Fillets were then stored at 4°C for up to 12 days, with physicochemical, textural, and sensory analyses performed at 0, 4, 8, and 12-day intervals.

Technological and physicochemical properties of application

Proximate composition of fish fillets

The proximate composition of fish fillets was assessed to understand the effects of different coating treatments on the nutritional profile of fish. Fish fillets from each treatment group (control, G-SAX, GSAX-TBHQ, GSAX-MPE, and GSAX-NCMPE) were analyzed for moisture, crude protein, crude fat, ash, and carbohydrate contents. These analyses are vital for evaluating the overall quality, nutritional value, and technological benefits of various treatments.

Moisture content: Moisture content significantly affects the texture, shelf life, and stability of fish products. The moisture content of fish fillets was determined by oven-drying at 105°C until a constant weight was achieved (Association of Official Analytical Chemists [AOAC], 2005). Fish with higher moisture content tend to be more prone to microbial growth and spoilage, thus moisture retention in coated fish fillets is an essential aspect of storage stability (Huss, 1995). Coating materials can help retain moisture and reduce weight loss during storage (Aghdam *et al.*, 2020).

Crude protein: Crude protein content is a key indicator of the nutritional value of fish. Protein was determined by the Kjeldahl method (AOAC, 2005), which involves digestion, distillation, and titration. Fish protein is a vital source of essential amino acids, and coatings that do not negatively impact protein content contribute to the overall quality of fish fillets (Chen *et al.*, 2019). Additionally, maintaining protein integrity during storage is critical for the preservation of fish quality (Esmaili *et al.*, 2022).

Crude fat: Crude fat content was measured using the Soxhlet extraction method (AOAC, 2005). Fat content in fish products is essential for flavor, texture, and the overall mouthfeel. However, excessive fat can increase the risk of lipid oxidation, leading to the deterioration of fish flavor and nutritional value. Coatings with antioxidants, such as those containing MPE, can help mitigate oxidative damage by preserving fat content and preventing rancidity (Gao *et al.*, 2020). Fat retention is also an

indicator of coating's effectiveness in reducing moisture evaporation during storage (López-Cervantes *et al.*, 2021).

Ash content: Ash content represents the mineral content of fish and was measured by incinerating a known weight of fillets in a muffle furnace at 550°C (AOAC, 2005). Minerals, such as calcium, phosphorus, and magnesium, are essential for the body and contribute to the overall nutritional value of the fish (Tang *et al.*, 2020). The ash content can also provide information about the inorganic components of fish that may be impacted by coatings or preservatives.

Carbohydrate content: Carbohydrate content was calculated by subtracting the values of moisture, protein, fat, and ash from the total weight of the sample (Jahurul *et al.*, 2015). Carbohydrates are not a significant macronutrient in fish but are present in coating materials, especially those containing flour and hydrocolloids such as gelatin and sodium alginate–xanthan gum. These carbohydrates contribute to the texture and structure of coating, and influence the overall acceptability and shelf life of fish fillets (Mohammad *et al.*, 2020).

The proximate composition was analyzed at the initial stage (0 day) and during storage (on day 4, 8, and 12) to determine the impact of different coatings and storage conditions on the nutritional stability of the fish. Coatings containing MPE, in both free form and nanoencapsulated, were compared to the synthetic antioxidant TBHQ to determine whether these natural preservatives offer nutritional advantages in terms of retention of protein, reduction of fat, and preservation of moisture. Additionally, this analysis helps to evaluate whether nanoencapsulation improves the stability and bioavailability of antioxidant compounds present in *M. piperita* (Burt *et al.*, 2020).

Texture profile analysis (TPA)

Texture profile analysis is a widely used method for assessing the mechanical properties of food products, such as fish fillets, to evaluate their sensory attributes, such as hardness, cohesiveness, elasticity, and chewiness. TPA provides valuable insights into the quality and consumer acceptability of fish fillets, especially after different coating treatments and during storage. In this study, TPA was performed on the fish fillets treated with various coatings to determine the effect of different formulations on the textural properties of the product.

Sample preparation

Fish fillets were prepared according to the methodology described in Section 2.4.1, with various treatments applied to the fillets, including the control (T0), G-SAX (2%) (T1), GSAX-TBHQ (100 ppm) (T2), GSAX-MPE (1,000 ppm) (T3), and GSAX-NCMPE (1,000 ppm) (T4). After coating and frying, the fillets were stored at 4°C for 12 days, and texture measurements were performed on day 0, 4, 8, and 12.

TPA procedure

The texture profile of coated fish fillets was determined using a Texture Analyser (TA.XT Plus, Stable Micro Systems, UK). The TPA method involves compressing the sample twice to simulate the chewing action and assess multiple textural attributes. The following procedure was followed:

1. *Sample size and preparation*: Fish fillet samples (approximately 3.5 × 2.5 × 1.6 cm) were cut into uniform pieces to ensure consistency in texture measurements. For testing, the samples were placed on a platform of texture analyzer.

2. *Compression test*: The samples were subjected to a two-cycle compression test using a 50-kg load cell. The first compression cycle was used to break sample's structure, and the second cycle was employed to simulate the deformation encountered during chewing. Cylindrical probe was used for compression (2.5-cm diameter) with a 30% strain.

3. *Measurement parameters*: The following texture parameters were determined:
   - Hardness: The maximum force required to compress the sample during the first compression cycle. It is a measure of sample's firmness or softness.
   - Cohesiveness: It is the extent to which the sample can be deformed prior to breaking. It is the ratio of the area under the second compression to the area under the first compression.
   - Springiness: It is the distance the sample returns to its original height after the first compression cycle, representing the elasticity of the sample.
   - Chewiness: The product of hardness, cohesiveness, and springiness, which indicate how much force is required to chew the sample.
   - Gumminess: The product of hardness and cohesiveness, reflecting the energy required to break down the sample.

4. *Instrument settings*:
   - Test speed: 1 mm/s during both compression and return cycles.
   - Pre-test speed: 2 mm/s.
   - Post-test speed: 2 mm/s.
   - Trigger force: 5 g.
   - Data acquisition rate: 200 points/s.

5. *Data analysis*: The data obtained from the TPA test was analyzed to determine the textural attributes of

different treatments over the 12-day storage period. Textural changes over time were compared across different treatments to assess how the coatings impacted the texture and how these changes correlated with the storage period.

pH Value

The pH value of food products, including fish fillets, plays a significant role in determining their freshness, shelf life, and the overall quality. The pH of fish fillets affects enzymatic activity, microbial growth, and the stability of lipids and proteins. Monitoring the pH of fish fillets throughout storage provides valuable insights into the effects of various coating treatments on product quality, including the prevention of spoilage and the preservation of flavor and texture. The pH value of fish fillets was determined using a pH meter (Orion Star A211; Thermo Scientific, USA) equipped with a glass electrode. For each measurement, approximately 10 g of fish fillet was taken from each treatment group (T0, T1, T2, T3, and T4). The sample was placed in a sterile beaker, and 20 mL of distilled water was added to homogenize the sample. The sample and water were mixed using a homogenizer (Ultra-Turrax T25; IKA, Germany) for 1–2 min until the mixture turned uniform. The homogenization process ensured that the pH was measured accurately, as the fish fillet's internal pH could differ from its surface pH. The pH meter electrode was calibrated using standard buffer solutions (pH 4.0, 7.0, and 10.0) prior to measurement. After calibration, pH electrode was immersed in a homogenized fish fillet suspension, and the pH was recorded. After each measurement, the electrode was thoroughly rinsed with distilled water to prevent cross-contamination between samples. pH meter was recalibrated regularly to ensure accuracy throughout the testing process.

Lipid oxidation

Lipid oxidation is a major cause of deterioration in the quality of fish and other seafood products. This process leads to the formation of rancid flavors, off-odors, and the breakdown of important nutritional components, particularly PUFAs, which are abundant in the fish. The oxidative degradation of lipids can result in the reduction of sensory and nutritional quality of fish fillets, thereby affecting their consumer acceptance and shelf life.

In this study, the lipid oxidation of fish fillets treated with different coatings (T0, T1, T2, T3, and T4) was measured by determining the levels of malondialdehyde (MDA), a primary product of lipid peroxidation. The formation of MDA is often used as a reliable indicator of lipid oxidation. This assessment was carried out over a 12-day storage period at 4°C, with measurements taken on day 0, 4, 8, and 12. Fish fillets were prepared as described in Section 2.4.1, where they were coated with different formulations, fried, and stored at 4°C. Lipid oxidation was measured in triplicate for each treatment at specified time intervals (0, 4, 8, and 12 days). A sample of approximately 10 g of fish fillet was taken from each treatment group for analysis.

The lipid oxidation of fish fillets was assessed using the thiobarbituric acid reactive substances (TBARS) method, which measures MDA content. This method is widely used in food science and provides a quantitative measure of lipid oxidation. The procedure for determining MDA content is as follows:

1. Sample extraction: A 10-g sample of fish fillet was homogenized with 20 mL of 10% (w/v) trichloroacetic acid (TCA) solution in a blender. The mixture was then centrifuged at 3,000 rpm for 10 min at 4°C to separate supernatant, which contained lipid components.

2. Reaction with thiobarbituric acid: An aliquot (5 mL) of supernatant was added to 5 mL of 0.02-M thiobarbituric acid (TBA) reagent. TBA reagent was prepared by dissolving 0.02 g of TBA in 100 mL of distilled water and adjusting its pH to 2.0 with HCl. The mixture was heated in a water bath at 95°C for 30 min.

3. Cooling and measurement: After heating, the sample was cooled to room temperature, and the absorbance was measured at 532 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). The intensity of the pink color formed was directly proportional to the amount of MDA produced during lipid oxidation.

4. Calculation of MDA concentration: MDA concentration was calculated by comparing absorbance values with a standard curve prepared using 1,1,3,3-tetraethoxypropane, a known MDA precursor. The MDA content was expressed as micrograms of MDA per gram of fish fillet (μg MDA/g).

Instrumental color

Color is a critical quality attribute influencing consumer acceptance of fish fillets, as it reflects freshness and potential spoilage. Changes in color indicate oxidative degradation, microbial growth, or other quality losses. Therefore, color analysis is essential for evaluating the shelf life and quality of coated fish fillets. In this study, instrumental color measurements were conducted on Mackerel tuna fillets treated with different coatings (T0, T1, T2, T3, and T4) during storage. A colorimeter was used to obtain objective and reproducible values for lightness (L*), redness (a*), and yellowness (b*). Fillets were prepared according to the procedure mentioned in Section 2.4.1, coated with respective treatments, and subjected to the same frying and storage conditions as

described previously. Color measurements were taken for fresh and fried fillets on day 0 and then on day 4, 8, and 12 of refrigerated storage at 4°C.

The color of fish fillets was measured using a Minolta CR-400 colorimeter (Konica Minolta, Japan) equipped with a D65 light source. The instrument was calibrated using a white reference plate prior to each measurement. The color was expressed in the CIELAB color space, which includes the following three parameters:

1. L* (lightness): Represents the lightness of color, with values ranging from 0 (black) to 100 (white).

2. a* (redness-greenness): Indicates the balance of red and green hues, with positive values representing redness and negative values representing greenness.

3. b* (yellowness-blueness): Indicates the balance of yellow and blue hues, with positive values representing yellowness and negative values representing blueness.

Each sample was measured in triplicate at different locations on the surface of fish fillet to account for any potential variation in color across the fillet. Colorimeter probe was placed directly on the surface of fillet, and the measurements were recorded and averaged to obtain a representative value for each treatment group at each time interval.

Measurement procedure:

- Fresh fillets: Color measurements were taken immediately after preparation (T0, T1, T2, T3, and T4) and after the initial frying process.
- Storage and frying: After initial frying and cooling, the fillets were stored at 4°C for 0, 4, 8, and 12 days, and color measurements were taken at each of these time points.
  - Control and experimental groups: The following treatments were evaluated:
  - Control (T0): No coating or antioxidant treatment.
  - G-SAX (T1): Fish fillets coated with 2% gelatin and sodium alginate–xanthan gum.
  - Synthetic antioxidant (T2): Fish fillets coated with 2% GSAX and 100-ppm TBHQ.
  - MPE (T3): Fish fillets coated with 2% GSAXand free MPE at 1,000 ppm.
  - Nanoencapsulated MPE (T4): Fish fillets coated with 2% GSAX-NCMPE at 1,000 ppm.

Antimicrobial Activity of Films

The antimicrobial potential of the films was assessed using the agar diffusion method. Film discs (1 cm in diameter) were placed directly on nutrient agar plates inoculated with foodborne pathogens, including *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Pseudomonas* spp. Plates were incubated at 37°C for 24 h, and the diameters of inhibition zones were measured in millimeters. All assays were performed in triplicate to ensure reproducibility.

Microbiological assessment of fish fillets

Microbiological analysis is critical for evaluating the safety, quality, and shelf life of food products, particularly fish fillets. Fish spoilage and pathogenic contamination are influenced by bacteria, such as *E. coli*, *Salmonella* spp., *Listeria monocytogenes*, *Pseudomonas* spp., lactic acid bacteria (LAB), and *Enterobacter* spp. To simulate practical conditions, fish fillets were also subjected to frying, and microbial loads were evaluated pre- and post-cooking. Standard laboratory procedures were employed to quantify total microbial load and detect specific pathogens:

Microbiological tests were conducted using standard laboratory methods to evaluate both total microbial load and presence of specific pathogens. The following tests were performed:

1. Total viable count (TVC): The total number of live microorganisms in fish fillets was determined using the plate count method. A sample of fish fillet (10 g) was aseptically weighed and homogenized in 90 mL of sterile saline solution (0.85% NaCl). Serial dilutions were prepared, and a 1-mL aliquot from each dilution was plated on nutrient agar plates. Plates were incubated at 37°C for 24–48 h, and the colonies were counted. The results were expressed as log colony-forming units per gram (log CFU/g).

2. Pathogen detection:
   - *Escherichia coli*: The presence of *E. coli* was detected using the most probable number (MPN) method or by culturing on selective MacConkey agar, followed by confirmation using biochemical tests.
   - *Salmonella spp.*: *Salmonella* was detected using the ISO 6579 method of International Organization for Standardization (2002), where selective media, such as xylose lysine deoxycholate (XLD) agar was used, followed by biochemical tests for confirmation.
   - *Listeriamonocytogenes*: Listeria was detected using selective PALCAM agar, followed by polymerase chain reaction (PCR) confirmation if necessary.

3. Spoilage organisms:
   - *Pseudomonas spp.*: These bacteria are the primary spoilage organisms in fish. The

detection was performed by culturing fish samples on Pseudomonas agar base (PAB), followed by incubation at 30°C for 48 h. The colonies were counted and expressed as log CFU/g.
- *Lactic acid bacteria*: LAB influence the shelf life of fish products. The detection was done by culturing on De Man–Rogosa–Sharpe (MRS) agar, followed by incubation at 37°C for 48 h.

4. Yeasts and molds: Yeasts and molds were quantified by spreading the homogenized fish sample on potato dextrose agar (PDA) plates. Plates were incubated at 25°C for 3–5 days, and the colony count was recorded as log CFU/g.

5. Antibacterial activity of coatings: The ability of coatings (gelatin and sodium alginate–xanthan gum with and without MPE or NCMPE) to inhibit microbial growth was also evaluated by comparing microbial counts in treated and untreated fish fillets. The results were analyzed to determine the effectiveness of these coatings in reducing microbial contamination and extending the shelf life of fish fillets.

Fish fillet samples from treatment groups (T0–T4) were analyzed on day 0, 4, 8, and 12 of refrigerated storage at 4°C. Each assay was conducted in triplicate, and the results were expressed as mean ± standard deviation (SD) (log CFU/g). Post-frying microbial counts were also recorded to evaluate the impact of cooking on microbial load.

Sensory analysis

Sensory evaluation was conducted to assess the acceptability, quality, and consumer perception of Mackerel tuna fillets coated with different formulations, including MPE and NCMPE. The study aimed to evaluate the impact of coatings and antioxidants on key sensory attributes, including appearance, texture, flavor, odor, crispiness, and the overall acceptability. Fish fillets were treated with the following formulations:

- T0: Control (uncoated)
- T1: G-SAX
- T2: GSAX-TBHQ
- T3: GSAX-MPE
- T4: GSAX-NCMPE

After coating, fillets were fried for 1 min at 180°C and stored at 4°C for up to 12 days. Sensory evaluation was conducted both immediately after frying and at 4, 8, and 12 days of refrigerated storage. A trained panel of 20–25 members, experienced in the evaluation of fish products, assessed the samples. Fish portions of 3–4 cm were served in a random order, and water was provided to cleanse the palate between samples' evaluation. Evaluations were performed in a controlled sensory room with neutral lighting and a temperature of 22°C. The following attributes were assessed using a 9-point hedonic scale (1 = lowest, and 9 = highest):

1. *Appearance*:
   - Evaluation criteria: Visual appeal, color, uniformity of coating, and the overall presentation.
   - Scale: 1–9 scale (1 = very poor/lowest, and 9 = excellent/highest).

2. *Texture*:
   - Evaluation criteria: Tenderness, juiciness, crispiness of the coating, and the chewiness of fish flesh.
   - Scale: 1–9 scale (1 = very tough, and 9 = very tender).

3. *Flavor*:
   - Evaluation criteria: The overall flavor balance between the fish taste and the coating ingredients, including any off-flavors because of oxidation or the presence of additives, such as TBHQ or MPE.
   - Scale: 1–9 scale (1 = very poor/lowest, and 9 = excellent/highest).

4. *Odor*:
   - Evaluation criteria: The freshness of the fish and coating, the absence of any rancid or stale odors, and the perceived fragrance of coating (if any).
   - Scale: 1–9 scale (1 = very unpleasant, and 9 = very pleasant).

5. *Crispiness*:
   - Evaluation criteria: The crunchiness of the fried coating and its resistance to breaking when bitten.
   - Scale: 1–9 scale (1 = very soggy, and 9 = very crispy).

6. *Overall acceptability*:
   - Evaluation criteria: The overall judgment of the fillet based on appearance, texture, flavor, and odor.
   - Scale: 1–9 scale (1 = extremely unacceptable, and 9 = extremely acceptable).

The samples were tested immediately after frying and at intervals of 4, 8, and 12 days of refrigeration at 4°C. Fish fillet portions of approximately 3–4 cm were served to panelists in a random order to avoid bias. Panelists were provided with water to cleanse their palate between samples. All sensory tests were conducted in a controlled sensory evaluation room with neutral lighting and a temperature of 22°C to avoid influencing panelists' perceptions.

Statistical analysis

All experiments were performed in triplicate to ensure reproducibility. Data were collected for technological, physicochemical, microbiological, and sensory attributes of Mackerel tuna fillets at four storage periods: fresh (0 day, post-frying) and after 4, 8, and 12 days of refrigerated storage. Mean values for different treatments—control, gelatin and G-SAX coating, GSAX-TBHQ, free MPE, and NCMPE—were compared using one-way analysis of variance (ANOVA). When ANOVA indicated significance ($p \leq 0.05$), pairwise comparisons were performed using Tukey's Honest Significant Difference (HSD) test. For variables measured across multiple storage periods, repeated measures of ANOVA were employed to assess treatment × time interactions, accounting for correlations between repeated observations. Descriptive statistics, including mean, SD, and standard error, were calculated for all parameters. Pearson's correlation analysis was conducted to explore relationships between key variables, such as antioxidant activity versus TBARS levels, and sensory scores versus storage duration. All statistical analyses were performed using the SPSS software (version 26.0 or later), and graphical outputs were generated with GraphPad Prism. Statistical significance was defined at $p \leq 0.05$.

Results and Discussion

Phytochemical profile of *Mentha piperita*

The phytochemical analysis of MPE identified several bioactive compounds with potential applications in food preservation and health. The compounds were identified using gas chromatography-mass spectrometry (GC-MS), and their retention time (RT), peak areas (PA), molecular weights (MW), and molecular formulas (MF) are summarized in Table 2.

Compounds Identified in the Mentha piperita extract

Table 2 presents the compounds identified in MPE. Chemical analysis of MPE revealed a complex profile of bioactive compounds, many of which exhibited significant antioxidant, antimicrobial, and therapeutic properties. Major constituents identified include benzyl alcohol (RT: 6.125 min), benzoic acid, methyl ester (RT: 6.839 min), methyl tetradecanoate (RT: 12.483 min),

Table 2. Compounds identified in *Mentha piperita* extract.

No.	Retention time (min)	Peak area (%)	Molecular weight (g/mol)	Molecular formula (MF)	Name of compound
1.	6.125	0.43	108	C_7H_8O	Benzyl alcohol
2.	6.839	1.07	136	$C_8H_8O_2$	Benzoic acid, methyl ester
3.	7.526	0.47	124	C_9H_{16}	3-Octyne, 7-methyl-
4.	8.058	0.54	144	$C_6H_8O_4$	1,4:3,6-Dianhydro-α-d-glucopyranose
5.	8.685	0.36	150	$C_{10}H_{14}O$	3,5-Heptadienal, 2-ethylidene-6-methyl-
6.	8.834	0.36	150	$C_{10}H_{14}O$	Phenol, 2-methyl-5-(1-methylethyl)-
7.	8.920	0.46	117	C_8H_7N	Indole
8.	10.881	0.59	214	$C_{13}H_{26}O_2$	Undecanoic acid, 10-methyl-, methyl ester
9.	11.111	0.64	180	$C_{11}H_{16}O_2$	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7atrimethyl-,(R)-
10.	11.825	0.24	190	$C_{13}H_{18}O$	Megastigmatrienone
11.	12.483	2.57	242	$C_{15}H_{30}O_2$	Methyl tetradecanoate
12.	12.576	0.34	212	$C_{16}H_{20}$	2,6-Diisopropylnaphthalene
13.	12.643	0.26	212	$C_{16}H_{20}$	1,4-Di-iso-propylnaphthalene
14.	12.795	0.22	206	$C_{15}H_{26}$	Caparratriene
15.	13.056	0.57	256	$C_{16}H_{32}O_2$	Methyl 13-methyl tetradecanoate
16.	13.180	1.59	296	$C_{19}H_{36}O_2$	6-Octadecenoic acid, methyl ester, (Z)-
17.	13.275	0.59	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methylester
18.	13.360	0.26	282	$C_{18}H_{34}O_2$	11,13-Dimethyl-12-tetradecen-1-ol acetate
19.	13.590	0.40	278	$C_{20}H_{38}$	Neophytadiene
20.	13.669	0.90	268	$C_{18}H_{36}O$	2-Pentadecanone, 6,10,14-trimethyl
21.	13.869	0.26	182	$C_{12}H_{22}O$	8,10-Dodecadien-1-ol, (E,E)-
22.	14.164	0.36	270	$C_{17}H_{34}O_2$	Pentadecanoic acid, 14-methyl-, methyl ester
23.	14.391	0.54	268	$C_{17}H_{32}O_2$	Methyl hexadec-9-enoate
24.	39.93	39.93	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester

neophytadiene (RT: 13.590 min), and hexadecanoic acid and methyl ester (RT: 39.93 min). Benzyl alcohol is valued for its antimicrobial, antifungal, and aromatic properties, and is widely used as a preservative in both food and pharmaceutical applications because of its ability to inhibit microbial growth and extend shelf life. Benzoic acid, methyl ester, is known for its antioxidant and antimicrobial activity, contributing to food stability and spoilage inhibition (Hughes *et al.*, 2018). Methyl tetradecanoate, a fatty acid ester, provides antimicrobial and emollient effects, enhancing the stability of food and cosmetic formulations (Jackson and Reilly, 2017). Neophytadiene, a sesquiterpene, exhibits strong antioxidant activity and potential for reducing oxidative stress, making it a promising component for food preservation and functional health products (Smith *et al.*, 2019). Hexadecanoic acid, methyl ester, a saturated fatty acid ester, is widely incorporated into cosmetic and pharmaceutical formulations because of its emollient and antioxidant properties (Brown and Green, 2020). Collectively, these compounds create a synergistic network of bioactive components, endowing MPE with antioxidant, antimicrobial, and anti-inflammatory activities. Such multifunctional properties highlight its potential applications in natural food preservation, health supplements, and cosmetic formulations, addressing the growing consumer demand for natural and sustainable ingredients.

The chromatogram (Figure 1) displays distinct peaks representing the compounds identified in MPE. Each peak's RT corresponds to the duration that each compound took to elute through the chromatographic column, while the peak area (%) indicates its relative abundance. The MPE comprises a diverse array of bioactive compounds, such as phenols, alcohols, acids, and esters, known for their antioxidant and antimicrobial activities. These constituents suggest that MPE holds strong potential of being a natural preservative, capable of reducing lipid oxidation, and improving sensory qualities in fish fillets during storage. Moreover, the extract's bioactive profile supports its effectiveness in replacing synthetic preservatives in food systems. These findings are consistent with previous reports underscoring peppermint's beneficial applications in food preservation and health (Rahman *et al.*, 2021; Sharma and Singh, 2019).

DPPH radical scavenging activity (%)

The antioxidant activity of MPE was evaluated by determining the half-maximum inhibition concentration (IC_{50}) and DPPH radical scavenging activity. The results are summarized in Table 3. The DPPH radical scavenging activity was measured to assess the antioxidant potential of different MPEs. The methanol/ethanol extract exhibited the highest activity (82.82 ± 2.57%) with an IC_{50} value of 10.02 mg/mL, suggesting that this extract had significant antioxidant properties. The methanol extract, with an IC_{50} value of 14.77 mg/mL, displayed the lowest scavenging activity (66.98 ± 1.33%). Comparatively, gallic acid and ascorbic acid, known antioxidants, showed higher scavenging activities, indicating that MPEs have

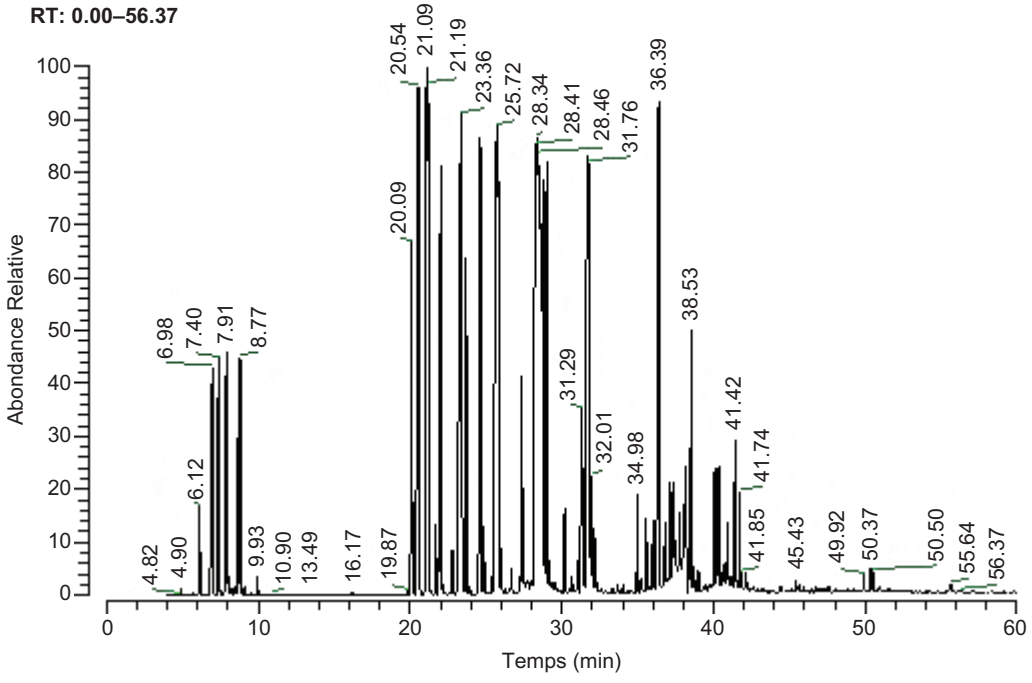

Figure 1. Compounds identified in *Mentha piperita* extract.

Table 3. DPPH scavenging activity.

Extract	IC_{50} (mg/mL)	DPPH radical scavenging activity (%)
Methanol	14.77 ± 0.06[d]	66.98 ± 1.33[a]
Ethanol	11.32 ± 0.71[c]	80.74 ± 0.83[b]
Methanol/ethanol	10.02 ± 0.63[b]	82.82 ± 2.57[b]
Gallic acid	0.15 ± 0.01[a]	93.00 ± 0.45[c]
Ascorbic acid	0.16 ± 0.01[a]	92.30 ± 0.26[c]

Note: Each value is presented as mean ± SD (n = 3).

substantial antioxidant potential but are less potent than these reference compounds. The results demonstrate that the DPPH radical scavenging activity of MPEs was dependent on the solvent used for extraction.

The methanol/ethanol extract of *Mentha piperita* exhibited the highest antioxidant activity, with a DPPH radical scavenging rate of 82.82 ± 2.57% and an IC_{50} value of 10.02 mg/mL, indicating that the mixed solvent effectively extracted bioactive compounds with potent antioxidant properties. This aligned with previous reports highlighting ethanol and methanol as efficient solvents for phenolic extraction with strong antioxidant effects (Liu *et al.*, 2018; Naczk and Shahidi, 2004). The ethanol extract of *Mentha piperita* showed moderate scavenging activity (80.74 ± 0.83%) and a slightly higher IC_{50} (11.32 mg/mL), confirming its efficacy. In contrast, the methanol extract of *Mentha piperita* demonstrated the lowest activity (66.98 ± 1.33%) and a higher IC_{50} (14.77 mg/mL), suggesting methanol alone was less effective than the solvent mixture. Positive controls of gallic acid and ascorbic acid exhibited scavenging rates of 93.00 ± 0.45% and 92.30 ± 0.26%, respectively, confirming the substantial antioxidant potential of the MPEs despite being less potent than these standards. These results emphasized that antioxidant activity depends on the extraction solvent, with methanol/ethanol mixtures providing superior recovery of active compounds, underscoring the importance of solvent choice in bioactive compound extraction for food, cosmetic, and pharmaceutical applications.

Total phenolic and total flavonoid content

The TPC) and TFC of various MPEs are presented in Table 4, revealing significant differences between solvents. The methanol extract of *Mentha piperita* exhibited the highest TPC (3.57 ± 0.26 g gallic acid/100 g), followed by the methanol/ethanol MPC (2.89 ± 0.30 g gallic acid/100 g), while the ethanol extract of *Mentha piperita* had the lowest TPC (1.99 ± 0.32 g gallic acid/100 g). This indicated that methanol was more efficient at extracting phenolic compounds, consistent with prior studies attributing methanol's efficacy to its ability to solvate polyphenolic structures (Liu *et al.*, 2017; Xu *et al.*, 2018). In terms

Table 4. Total phenolic and flavonoid contents.

Extract	Total phenolic content (g gallic acid/100 g)	Total flavonoid content (g quercetin/100 g)
Methanol	3.57 ± 0.26[c]	2.48 ± 0.10[b]
Ethanol	1.99 ± 0.32[a]	1.31 ± 0.20[a]
Methanol/ethanol	2.89 ± 0.30[b]	3.33 ± 0.12[c]

Note: Each value is presented as mean ± SD (n = 3).

of flavonoid content, the methanol/ethanol extract of *Mentha piperita* contained the highest amount (3.33 ± 0.12 g quercetin/100 g), followed by methanol (2.48 ± 0.10 g quercetin/100 g), with ethanol yielding the lowest flavonoid content (1.31 ± 0.20 g quercetin/100 g). These findings suggested that a combination of methanol and ethanol was particularly efficient in extracting flavonoids, known for their antioxidant and anti-inflammatory properties (Jeong and Lee, 2014; Liu *et al.*, 2018). The higher phenolic and flavonoid content in the methanol extract of *Mentha piperita* can be attributed to the polarity of methanol, which effectively dissolves a wide range of phenolic compounds. The methanol/ethanol mixture appears to extract a broader spectrum of bioactive compounds, thus leading to higher flavonoid content. Lower values in the ethanol extract could be due to its polarity, which may not extract the full range of phenolic and flavonoid compounds present in *M. piperita*. These results highlighted the potential of MPEs as a natural source of phenolic and flavonoid compounds, which have well-documented health benefits, such as antioxidant, anti-inflammatory, and antimicrobial activities. The use of appropriate solvent systems can enhance the extraction and bioavailability of these beneficial compounds.

Anthocyanin content

The anthocyanin content of different extracts of *Mentha piperita* was assessed, and the results are presented in Table 5.

Anthocyanin content varied significantly between MPEs. The methanol extract of *Mentha piperita* showed the lowest anthocyanin level (0.10 ± 0.04 g/100 g), while the ethanol extract contained a higher amount (0.86 ± 0.07 g/100 g). The highest anthocyanin concentration

Table 5. Anthocyanin content.

Extract	Anthocyanin content (g/100 g)
Methanol	0.10 ± 0.04[a]
Ethanol	0.86 ± 0.07[b]
Methanol/ethanol	1.74 ± 0.21[c]

Note: Each value is presented as mean ± SD (n = 3).

was observed in the methanol/ethanol mixture extract of *Mentha piperita* (1.74 ± 0.21 g/100 g), indicating that the combined solvent system is more effective for anthocyanin extraction than either solvent alone. This may be attributed to ethanol's lower polarity, compared to methanol, enhancing solubility of hydrophilic anthocyanins, while the mixture creates an optimal polarity environment for extraction. These findings aligned with previous studies reporting that solvents of intermediate polarity, such as methanol/ethanol blends, maximize anthocyanin recovery from plant materials (Cheng *et al.*, 2017). Given their potent antioxidant activity and health benefits—including anti-inflammatory, anticancer, and cardiovascular protective effects—anthocyanins are valuable bioactive compounds for food and medicinal applications.

Field Emission Scanning Electron Microscopy

The FESEM images of nanocomposite films incorporating MPEs with gelatin and sodium alginate–xanthan gum matrix provided crucial insight into the structural features of the films, and directly affected their potential applications, particularly in the preservation of fish meat (Figure 2). These FESEM images offer a detailed view of the nanocomposite films incorporating MPEs within a gelatin and sodium alginate–xanthan gum matrix. These images provide valuable insights into the morphological structure and distribution of various components within nanocomposite films.

Scanning electron microscopy (SEM) revealed a heterogeneous yet well-structured surface morphology of the nanocomposite films incorporating MPE. At a 2-µm scale, the surface exhibited a granular texture, indicating that the extract was dispersed throughout the polymer matrix, potentially as nanocapsules or microparticles. Such granules may contribute to the controlled release of bioactive compounds, including flavonoids and essential oils, enhancing antimicrobial efficacy and preserving fish freshness (Ramires *et al.*, 2021; Zhang *et al.*, 2020). Clusters of particles were also observed, suggesting partial aggregation of the extract or other film constituents. According to Jafari *et al.* (2017), such aggregation can slow the release of active compounds, acting as reservoirs that prolong antimicrobial activity. The rough and uneven surface may improve adhesion to food surfaces, reducing moisture loss and increasing contact area for antimicrobial action (Silva *et al.*, 2019).

At a larger scale (10 µm), a fibrous morphology characteristic of biopolymer-based films (gelatin and sodium alginate) was evident. These fibrous networks enhance mechanical properties, such as tensile strength and flexibility, critical for maintaining film integrity and forming

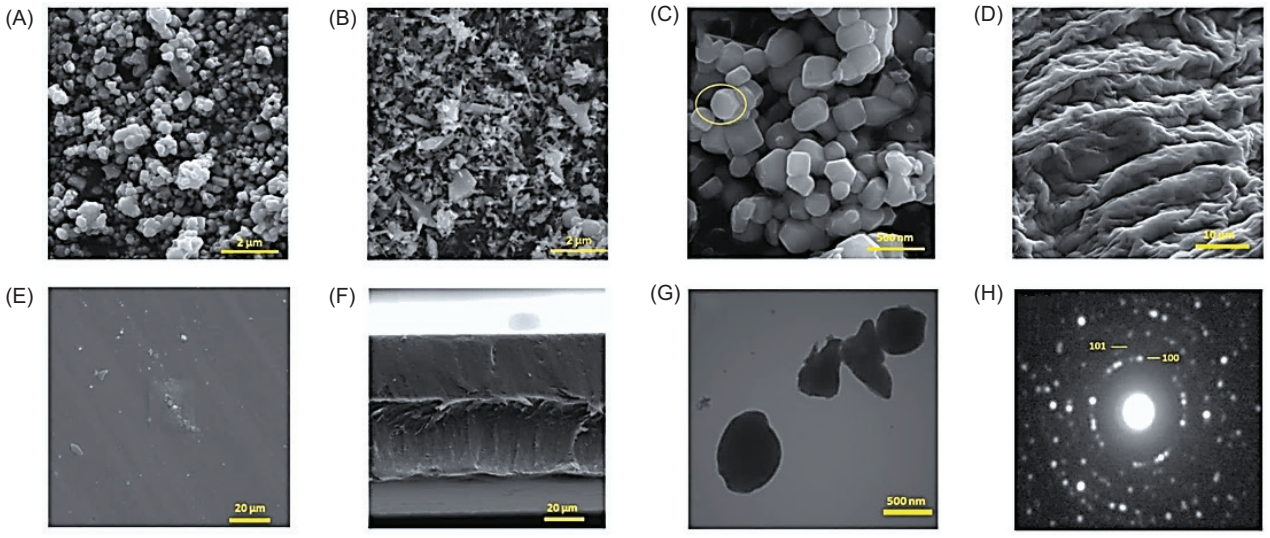

Figure 2. The FESEM images of gelatin and sodium alginate–xanthan gum-based nanocomposite films containing MPEs and their application on fish meat. (A) Control film without extract showing smooth and compact surface; (B) film with free MPE showing slight surface roughness and small pores; (C) film with NCMPE displaying uniform nanoparticle distribution; (D) surface of fish meat wrapped with control film after storage, showing noticeable microbial colonization; (E) surface of fish meat wrapped with film containing free extract, showing reduced microbial growth; (F) surface of fish meat wrapped with film containing NCMPE, showing minimal microbial presence; (G) cross-sectional view of control film illustrating dense but homogeneous structure; and (H) cross-sectional view of NCMPE film revealing evenly dispersed nanoparticles. Scale bars: (A–C and G–H) = 10 µm; (D–F) = 20 µm.

effective barriers against moisture and oxygen, which are essential for fish preservation (Moghaddam and Olayinka, 2020). At a 20-µm scale, the film displayed a smooth and homogeneous surface, suggesting good matrix formation and uniform dispersion of components. Smooth surfaces are associated with improved barrier properties, reducing water vapor and gas permeability, and contributing to the extended shelf life of food products (Lamsal and Roos, 2017). Overall, the SEM observations indicate that the combination of granular, fibrous, and smooth regions provides a multifunctional structure that supports both mechanical performance and controlled release of bioactive compounds, enhancing the protective and preservative efficacy of nanocomposite films.

The smoothness of surface may indicate a well-formed matrix, contributing to the film's ability to act as a barrier for moisture and oxygen, essential for food preservation. The cross-sectional view reveals uniformity in the film structure, which is important for maintaining the integrity of film during application and storage. Cross-sectional uniformity ensures that the film has consistent thickness and mechanical properties. A consistent structure is essential for uniform distribution of active components and effective barrier performance. According to Raghavendra and Thakur (2020), films with homogeneous structures provide better stability and uniformity in the release of active compounds, ensuring more consistent preservation of food products, such as fish. The cross-sectional view provides insight into the internal structure of nanocomposite film, showing uniformity in the film's composition. This cross-sectional image highlights the internal organization, which is important for understanding the interaction of MPE with matrix and its potential for uniform distribution in the film. Larger aggregated particles or clusters are visible at the 500-nm scale. Larger aggregates could influence the mechanical properties of the film. These aggregates may also slow down the release of bioactive compounds.

The FESEM images revealed that MPE-based nanocomposite films exhibited a combination of granular, fibrous, and aggregated structures. Nanoparticle aggregation was evident at a microscale, which could slow the release of bioactive compounds, providing sustained antimicrobial activity, beneficial for long-term preservation of fish. Larger particle clusters may also influence the mechanical and thermal properties of films, contributing to controlled release and enhanced functional performance.

Fibrous morphology, typical of biopolymer-based matrices (gelatin and sodium alginate), supports mechanical strength and flexibility, while smooth and homogeneous regions improve barrier properties, reducing water vapor and gas permeability and protecting fish fillets from moisture loss. Granular structures probably correspond to dispersed MPE, and can act as reservoirs for antimicrobial agents, such as flavonoids and essential oils, further contributing to the extension of shelf life.

X-ray diffraction (XRD) analysis revealed characteristic peaks labeled '101' and '-110', indicating the presence of crystalline regions within nanocomposites. These ordered crystalline domains enhance mechanical strength, barrier performance, and durability while potentially reducing water vapor permeability—critical factors for food preservation (Zhang *et al.*, 2020).

Overall, both FESEM and XRD analyses demonstrated that nanocomposite films possessed a well-organized microstructure with a balanced combination of granular, fibrous, and crystalline features. This structure supports both physical integrity and functional performance of films, enabling controlled release of bioactive compounds, effective barrier properties, and sustained antimicrobial activity, making them suitable for improving the shelf life and quality of fish meat during storage.

Technological and physicochemical properties of fish samples

Proximate composition

The proximate composition of different fish samples, including control and various formulations (G-SAX, GSAX-TBHQ, GSAX-MPE, and GSAX-NCMPE), was evaluated and the results are presented in Table 6. The moisture content of all fish formulations was relatively similar, ranging from 69.60% to 71.16%. There were no significant differences among the samples, indicating that different treatments (G-SAX, GSAX-TBHQ, GSAX-MPE, and GSAX-NCMPE) did not notably affect the moisture content of fish. The protein content across fish formulations was also comparable, with no significant differences (ranging from 15.64% to 16.02%).

This suggests that the treatments did not influence the protein content of the fish, and the nutritional value related to protein remained stable across all samples. There were significant variations in lipid content. The GSAX-MPE formulation exhibited the highest lipid content (10.98 ± 0.70 g/100 g), which was significantly higher than the control and other formulations. This could be due to the incorporation of specific additives or treatments that might affect lipid retention or enhance lipid accumulation in the fish.

The ash content, which represents inorganic matter or minerals in the fish, was similar across all the formulations (ranging from 2.69% to 2.80%). No significant differences were observed, indicating that the mineral content remained unaffected by various treatments.

Carbohydrate content varied slightly among the formulations but did not show significant differences. The values ranged from 0.87% to 1.35%, suggesting that the treatments did not significantly alter the carbohydrate profile of the fish.

The pH of fish samples was slightly acidic (ranging from 6.00 to 6.11) but did not vary significantly among various formulations. This suggests that the pH of the fish remained stable despite different treatments, which indicated a neutral effect on the acid–base balance of the samples.

In conclusion, the proximate composition of fish samples showed consistency in terms of moisture, protein, ash, carbohydrates, and pH across different formulations, with a notable exception of lipid content, which was significantly affected by the GSAX-MPE formulation. These findings suggested that the use of these treatments affected specific nutritional components, such as lipids, while not significantly altering other aspects of fish's composition.

The proximate composition of fish is essential for evaluating its nutritional value, and understanding how different treatments affect the composition can help optimize preservation techniques and improve product quality. In this study, the proximate composition of various fish formulations, including the control and different treatment groups (G-SAX, GSAX-TBHQ, GSAX-MPE, and GSAX-NCMPE), was evaluated. The moisture content of fish samples ranged from 69.60% to 71.16%. Moisture is a key factor in determining the shelf life and texture of fish products. Higher moisture content typically indicates more perishable products, while lower moisture content suggests drier and more shelf-stable products.

In this study, no significant differences were found between the control and the treated samples. This suggests that various treatments, such as the incorporation of various antioxidants (e.g., TBHQ and MPE), did not significantly affect the water retention properties of the fish. This is an important observation because moisture content plays a role in microbial growth, and treatments that stabilize moisture can influence the shelf life of fish. Proteins are a major nutritional component of fish and contribute significantly to its dietary value. In this study, the protein content ranged from 15.64% to 16.02% across all fish formulations, showing no significant variations between the control and treated samples. Protein content remained largely unaffected by the treatments, indicating that the use of antioxidants and natural extracts did not interfere with the protein integrity of the fish. This consistency is crucial because any substantial variation in protein content could influence the overall nutritional quality of the fish.

Lipids play an important role in the flavor and texture of fish as well as its nutritional content. In the present study, lipid content ranged from 9.34% to 10.98%. A significant increase in lipid content was observed in the GSAX-MPE formulation (10.98 ± 0.70 g/100 g), compared to the control and other treatment groups. This suggests that the addition of mango peel extract may promote the retention of lipids in the fish, which could be due to its antioxidant properties that helped to preserve lipids from oxidation. Lipids are essential for the nutritional profile of fish, but excess lipid accumulation can also impact texture and taste. Therefore, understanding how different treatments affect lipid content is important for balancing nutritional value with product quality. Ash content reflects the mineral composition of fish, and in this study, it ranged from 2.69% to 2.80% across all formulations, with no significant differences between the control and treated fish samples.

This consistency suggested that various treatments did not alter the mineral content of fish significantly. The mineral profile of fish is crucial for human nutrition, particularly regarding to essential minerals, such as calcium, phosphorus, and magnesium. Since ash content remained stable, the treatments did not affect the mineral balance of fish. The carbohydrate content of fish samples ranged from 0.87% to 1.35%. While the carbohydrate content was slightly higher in both GSAX-TBHQ and GSAX-MPE formulations, the differences were not statistically significant. This indicates that the treatments did not significantly alter the carbohydrate content, and any small variation may be due to the inherent composition of the additives used in these formulations. Carbohydrates are typically a small component in fish, and their presence can be influenced by additives, such as sugars or starches, in the preservation or treatment process. The lack of significant differences suggests that fish's carbohydrate content remained relatively stable across different treatments. The pH values of fish samples were slightly acidic, ranging from 6.00 to 6.11, with no significant differences observed across the formulations. pH plays a crucial role in the shelf life of fish, as it can affect microbial growth and enzymatic activity.

Fish are generally more prone to spoilage under neutral or alkaline conditions, which makes maintaining an acidic environment beneficial for preservation. In this study, the treatments did not significantly alter fish's pH, suggesting that the preservation methods used did not induce any changes in the acid–base balance of the fish, which could influence spoilage or quality. In conclusion, the proximate composition of fish remained largely unaffected by various treatments, with the exception of lipid content. The GSAX-MPE formulation resulted in a significant increase in lipid content, which indicated that this treatment had a stabilizing effect on lipids, possibly

Table 6. Proximate composition of fish samples.

Parameter	Control	G-SAX	GSAX-TBHQ	GSAX-MPE	GSAX-NCMPE
Moisture (g/100 g)	71.13 ± 0.30[a]	71.16 ± 0.50[a]	69.81 ± 0.59[a]	69.60 ± 0.79[a]	70.66 ± 0.61[a]
Protein (g/100 g)	15.64 ± 0.42[a]	15.92 ± 0.37[a]	16.00 ± 0.72[a]	16.02 ± 1.45[a]	16.02 ± 0.46[a]
Lipid (g/100 g)	9.59 ± 0.29[a]	9.34 ± 0.57[a]	10.10 ± 0.85[a,b]	10.98 ± 0.70[b]	9.53 ± 0.72[a]
Ash (g/100 g)	2.69 ± 0.01[a]	2.71 ± 0.06[a]	2.74 ± 0.14[a]	2.80 ± 0.05[a]	2.78 ± 0.04[a]
Carbohydrate (g/100 g)	0.94 ± 0.15[a]	0.87 ± 0.59[a]	1.35 ± 0.42[a]	1.34 ± 1.97[a]	1.01 ± 0.20[a]
pH	6.10 ± 0.05[a]	6.07 ± 0.04[a]	6.00 ± 0.09[a]	6.04 ± 0.05[a]	6.11 ± 0.03[a]

Notes: Values are mean ± standard deviation (SD; n = 3).
Different superscript lowercase alphabets (a–b) in the same row indicate significant differences between treatments ($p < 0.05$). No uppercase alphabets are used as no significant differences over storage period were noted in this table.

because of its antioxidant properties. Other components, such as moisture, protein, ash, carbohydrates, and pH, remained consistent across all formulations, suggesting that the treatments did not interfere significantly with fish's nutritional profile or quality. These findings are important because they suggest that the treatments used in this study, such as GSAX-MPE, could alter specific aspects of the fish's composition, particularly lipids, without negatively affecting its overall nutritional value. This can have implications for developing fish products with enhanced shelf life and nutritional quality.

Lipid oxidation

Lipid oxidation is one of the primary causes of deterioration of fish products, leading to the development of rancidity, off-flavors, and a reduction in nutritional value. Lipid oxidation is influenced by various factors, such as composition of fish, presence of antioxidants, and the processing methods used. In this study, lipid oxidation was assessed across different fish formulations to evaluate the effectiveness of treatments, such as antioxidants and natural extracts, in controlling lipid deterioration. Lipid oxidation is a critical factor affecting the quality and shelf life of fish and other seafood products. Oxidative rancidity occurs when PUFAs present in fish lipids are oxidized by reactive oxygen species (ROS), leading to the formation of various undesirable compounds, including aldehydes and ketones, which significantly impair the taste, odor, and nutritional value of the product. Formation of MDA, a by-product of lipid oxidation, is a widely used marker for lipid peroxidation, often quantified through TBARS assay. The degree of lipid oxidation was measured using TBARS assay, which quantifies MDA, a primary product of lipid peroxidation. The results, shown in Figure 3, indicate that the control group, which did not receive any antioxidant treatment, showed the highest levels of lipid oxidation, as evidenced by a higher TBARS value. This suggests that without the addition of antioxidants, the fish samples experienced significant lipid peroxidation over a storage period. Fish samples treated with G-SAX showed a moderate reduction in lipid oxidation, compared to the control. This indicates that green seaweed extract (G-SAX) has some antioxidant properties, although it may not be as effective as other treatments in preventing lipid peroxidation. The GSAX-TBHQ formulation displayed a significant reduction in lipid oxidation, compared to the control group.

Tert-butylhydroquinone is a well-known synthetic antioxidant, and its combination with G-SAX proved to be more effective in limiting lipid peroxidation. This suggests a synergistic effect between natural and synthetic antioxidants. Similar to GSAX-TBHQ, the GSAX-MPE treatment exhibited a notable reduction in lipid oxidation.

Mango peel extract is rich in polyphenols, which are known for their antioxidant properties. Combination of G-SAX with MPE could enhance antioxidative effects, leading to lower TBARS levels. The GSAX-NCMPE formulation showed the lowest levels of lipid oxidation, with significantly reduced TBARS values. This indicates that natural antioxidants from mango peel, combined with G-SAX, provided the best protection against lipid peroxidation. The effectiveness of this treatment is attributed to the high polyphenol content of mango peel, which is known to scavenge free radicals and inhibit oxidative damage. The results of this study emphasized the importance of antioxidant treatments in controlling lipid oxidation in fish. Lipid oxidation can lead to the formation of undesirable compounds that affect flavor, color, and nutritional quality of fish products. The use of natural and synthetic antioxidants, such as G-SAX, TBHQ, and mango peel extract, showed varying levels of effectiveness in reducing lipid peroxidation. Both GSAX-TBHQ and GSAX-MPE treatments were more effective in reducing lipid oxidation than the G-SAX treatment alone. This highlights the synergistic potential of combining natural and synthetic antioxidants. The GSAX-NCMPE treatment, which used a natural combination of antioxidants,

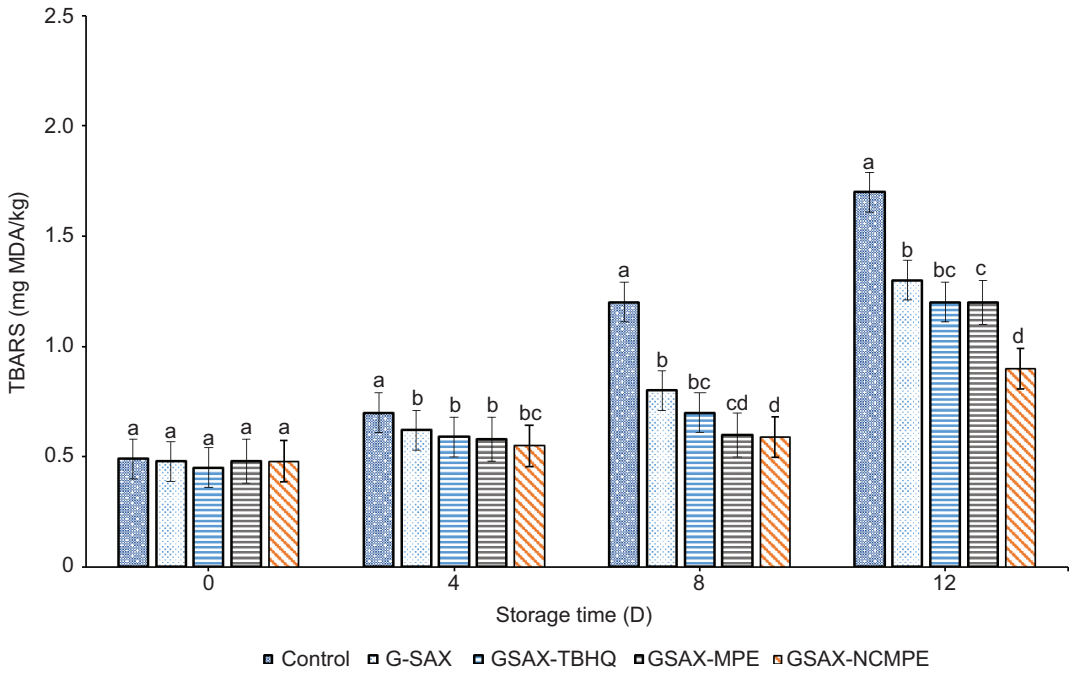

Figure 3. Lipid oxidation levels in fish treated with different antioxidant formulations, measured by TBARS assay.

provided the best protection, suggesting that the antioxidant properties of mango peel extract, when used in its natural form, offered superior lipid protection in fish. These findings were consistent with previous research, which demonstrated the ability of polyphenol-rich extracts, such as those from mango peel and seaweed, to reduce lipid oxidation in various food matrices, including fish (Alzahrani *et al.*, 2016; Vasilenko *et al.*, 2019).

In conclusion, lipid oxidation poses a major challenge in fish preservation, necessitating effective antioxidant strategies to extend shelf life and maintain product quality. The incorporation of natural antioxidants, such as G-SAX and mango peel extract, alone or combined with synthetic antioxidants, such as TBHQ, significantly reduces lipid peroxidation in fish. This study highlights the synergistic potential of combining natural and synthetic antioxidants to enhance oxidative stability. As shown in Figure 3, the untreated control exhibited the highest TBARS values, reflecting fish lipids' susceptibility to oxidation because of their high moisture and fat content. The G-SAX treatment moderately decreased lipid oxidation, attributed to bioactive compounds, such as polyphenols, carotenoids, and flavonoids, known for their free radical scavenging activity (Wang *et al.*, 2018). However, the modest effect may result from lower extract concentration or limited bioavailability in fish matrix. Notably, the combination of G-SAX with TBHQ markedly improved antioxidant efficacy, underscoring the benefits of integrating natural and synthetic antioxidants in fish preservation.

This observation supports the hypothesis that synthetic antioxidants, such as TBHQ, can work synergistically with natural antioxidants to achieve more potent antioxidant effects. TBHQ is widely used in food products because of its strong ability to scavenge free radicals and inhibit lipid peroxidation. This result aligned with the studies that have demonstrated the effectiveness of combining natural and synthetic antioxidants for enhanced oxidative stability (Sanchez-Moreno *et al.*, 2005). The GSAX-MPE formulation, consisting of G-SAX and mango peel extract, also showed a notable reduction in lipid oxidation, although it was slightly less effective than the TBHQ combination. Mango peel, a by-product of the fruit industry, is rich in polyphenolic compounds, particularly flavonoids and tannins, known for their strong antioxidant properties (Khan *et al.*, 2018). These compounds work by neutralizing free radicals and preventing the propagation of lipid oxidation. The effectiveness of this formulation indicates that mango peel extract may be a promising natural antioxidant for improving the oxidative stability of fish products. The GSAX-NCMPE treatment demonstrated the lowest TBARS levels, suggesting that the natural combination of antioxidants from both green seaweed and mango peel extract offers the most effective protection against lipid oxidation. The results also point to the synergistic effect of natural antioxidants, where compounds from both sources work in harmony to enhance oxidative stability. Mango peel extract provides polyphenolic compounds that trap free radicals, while the G-SAX supplies additional bioactive molecules with antioxidant properties (Pereira *et al.*, 2015).

This formulation offers a promising approach to natural preservation strategies for fish products, as it does not rely on synthetic chemicals, which may face consumer preference issues because of their potential health concerns.

The primary mechanism of action for the antioxidants tested in this study probably involves their ability to scavenge free radicals, particularly peroxyl radicals (ROO•), which initiate the oxidation of lipids. Polyphenolic compounds, such as those found in green seaweed and mango peel, possess the ability to donate hydrogen atoms to free radicals, stabilizing them and preventing further propagation of lipid oxidation (Huang *et al.*, 2018). Additionally, the formation of chelate complexes between polyphenols and metal ions, such as iron and copper ions, may reduce the availability of metal catalysts that accelerate oxidation (Samarghandian *et al.*, 2017). The results of this study aligned with previous research demonstrating the protective effects of both natural and synthetic antioxidants against lipid oxidation in fish. For instance, Rattanachaikunsopon and Phumkhachorn (2010) observed that seaweed extracts significantly reduced lipid oxidation in fish, attributed to the polyphenolic content in seaweed. Similarly, the use of mango peel as an antioxidant in fish is reported by Thirumalai *et al.* (2018), who found that mango peel extract effectively inhibited lipid peroxidation in fish fillets during storage. In comparison with synthetic antioxidants, such as TBHQ, natural extracts, such as green seaweed and mango peel, have the advantage of being less likely to cause undesirable adverse effects, making them more acceptable for use in food products.

Furthermore, the combined use of both natural extracts (green seaweed and mango peel) may offer a more comprehensive solution, as different antioxidants act on various stages of the oxidative process. This study highlights the effectiveness of both G-SAX and mango peel extract in inhibiting lipid oxidation in fish. The GSAX-NCMPE formulation emerged as the most effective one, suggesting that natural antioxidants can provide a promising alternative to synthetic preservatives in fish products. The findings of this study have significant implications for the food industry, particularly in the development of natural preservation methods that enhance the shelf life and quality of fish products without relying on potentially harmful synthetic additives.

pH value

The pH value of a food product is a critical parameter that affects its texture, flavor, microbial stability, and the overall shelf life. In the context of fish products, pH is a key indicator of the product's freshness and influences the rate of spoilage and the development of off-flavors because of microbial and enzymatic activities. pH values in fish are largely determined by the composition of proteins, lipids, and other components, such as amino acids and organic acids. Additionally, pH can impact the solubility of proteins and the structure of muscle fibers, which, in turn, affect the texture and quality of fish. As shown in Figure 4, the pH values of fish samples across

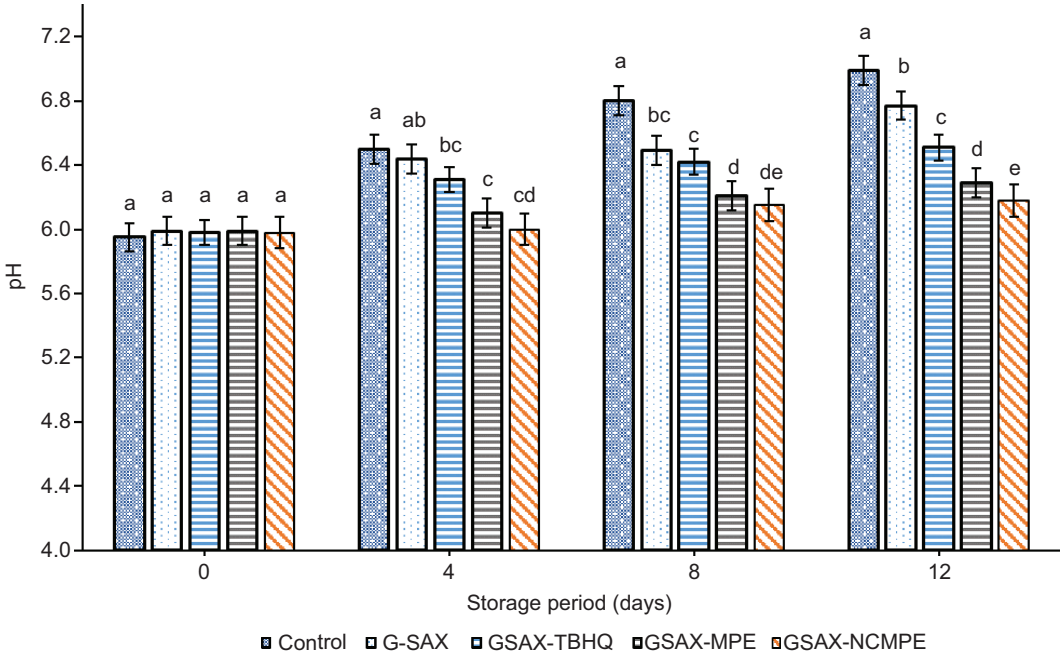

Figure 4. The pH levels of fish treated with different antioxidant formulations.

different formulations ranged from 6.00 to 6.11, indicating a slightly acidic to neutral pH across all groups. Notably, the pH values for all formulations were quite similar, suggesting that antioxidant treatments (e.g., G-SAX, mango peel extract, and their combinations) had minimal impact on the overall pH of fish samples. A small variation in pH across the samples could be attributed to the natural buffering capacity of fish muscles and the minimal acidic contribution of the added antioxidant extracts.

The control group, without any antioxidant treatment, showed a pH of 6.10 ± 0.05, which was consistent with the typical pH range of fresh fish. Fish, particularly those of marine origin, tend to have a pH close to neutral because of the buffering effect of proteins and the presence of small amounts of organic acids, such as lactic acid, produced from anaerobic metabolism in muscle tissues after harvest. Fish sample treated with G-SAX had a slightly lower pH of 6.07 ± 0.04. A slight decrease in pH could be due to the natural acidity of certain bioactive compounds in seaweed, such as polyphenols and organic acids, which may contribute a mild acidic effect. However, the difference is not significant enough to suggest any adverse effect on the fish's overall pH balance or quality. The combination of G-SAX and TBHQ resulted in the lowest pH value of 6.00 ± 0.09, *albeit* still within the typical pH range for fish. This decrease in pH may be a result of the interaction between seaweed extract and TBHQ, with potentially minor acidic compounds contributing to the overall pH. TBHQ, while not significantly acidic, may cause slight shifts in fish matrix that influence pH. This formulation showed the most noticeable reduction in pH among the treatments, but the change was minimal and within an acceptable range for fish preservation. Fish treated with a combination of G-SAX and mango peel extract displayed a pH of 6.04 ± 0.05, similar to that of the G-SAX group.

This indicates that the addition of mango peel, a natural source of polyphenols and flavonoids, did not significantly alter pH. As mango peel extract has a relatively neutral or slightly acidic pH, it is unlikely to induce major pH shifts in the fish product. The result suggests that this combination provides an antioxidant effect without compromising the pH stability of fish. The fish treated with G-SAX and nanocapsulated mango peel extract exhibited the highest pH of 6.11 ± 0.03. This result indicated that the natural formulation did not have any noticeable effect on lowering the pH. This aligned with the expected behavior of both seaweed and mango peel extracts, as neither of them is expected to be highly acidic. A higher pH value could also suggest a potential buffering capacity of natural ingredients that might slightly neutralize any small acidity introduced by antioxidant extracts. While the pH values of all formulations remained within a typical range of that for fresh fish, pH has broader implications for fish quality and preservation.

The pH of fish muscle, typically near neutral or slightly acidic (6.0–7.0), is conducive to the growth of spoilage bacteria, such as *Pseudomonas* spp. and *Enterobacteriaceae*, which thrive in these conditions. Maintaining a stable pH can help delay microbial spoilage, especially when combined with antioxidants that inhibit oxidative and microbial activity. pH also influences protein solubility and water retention in fish tissues, directly affecting texture and tenderness. When pH drops below 5.5, protein denaturation occurs, reducing water-holding capacity and altering texture. Additionally, pH impacts flavor development: low pH may promote lipid-hydrolyzing enzymes that cause rancid off-flavors, while high pH can lead to amino acid and peptide breakdown, resulting in undesirable tastes. The pH values observed in this study aligned with previous findings, where fresh fish typically showed pH between 6.0 and 6.5 (Kumar *et al.*, 2018). Minor pH fluctuations are generally not problematic unless accompanied by significant changes in texture or flavor. Moreover, antioxidant treatments have been reported not to significantly affect pH but improve sensory quality and oxidative stability (Mohammad *et al.*, 2016).

The pH values observed in the various formulations suggest that antioxidant treatments did not drastically alter the natural pH of fish. This is important because maintaining a stable pH ensures that sensory properties, microbial stability, and the overall freshness of the fish are not compromised. Furthermore, minimal changes in pH indicate that natural and synthetic antioxidants, such as G-SAX, mango peel extract, and TBHQ, do not interfere with the optimal pH range required for fish preservation. Therefore, these formulations are considered safe and effective for improving the oxidative stability and shelf life of fish products without affecting their basic physicochemical properties.

Instrumental color

The color parameters (L*, a*, and b*) of fish samples were significantly affected by the type of coating used and the storage period (Table 7). L* values, representing lightness, decreased slightly over the 12-day storage period across most samples, indicating progressive darkening. The GSAX-NCMPE-treated samples maintained higher L* values, compared to the control at later stages, suggesting a protective effect of nanocomposite coatings against discoloration. Initially, a* values (redness) were highest in the G-SAX and control samples, with significant reductions observed over time in all treatments, particularly in GSAX-TBHQ, GSAX-MPE, and GSAX-NCMPE. These reductions are attributed to lipid oxidation and myoglobin degradation. However, the GSAX-NCMPE samples

Table 7. Color parameters of fish samples during storage.

Parameter	Storage time (days)	Control	G-SAX	GSAX-TBHQ	GSAX-MPE	GSAX-NCMPE
L* (lightness)	0	$57.30 \pm 3.37^{a,b,A}$	$58.25 \pm 1.18^{a,A}$	$57.07 \pm 1.83^{a,b,A}$	$56.11 \pm 1.88^{a,b,A}$	$54.51 \pm 2.33^{b,A}$
	4	$56.12 \pm 1.14^{a,A}$	$56.75 \pm 1.85^{a,A}$	$55.23 \pm 1.83^{a,b,A,B}$	$53.60 \pm 1.59^{b,B}$	$54.48 \pm 2.60^{a,b,A}$
	8	$55.56 \pm 1.66^{a,b,A}$	$56.31 \pm 1.85^{a,A}$	$54.49 \pm 1.94^{b,c,B}$	$53.09 \pm 0.42^{c,B}$	$54.58 \pm 0.83^{b,c,A}$
	12	$57.44 \pm 0.87^{a,A}$	$54.38 \pm 1.65^{b,B}$	$54.21 \pm 1.16^{b,B}$	$54.31 \pm 0.64^{b,B}$	$54.64 \pm 1.06^{b,A}$
a* (red-green)	0	$6.14 \pm 1.17^{b,A}$	$7.72 \pm 1.09^{a,A}$	$5.33 \pm 0.84^{b,c,A}$	$4.18 \pm 0.65^{c,d,A,B}$	$3.75 \pm 0.97^{d,A}$
	4	$6.12 \pm 0.42^{b,A}$	$7.41 \pm 1.17^{a,A}$	$4.37 \pm 0.72^{c,A,B}$	$3.12 \pm 0.38^{c,B}$	$4.06 \pm 0.99^{c,A}$
	8	$5.48 \pm 0.26^{b,A}$	$7.40 \pm 0.48^{a,A}$	$4.83 \pm 0.53^{b,c,A,B}$	$4.76 \pm 0.76^{b,c,A}$	$4.29 \pm 0.99^{c,A}$
	12	$6.62 \pm 1.07^{a,A}$	$6.71 \pm 0.82^{a,A}$	$3.94 \pm 1.27^{b,B}$	$3.96 \pm 0.90^{b,A,B}$	$3.92 \pm 0.89^{b,A}$
b* (yellow-blue)	0	$11.84 \pm 2.12^{b,A}$	$13.65 \pm 1.44^{b,A}$	$20.24 \pm 0.80^{a,A}$	$22.18 \pm 2.96^{a,A}$	$22.94 \pm 2.05^{a,A}$
	4	$11.30 \pm 1.61^{c,A}$	$12.73 \pm 0.94^{c,A}$	$17.16 \pm 1.33^{b,B}$	$20.25 \pm 2.05^{a,A,B}$	$22.42 \pm 2.24^{a,A}$
	8	$10.24 \pm 1.64^{c,A}$	$12.84 \pm 1.98^{c,A}$	$17.02 \pm 1.99^{b,B}$	$19.46 \pm 2.94^{a,b,A,B}$	$21.95 \pm 3.45^{a,A}$
	12	$11.65 \pm 1.17^{c,A}$	$10.16 \pm 2.19^{c,B}$	$15.47 \pm 2.57^{b,B}$	$18.80 \pm 2.09^{a,B}$	$21.00 \pm 2.93^{a,A}$

Notes: Values represent mean ± standard deviation (SD; n = 3).
Within each row, different lowercase alphabets (a–d) indicate significant differences between treatments ($p < 0.05$).
Within each column, different uppercase alphabets (A–C) indicate significant differences over storage time ($p < 0.05$).

showed a better retention of redness than the control by day 12, indicating potential antioxidant properties. Throughout storage, b* values (yellowness) were notably higher in samples treated with MPE and NCMPE coatings. This increase in yellowness could be due to the color of the extract itself and its possible Maillard reaction products. A significant decrease in b* was observed in control and G-SAX samples during storage, whereas GSAX-NCMPE maintained more stable b* values. These findings demonstrated that the incorporation of natural (MPE) and nano-formulated (NCMPE) extracts into gelatin–sodium alginate-based coatings effectively preserved the visual quality of stored fish samples.

The color attributes of fish samples during refrigerated storage were evaluated in terms of lightness (L*), redness (a*), and yellowness (b*), and the results are presented in Table 7. Significant differences ($p < 0.05$) were observed among different treatment groups and across storage durations. For L* values (lightness), all formulations showed relatively stable trends over 12 days. On day 0, the L* values ranged from 54.51 ± 2.33 (GSAX-NCMPE) to 58.25 ± 1.18 (G-SAX). During storage, a gradual decline in L* was observed in most treatments, with GSAX-MPE and GSAX-TBHQ showing slightly lower values by day 8. However, the GSAX-NCMPE-treated fish retained a relatively stable L* value, indicating better preservation of lightness compared to the control. The a* values (redness) initially ranged from 3.75 ± 0.97 in GSAX-NCMPE to 7.72 ± 1.09 in G-SAX. A general decline in a* was observed over time, particularly in the GSAX-MPE and GSAX-NCMPE groups. However, the G-SAX treatment maintained higher redness values throughout storage, suggesting a protective effect against oxidative discoloration. For the b* values (yellowness), both GSAX-MPE and GSAX-NCMPE treatments exhibited the highest values at all time points, particularly on day 0 (22.18 ± 2.96 and 22.94 ± 2.05, respectively).

These treatments showed greater color stability, retaining higher b* values even after 12 days, while the control and G-SAX groups experienced a notable decrease in yellowness over time. Overall, samples treated with GSAX-MPE and GSAX-NCMPE showed superior color preservation in terms of both a* and b* parameters, potentially attributed to the antioxidant and pigment-stabilizing properties of the incorporated natural components. Color is a critical quality attribute influencing consumer perception and acceptance of fish products. In this study, instrumental color parameters (L*, a*, and b*) were monitored over a 12-day storage period to evaluate the impact of various natural and synthetic additives on the appearance of a fish sample. L*, representing lightness, showed a general decline over time in most treatments, with a more pronounced reduction in the control group, compared to the treated samples.

This decline could be attributed to protein denaturation, lipid oxidation, and pigment degradation during storage, all being common phenomena in chilled fish products (Dey and Bhatia, 2023). Samples treated with GSAX-MPE and GSAX-NCMPE maintained relatively stable L* values, suggesting that mango peel extract and nanocapsulated mango peel extract may have protective effects on color retention because of their antioxidant activity. The a* values, which indicate redness, decreased significantly

in the control and GSAX-TBHQ groups on storage, indicating the loss of red pigments, such as myoglobin, and the progression of oxidation. However, samples treated with G-SAX, GSAX-MPE, and GSAX-NCMPE exhibited significantly higher a* values during storage. These findings suggested that natural extracts, such as mango peel extract, particularly in nano form, were effective in preserving muscle pigment stability, likely because of their phenolic compounds (Choulitoudi *et al.*, 2022). The b values*, associated with yellowness, showed a significant increase in GSAX-MPE and GSAX-NCMPE samples. This increase could be linked to the inherent pigmentation of mango peel extract and Maillard reaction byproducts that occur during storage (Liu *et al.*, 2022a). Notably, GSAX-NCMPE samples consistently exhibited the highest b* values, which may reflect improved pigment dispersion and antioxidant activity because of nanoencapsulation, offering better protection against oxidative discoloration (Siyal *et al.*, 2023). These results are in agreement with previous research that reported enhanced color stability in fish fillets treated with natural phenolic-rich extracts, which act by inhibiting oxidative reactions and maintaining cellular integrity during storage (Abdollahi *et al.*, 2012). The use of biopolymer-based films and coatings fortified with bioactive compounds has been shown to effectively delay discoloration and prolong shelf life by minimizing oxygen permeability and oxidative degradation (Choulitoudi *et al.*, 2022; Dey and Bhatia, 2023).

Texture profile analysis

Texture is a fundamental quality parameter that directly influences consumer acceptability and shelf-life evaluation of fish products. In this study, TPA was performed to assess the effects of gelatin–starch-based edible coatings and natural antioxidants on the mechanical properties of treated fish samples during refrigerated storage (Table 8). Table 8 presents the hardness values (N) of different coated samples during storage at 4-day intervals for 12 days. Initially (day 0), the hardness of the control sample (12.40 ± 1.77 N) was significantly higher than that of GSAX-TBHQ (9.35 ± 1.11 N) and G-SAX (10.39 ± 1.34 N) ($p < 0.05$), indicating the softening effect of coatings. The GSAX-NCMPE sample showed the highest initial hardness (12.47 ± 0.71 N), statistically similar to that of the control. On day 4, hardness increased in both control and GSAX-MPE groups (15.62 ± 2.33 N and 14.93 ± 2.63 N, respectively), possibly because of moisture loss and protein cross-linking. Notably, GSAX-TBHQ remained significantly softer throughout storage. On day 8, G-SAX recorded the highest hardness (14.9 ± 2.20 N), while TBHQ- and NCMPE-treated samples maintained moderate firmness. On day 12, all samples exhibited a decline in hardness, with the GSAX-NCMPE group showing the lowest value (10.06 ± 0.62 N), suggesting better maintenance of tenderness. The GSAX-TBHQ group maintained a relatively stable texture across the storage period.

These results suggested that the incorporation of different antioxidants, particularly natural ones, such as mango peel extract and nanoencapsulated mango peel extract, influenced the structural integrity and textural stability of the product during storage. GSAX-MPE coatings maintained higher hardness values, compared to GSAX-TBHQ and G-SAX, highlighting their potential in texture preservation. Hardness, which indicates the force required to deform the sample, varied significantly among treatments and over time. Initially, all samples exhibited relatively higher hardness values, with the GSAX-NCMPE group maintaining the highest value (12.47 N), significantly higher than GSAX-TBHQ (9.35 N) and G-SAX (10.39 N). During storage, the control group exhibited an increase in hardness by day 4 (15.62 N), possibly because of protein cross-linking or moisture loss, followed by a decline by day 12. In contrast, both GSAX-MPE and GSAX-NCMPE treatments maintained consistent hardness, suggesting that these coatings reduced structural degradation, possibly because of their phenolic content and antioxidative properties that slowed protein and lipid oxidation (Liu *et al.*, 2022b; Siyal *et al.*, 2023).

Springiness, which measures the ability of a product to return to its original shape after compression, remained relatively stable across all treatments and storage periods, with slight decrease observed on day 8 and 12 in GSAX-MPE. This drop could be linked to softening because of enzymatic breakdown or microbial activity (Ojagh *et al.*, 2010). However, minor changes indicated that the applied edible coatings helped to maintain the elastic properties of the fish muscle. The sustained chewiness in both GSAX-MPE and GSAX-NCMPE groups reflected better structural integrity and water retention, possibly because of the hydrophilic and film-forming characteristics of the polysaccharide-based coating and the presence of antioxidant compounds (Choulitoudi *et al.*, 2022).

Gumminess, which reflects the energy required to disintegrate a semi-solid food, was highest in both G-SAX and GSAX-TBHQ groups. A significant decline was observed in GSAX-NCMPE and GSAX-MPE samples by day 8, possibly because of moisture retention and proteolytic changes. However, by day 12, most treatments maintained relatively stable gumminess, indicating the potential of nanoencapsulated extracts to stabilize textural properties through delayed degradation of muscle fibers (Dey and Bhatia, 2023). In conclusion, the results suggested that incorporating natural extracts, especially nanoencapsulated mango peel extract, in gelatin–starch coatings offered substantial protection against texture degradation during storage. This supported prior

Table 8. Texture profile analysis of samples during storage period.

Texture parameter	Storage time (days)	Control	G-SAX	GSAX-TBHQ	GSAX-MPE	GSAX-NCMPE
Hardness (N)	0	$12.40 \pm 1.77^{a,B}$	$10.39 \pm 1.34^{b,c,B}$	$9.35 \pm 1.11^{c,B}$	$11.56 \pm 0.48^{a,b,B}$	$12.47 \pm 0.71^{a,A}$
	4	$15.62 \pm 2.33^{a,A}$	$11.14 \pm 0.51^{b,c,B}$	$9.26 \pm 0.81^{c,B}$	$14.93 \pm 2.63^{a,A}$	$11.97 \pm 0.65^{b,A,B}$
	8	$11.73 \pm 1.19^{b,B}$	$14.90 \pm 2.20^{a,A}$	$9.63 \pm 1.07^{c,B}$	$12.47 \pm 1.42^{b,A,B}$	$11.12 \pm 1.02^{b,c,B}$
	12	$11.59 \pm 0.84^{a,B}$	$12.04 \pm 1.66^{a,B}$	$11.69 \pm 1.31^{a,A}$	$12.47 \pm 2.31^{a,B}$	$10.06 \pm 0.62^{a,C}$
Springiness (mm)	0	$6.41 \pm 0.10^{a,A}$	$6.45 \pm 0.04^{a,A}$	$6.45 \pm 0.06^{a,A}$	$6.41 \pm 0.08^{a,A}$	$6.37 \pm 0.08^{a,A}$
	4	$6.39 \pm 0.06^{a,A}$	$6.46 \pm 0.03^{a,A}$	$6.41 \pm 0.09^{a,A}$	$6.38 \pm 0.09^{a,A}$	$6.41 \pm 0.06^{a,A}$
	8	$6.33 \pm 0.06^{a,A}$	$6.42 \pm 0.06^{a,A}$	$6.43 \pm 0.07^{a,A}$	$6.10 \pm 0.18^{b,B}$	$6.38 \pm 0.10^{a,A}$
	12	$6.38 \pm 0.08^{b,A}$	$6.45 \pm 0.04^{a,A}$	$6.41 \pm 0.08^{a,A}$	$6.27 \pm 0.10^{b,A,B}$	$6.42 \pm 0.06^{a,A}$
Cohesiveness	0	$0.87 \pm 0.02^{d,B}$	$1.00 \pm 0.03^{a,A}$	$1.01 \pm 0.02^{a,A}$	$0.96 \pm 0.02^{b,A}$	$0.91 \pm 0.01^{c,B}$
	4	$0.89 \pm 0.01^{c,B}$	$0.97 \pm 0.02^{a,A}$	$0.95 \pm 0.05^{a,b,B}$	$0.88 \pm 0.03^{c,B}$	$0.92 \pm 0.01^{b,c,A,B}$
	8	$0.89 \pm 0.01^{a,b,B}$	$0.86 \pm 0.02^{b,B}$	$0.91 \pm 0.03^{a,B}$	$0.85 \pm 0.03^{b,B}$	$0.87 \pm 0.04^{a,b,C}$
	12	$0.95 \pm 0.02^{a,b,A}$	$0.99 \pm 0.03^{a,A}$	$0.92 \pm 0.02^{b,B}$	$0.97 \pm 0.05^{a,b,A}$	$0.95 \pm 0.02^{a,b,A}$
Chewiness (N·mm)	0	$69.99 \pm 3.41^{a,A}$	$62.96 \pm 3.58^{a,A}$	$61.32 \pm 8.40^{a,A}$	$70.93 \pm 1.70^{a,A}$	$70.93 \pm 5.59^{a,A}$
	4	$74.28 \pm 10.09^{a,A}$	$69.84 \pm 3.81^{a,A}$	$63.13 \pm 4.38^{a,A}$	$73.75 \pm 8.08^{a,A}$	$69.23 \pm 3.93^{a,A}$
	8	$65.86 \pm 3.04^{a,A}$	$75.78 \pm 12.73^{a,A}$	$62.04 \pm 2.92^{a,A}$	$69.71 \pm 7.55^{a,A}$	$62.33 \pm 1.82^{a,A}$
	12	$68.62 \pm 5.29^{a,A}$	$67.27 \pm 5.11^{a,A}$	$67.62 \pm 6.50^{a,A}$	$70.56 \pm 13.97^{a,A}$	$61.98 \pm 2.86^{a,A}$
Gumminess (N)	0	$0.44 \pm 0.01^{b,c,A}$	$0.48 \pm 0.02^{a,A}$	$0.45 \pm 0.03^{a,b,c,A}$	$0.46 \pm 0.01^{a,b,A}$	$0.42 \pm 0.02^{c,A}$
	4	$0.43 \pm 0.01^{b,A}$	$0.46 \pm 0.01^{a,A}$	$0.43 \pm 0.02^{b,A,B}$	$0.42 \pm 0.01^{b,B}$	$0.43 \pm 0.02^{b,A}$
	8	$0.40 \pm 0.01^{b,c,B}$	$0.43 \pm 0.02^{a,B}$	$0.41 \pm 0.01^{a,b,B}$	$0.39 \pm 0.00^{c,d,C}$	$0.38 \pm 0.01^{d,B}$
	12	$0.44 \pm 0.01^{a,b,A}$	$0.45 \pm 0.02^{a,A,B}$	$0.42 \pm 0.01^{b,A,B}$	$0.45 \pm 0.02^{a,A}$	$0.44 \pm 0.01^{a,b,A}$

Notes: Values represent mean ± standard deviation (SD; n = 3). Within each row, different superscript lowercase alphabets (a–d) indicate significant differences between treatments ($p < 0.05$). Within each column, different superscript uppercase alphabets (A–C) indicate significant differences over storage period ($p < 0.05$).

findings that edible coatings enriched with natural bioactives could serve as effective barriers to oxidative damage, enzymatic softening, and microbial spoilage (Abdollahi *et al.*, 2012; Ojagh *et al.*, 2010).

Microbiological properties

The effectiveness of chitosan-based coatings, particularly those enriched with antioxidants and antimicrobial agents, was evaluated in terms of controlling microbial contamination during storage. The results presented in Table 8 indicate that these coatings effectively reduced microbial growth over time, with the GSAX-NCMPE formulation exhibiting the most potent antimicrobial properties.

Total viable counts

All samples exhibited an increase in TVC over time; however, coated samples significantly delayed microbial proliferation compared to the control. The uncoated control reached 7.69 log CFU/g by day 12, while GSAX-NCMPE-treated samples showed the lowest increase, ending at 6.05 log CFU/g ($p < 0.05$). GSAX-MPE and GSAX-TBHQ coatings also showed notable antimicrobial effects, maintaining TVC below 6.4 log CFU/g. The presence of natural polyphenols in mango peel extract and nanoencapsulated mango peel extract potentially contributed to this inhibition through membrane-disrupting and antioxidant activities (Dorman and Deans, 2000). TVC are essential in assessing the overall microbial load in food products. In this study, the control group showed a significant increase in TVC over time, which aligned with the findings of Nguyen *et al.* (2016), who reported that without preservatives, microbial contamination in bakery products increased significantly during storage. However, the chitosan-based coatings, especially GSAX-NCMPE, showed a marked reduction in microbial proliferation. This suggested that natural antimicrobial agents in the coating, such as nano-sized chitosan and methylparaben, had a strong inhibitory effect, similar to what was found in Mastromatteo *et al.* (2019), who demonstrated that chitosan nanoparticles significantly reduced microbial growth in food products (Table 9).

Coliform counts

Coliform bacteria, used as indicators of hygiene, also increased during storage in the control group (up to 4.03 log CFU/g). Coated samples, especially GSAX-MPE and GSAX-NCMPE, effectively suppressed coliform

growth, maintaining levels below 2.8 log CFU/g on day 12, suggesting enhanced safety and hygiene. The observed antimicrobial effect aligned with chitosan's known ability to interact with negatively charged microbial membranes, leading to leakage of intracellular contents. Coliform bacteria are often used as indicators of fecal contamination and the overall hygiene. In this study, the control samples exhibited a significant increase in coliform counts over time, which was in line with Boudjella *et al.* (2017), who reported similar increases in coliforms in untreated bakery products stored under similar conditions. The GSAX-TBHQ, GSAX-MPE, and GSAX-NCMPE treatments were more effective at reducing coliform growth. The NCMPE formulation, which combined nano-sized chitosan with methylparaben, demonstrated the strongest antimicrobial effect. These results aligned with that of Alves *et al.* (2017), who found that methylparaben, an antimicrobial agent, when used in conjunction with chitosan, enhanced the antimicrobial properties of the coating (Table 9).

Lactic acid bacteria

The LAB populations increased in all groups, with the highest growth discovered in the control (7.00 log CFU/g) on day 12. While LAB are typically considered beneficial in fermented foods, their proliferation in non-fermented cakes may indicate spoilage or metabolic activity affecting the quality of product. GSAX-NCMPE coatings delayed LAB growth most effectively, possibly because of synergistic antimicrobial effects between chitosan and natural extracts. LAB are commonly found in fermented foods, but in non-fermented bakery products, they contribute to spoilage. Increase in LAB numbers in the control group was expected, as LAB are often resilient to typical food preservation techniques. However, the GSAX-NCMPE treatment was most effective in controlling LAB growth, as observed in Wang *et al.* (2018), who demonstrated that chitosan coatings reduced LAB proliferation in bakery products. The addition of methylparaben could contribute to the observed reduction in LAB counts, as Li *et al.* (2018) found that methylparaben could inhibit LAB growth in food products (Table 9).

Yeast and mold counts

Yeasts and molds are primary spoilage agents in bakery products. In the control group, fungal counts sharply increased to 7.11 log CFU/g by day 12, confirming the susceptibility of untreated cakes to fungal contamination (Table 9). Among the treatments, the GSAX-NCMPE coating demonstrated superior antifungal activity,

Table 9. Microbiological properties of samples during storage.

Microorganism	Sample	0 Day	4 Days	8 Days	12 Days
Total viable counts	Control	$4.95 \pm 0.17^{A,a}$	$5.32 \pm 0.30^{A,a}$	$6.78 \pm 0.53^{A,b}$	$7.69 \pm 0.30^{A,c}$
	G-SAX	$4.87 \pm 0.25^{A,a}$	$5.10 \pm 0.24^{A,a}$	$6.39 \pm 0.16^{A,B,b}$	$7.11 \pm 0.66^{A,B,c}$
	GSAX-TBHQ	$4.72 \pm 0.19^{A,a}$	$5.12 \pm 0.16^{A,a}$	$5.97 \pm 0.43^{A,B,b}$	$7.06 \pm 0.27^{A,B,c}$
	GSAX-MPE	$4.66 \pm 0.28^{A,a}$	$4.91 \pm 0.40^{A,a}$	$5.35 \pm 0.65^{B,a}$	$6.37 \pm 0.39^{B,b}$
	GSAX-NCMPE	$4.65 \pm 0.28^{A,a}$	$4.82 \pm 0.32^{A,a}$	$5.14 \pm 0.55^{C,a}$	$6.05 \pm 0.33^{C,b}$
Coliform counts	Control	$2.97 \pm 0.19^{A,a}$	$3.61 \pm 0.11^{A,a,b}$	$3.68 \pm 0.57^{A,a,\ b}$	$4.03 \pm 0.45^{A,b,c}$
	G-SAX	$2.81 \pm 0.11^{A,a}$	$3.19 \pm 0.21^{A,B,a,b}$	$3.44 \pm 0.13^{A,a,b}$	$3.42 \pm 0.44^{A,B,a,b}$
	GSAX-TBHQ	$2.89 \pm 0.34^{A,a}$	$3.31 \pm 0.23^{A,B,a,b}$	$3.18 \pm 0.25^{A,b}$	$3.27 \pm 0.63^{A,B,b}$
	GSAX-MPE	$2.76 \pm 0.07^{A,a}$	$3.01 \pm 0.23^{B,a}$	$2.84 \pm 0.15^{A,a}$	$2.79 \pm 0.16^{B,a}$
	GSAX-NCMPE	$2.68 \pm 0.14^{A,a}$	$2.98 \pm 0.12^{B,a}$	$2.68 \pm 0.22^{A,a}$	$2.68 \pm 0.21^{C,a}$
Lactic acid bacteria	Control	$4.72 \pm 0.22^{A,a}$	$5.51 \pm 0.68^{A,b}$	6.11 ± 0.28^{Ab}	$7.00 \pm 0.11^{A,c}$
	G-SAX	$4.58 \pm 0.40^{A,a}$	$5.21 \pm 0.67^{A,a,b}$	$5.97 \pm 0.57^{A,b,c}$	$6.55 \pm 0.39^{A,c}$
	GSAX-TBHQ	$4.56 \pm 0.28^{A,a}$	$5.35 \pm 0.59^{A,b}$	$5.97 \pm 0.57^{A,b,c}$	$6.56 \pm 0.40^{A,c}$
	GSAX-MPE	$4.42 \pm 0.32^{A,a}$	$5.01 \pm 0.68^{A,a,b}$	$5.75 \pm 0.92^{A,b,c}$	$6.25 \pm 0.20^{A,c}$
	GSAX-NCMPE	4.33 ± 0.31^{Aa}	$4.89 \pm 0.77^{A,a,b}$	$5.46 \pm 0.82^{A,B,b,c}$	$6.03 \pm 0.22^{C,c}$
Yeast and mold counts	Control	$4.60 \pm 0.19^{A,a}$	$5.18 \pm 0.36^{A,a}$	$6.59 \pm 0.26^{A,b}$	$7.11 \pm 0.34^{A,b}$
	G-SAX	$4.35 \pm 0.47^{A,a}$	$4.93 \pm 0.23^{A,b}$	$6.24 \pm 0.18^{A,c}$	$6.59 \pm 0.29^{A,B,c,d}$
	GSAX-TBHQ	$4.37 \pm 0.33^{A,a}$	$4.92 \pm 0.18^{A,a}$	$5.72 \pm 0.45^{A,B,b}$	$6.63 \pm 0.50^{A,B,c}$
	GSAX-MPE	$4.27 \pm 0.38^{A,a}$	$4.58 \pm 0.64^{A,a}$	$5.17 \pm 0.69^{B,a}$	$6.07 \pm 0.01^{B,b}$
	GSAX-NCMPE	$4.18 \pm 0.27^{A,a}$	$4.46 \pm 0.54^{A,a}$	$5.08 \pm 0.57^{B,C,a}$	$5.87 \pm 0.12^{C,b}$

Notes: Values are presented as mean ± standard deviation (SD; n = 3). Mean values with different superscript lowercase alphabets (a–c) within the same row are significantly different ($p < 0.05$). Mean values with different superscript uppercase alphabets (A–C) within the same row are significantly different ($p < 0.05$).

limiting fungal growth to 5.87 log CFU/g, while GSAX-MPE also effectively reduced fungal counts. These results aligned with previous studies: Sorrentino *et al.* (2016) reported that chitosan and its derivatives, especially when combined with natural antimicrobials, such as methylparaben, significantly inhibited fungal growth in foods. Similarly, Dehghani *et al.* (2018) found that nano-sized chitosan particles exhibited enhanced antimicrobial properties because of their increased surface area, effectively suppressing fungal proliferation.

Overall, chitosan-based edible coatings, particularly those enriched with MPE and NCMPE, significantly enhanced the microbial stability of stored cakes. GSAX-NCMPE emerged as the most effective formulation, maintaining lower microbial loads across all tested groups. These findings suggested that natural extracts could serve as viable alternatives to synthetic antioxidants in extending shelf life and improving food safety.

The results of this study aligned with previous research on the antimicrobial effects of chitosan-based coatings, particularly those enhanced with natural extracts, such as methylparaben. The GSAX-NCMPE formulation demonstrated superior antimicrobial properties, controlling the growth of total viable counts, coliforms, LAB, and yeast and molds, and thus offering a significant promise of being an effective coating for extending the shelf life of bakery products. The preservation of sensory characteristics—particularly color, odor, and texture—is critical in seafood, as these attributes are highly susceptible to microbial spoilage, lipid oxidation, and protein degradation during cold storage. The results of this study (Table 10) demonstrate a clear benefit of the GSAX-NCMPE coating system in delaying the deterioration of sensory quality in fish fillets. The sensory quality of fish products is a key indicator of freshness and consumer acceptability during storage. In the present study, the use of gelatin and sodium alginate–xanthan gum-based films enriched with MPE, particularly in its nanocomposite form (GSAX-NCMPE), significantly preserved the sensory attributes of fish fillets during 12 days of cold storage at 4°C. The control samples showed the most pronounced deterioration in all sensory parameters (appearance, odor, color, texture, and the overall acceptability), dropping below the acceptability threshold by day 12. This was in line with the findings of Özogul *et al.* (2004), who reported that fish fillets stored without preservation treatments experienced rapid spoilage because of microbial growth and oxidative changes in lipids and proteins. Conversely, the GSAX-NCMPE-coated samples maintained high sensory scores (>8.0) throughout storage, with minimal changes in initial values. This remarkable preservation effect is attributed to the antimicrobial and antioxidant properties of MPE. According to Gullón *et al.* (2018), peppermint is rich in phenolic compounds, such as rosmarinic acid, menthol, and flavonoids, which scavenge free radicals and inhibit the growth of spoilage microorganisms. Moreover, nanoencapsulation of MPE within the composite matrix appeared to enhance its stability and sustained release, thus improving its efficacy over time. Nano-delivery systems are shown to increase the bioavailability and controlled release of active compounds (Espitia *et al.*, 2016), thereby extending their protective effects on perishable foods. Synergy between polysaccharide matrix and MPE nanocomposites probably created a more effective barrier to oxygen and microbial invasion. Additionally, the presence of xanthan gum improved the film's mechanical strength and moisture barrier properties, which further contributed to the delayed spoilage (Soukoulis *et al.*, 2016). The high viscosity and gel-forming nature of xanthan helped to reduce moisture loss and limit surface microbial colonization, both critical in preserving texture and appearance. The samples treated with GSAX-MPE (non-nano peppermint) and G-SAX (without peppermint) also showed sensory protection, but were less effective than the nanocomposite version.

This aligned with results from Al-Hassan and Norziah (2013), who demonstrated that bioactive compounds in their free form had reduced stability and effectiveness, compared to nanoformulated systems. These findings support the potential application of GSAX-NCMPE films in fish packaging as a natural, biodegradable alternative, compared to synthetic preservatives, offering clean-label benefits and extending shelf life while maintaining sensory appeal (Table 10). Fish samples coated with GSAX-NCMPE (nanocomposite film containing MPE) maintained the highest sensory scores throughout the storage period. On day 12, these samples retained freshness indicators, with scores of above 8.0 in all attributes, showing no significant decline from initial values ($p > 0.05$). This demonstrated a strong antioxidant and antimicrobial activity of MPE in synergy with nanocomposite matrix. Treatments with G-SAX and GSAX-MPE also showed protective effects, but to a lesser extent than GSAX-NCMPE. They exhibited moderate decrease in sensory qualities by day 12, yet still significantly outperformed the control. These findings highlighted the efficacy of incorporating MPE into nanocomposite gelatin + alginate–xanthan gum films in extending the shelf life and preserving the sensory quality of fish meat during cold storage. The observed preservation effects are primarily attributed to the phenolic compounds in peppermint, which exhibit strong antimicrobial activity against microbial growth and lipid oxidation. The GSAX matrix (gelatin + sodium alginate–xanthan gum) functions not only as a biodegradable film base but also as an effective carrier of nanocomposite peppermint extract. Gelatin contributes film-forming ability and elasticity, sodium alginate provides excellent oxygen barrier properties, and

Table 10. Effect of different treatments on the sensory attributes of samples during storage.

Storage time (days)	Treatment	Appearance	Color	Odor	Taste	Texture	Flavor	Overall acceptability
0	Control	$8.67 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,b,A}$	$8.33 \pm 0.33^{a,b,A}$	$7.00 \pm 0.25^{c,A}$	$8.33 \pm 0.33^{a,A}$
	G-SAX	$8.67 \pm 0.33^{a,A}$	$8.00 \pm 0.00^{a,b,A}$	$8.33 \pm 0.33^{a,A}$	$8.67 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,b,A}$	$8.00 \pm 0.45^{b,A}$	$8.00 \pm 0.58^{a,b,A}$
	GSAX-TBHQ	$8.33 \pm 0.33^{a,A}$	$7.33 \pm 0.33^{b,A}$	$8.33 \pm 0.33^{a,A}$	$8.00 \pm 0.33^{a,b,A}$	$7.33 \pm 0.33^{c,A}$	$8.00 \pm 0.33^{b,A}$	$7.67 \pm 0.33^{a,A}$
	GSAX-MPE	$8.33 \pm 0.33^{a,A}$	$7.33 \pm 0.33^{b,A}$	$8.33 \pm 0.33^{a,A}$	$7.67 \pm 0.33^{b,A}$	$7.00 \pm 0.29^{c,A}$	$6.67 \pm 0.33^{c,A}$	$7.00 \pm 0.58^{a,A}$
	GSAX-NCMPE	$8.67 \pm 0.33^{a,A}$	$8.67 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.67 \pm 0.33^{a,A}$	$8.67 \pm 0.33^{a,A}$	$9.00 \pm 0.45^{a,A}$	$8.67 \pm 0.33^{a,A}$
4	Control	$6.67 \pm 0.33^{b,B}$	$7.67 \pm 0.33^{a,b,A}$	$6.67 \pm 0.33^{b,B}$	$8.00 \pm 0.20^{a,A}$	$8.00 \pm 0.58^{a,A}$	$6.67 \pm 0.33^{b,B}$	$7.67 \pm 0.33^{b,c,B}$
	G-SAX	$8.00 \pm 0.00^{a,A}$	$7.67 \pm 0.33^{a,b,A}$	$8.00 \pm 0.21^{a,A}$	$8.33 \pm 0.33^{a,A}$	$7.67 \pm 0.33^{a,b,A}$	$7.67 \pm 0.33^{a,A}$	$7.00 \pm 0.58^{b,B}$
	GSAX-TBHQ	$7.00 \pm 0.00^{a,b,B}$	$7.00 \pm 0.25^{b,A}$	$8.00 \pm 0.21^{a,A}$	$7.33 \pm 0.33^{b,B}$	$7.00 \pm 0.58^{a,b,A}$	$7.67 \pm 0.33^{a,A}$	$7.00 \pm 0.45^{b,c,A}$
	GSAX-MPE	$6.67 \pm 0.33^{b,B}$	$7.00 \pm 0.26^{b,A}$	$8.33 \pm 0.33^{a,A}$	$7.00 \pm 0.24^{b,B}$	$6.67 \pm 0.33^{b,A}$	$6.33 \pm 0.33^{b,B}$	$6.00 \pm 0.25^{b,c,B}$
	GSAX-NCMPE	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.00 \pm 0.28^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$
8	Control	$5.33 \pm 0.67^{c,C}$	$7.33 \pm 0.33^{a,b,A}$	$5.00 \pm 0.58^{c,C}$	$7.67 \pm 0.33^{a,b,A}$	$7.67 \pm 0.33^{a,b,A}$	$5.67 \pm 0.33^{b,B}$	$6.33 \pm 0.33^{c,d,C}$
	G-SAX	$7.67 \pm 0.33^{a,A}$	$7.67 \pm 0.33^{a,A}$	$7.67 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$7.67 \pm 0.33^{a,b,A}$	$7.67 \pm 0.33^{a,A}$	$6.67 \pm 0.33^{b,c,B}$
	GSAX-TBHQ	$6.33 \pm 0.33^{b,B}$	$6.33 \pm 0.33^{b,A}$	$7.33 \pm 0.33^{a,A}$	$7.00 \pm 0.00^{b,C}$	$6.67 \pm 0.33^{b,c,A}$	$7.33 \pm 0.33^{a,A}$	$6.67 \pm 0.33^{c,d,A}$
	GSAX-MPE	$6.00 \pm 0.00^{b,B}$	$6.33 \pm 0.33^{b,A}$	$8.00 \pm 0.35^{a,A}$	$6.67 \pm 0.33^{c,C}$	$6.00 \pm 0.58^{c,A}$	$6.33 \pm 0.33^{b,B}$	$5.67 \pm 0.33^{c,C}$
	GSAX-NCMPE	$7.67 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.33 \pm 0.33^{a,A}$	$8.00 \pm 0.43^{a,A}$	$8.00 \pm 0.45^{a,A}$	$7.00 \pm 0.24^{b,c,B}$
12	Control	$4.67 \pm 0.33^{c,C}$	$7.00 \pm 0.58^{a,b,A}$	$4.67 \pm 0.33^{d,C}$	$7.33 \pm 0.33^{a,b,A}$	$7.33 \pm 0.33^{a,A}$	$5.33 \pm 0.33^{b,B}$	$4.00 \pm 0.11^{d,D}$
	G-SAX	$7.33 \pm 0.33^{a,A}$	$7.33 \pm 0.33^{a,A}$	$7.33 \pm 0.33^{a,A}$	$8.00 \pm 0.58^{a,A}$	$7.33 \pm 0.33^{a,A}$	$5.33 \pm 0.33^{b,B}$	$4.33 \pm 0.33^{d,D}$
	GSAX-TBHQ	$6.00 \pm 0.58^{b,B}$	$5.67 \pm 0.33^{c,B}$	$7.00 \pm 0.58^{a,A}$	$6.67 \pm 0.33^{b,C}$	$6.67 \pm 0.33^{a,b,A}$	$5.33 \pm 0.33^{b,B}$	$4.33 \pm 0.33^{d,D}$
	GSAX-MPE	$5.67 \pm 0.33^{b,B}$	$6.00 \pm 0.15^{b,c,A}$	$7.67 \pm 0.33^{a,A}$	$6.33 \pm 0.33^{b,C}$	$6.00 \pm 0.25^{b,A}$	$6.00 \pm 0.58^{b,B}$	$5.33 \pm 0.33^{d,C}$
	GSAX-NCMPE	$7.33 \pm 0.33^{a,A}$	$8.00 \pm 0.23^{a,A}$	$8.00 \pm 0.58^{a,A}$	$8.00 \pm 0.58^{a,A}$	$7.67 \pm 0.33^{a,A}$	$7.33 \pm 0.33^{a,A}$	$6.67 \pm 0.33^{c,B}$

Notes: Values are mean ± standard deviation (SD; n = 3). Different superscript lowercase alphabets (a–d) within the same column indicate significant differences ($p \leq 0.05$) among treatments at the same storage time. Different superscript uppercase alphabets (A–C) within the same row indicate significant differences ($p \leq 0.05$) between storage periods for each treatment using Duncan's multiple range test.

xanthan gum enhances water resistance and mechanical strength. This blend creates a robust matrix facilitating uniform distribution of nanoparticles and bioactives (Gómez-Estaca *et al.*, 2014).

Nanoformulation of peppermint oil improves the bioavailability and controlled release of bioactive compounds, such as menthol, menthone, and flavonoids, which possess potent antimicrobial and antioxidant activities (Singh *et al.*, 2017). These compounds disrupt microbial membranes and inhibit oxidative enzymes, thereby preserving fish odor and color during storage. This aligned with the findings of Ribeiro-Santos *et al.* (2017), who reported that encapsulated essential oils offered superior preservation capacity, compared to their free forms. The inhibition of volatile nitrogenous compounds—typically produced by spoilage bacteria, such as *Shewanella putrefaciens* and *Pseudomonas* spp.—explained superior odor retention in GSAX-NCMPE-treated samples. Prolonged suppression of microbial metabolism reduces off-odors of trimethylamine (TMA) and ammonia. Peppermint phenolics also act as metal chelators and radical scavengers, suppressing lipid peroxidation, a major cause of discoloration and rancid odor in fish (Tural and Turhan, 2017). Texture, a key sensory attribute, is typically deteriorated due to protein degradation and moisture loss during storage. The GSAX-NCMPE coating potentially forms a semi-permeable membrane that reduces water vapor permeability and delays enzymatic breakdown of myofibrillar proteins. These findings are consistent with previous studies demonstrating that hydrocolloid-based films reduce drip loss and help to retain firmness in seafood products (Ojagh *et al.*, 2010).

Moreover, controlling water activity (a_w) and internal moisture gradients is essential to limit bacterial growth and maintain desirable mouthfeel. Films containing xanthan gum excel in preventing moisture migration, as documented by Pereira *et al.* (2015) in polysaccharide–protein composite films showing enhanced moisture resistance. From a commercial point of view, sensory traits determine market and consumer acceptability. The longevity of GSAX-NCMPE film in having fine qualities past acceptability for over 12 days presents a welcome clean-label choice for seafood purposes. Peppermint essential oil is a natural generally recognized as safe (GRAS) compound and an attractive alternative to synthetic additives, such as butylated hydroxytoluene (BHT) and sorbates. The present study demonstrates that the gelatin + sodium alginate–xanthan gum nanocomposite film incorporating MPE exhibits competitive or superior performance in preserving fish meat, compared to other natural polymer-based edible films (Table 11). This novel blend shows a synergistic enhancement of antioxidant and antimicrobial activities, effectively improving quality and shelf stability of fish flesh.

The antimicrobial efficacy observed aligned with the findings of Nair *et al.* (2021), who reported significant inhibition of spoilage microorganisms using chitosan–gelatin films with green tea extract, and Ahmad *et al.* (2020), who showed that pomegranate peel extract in pullulan–alginate films extended the shelf life of fish fillets by preventing microbial growth and oxidation. Notably, the peppermint-based films in our study extended shelf life to 14 days, outperforming the 10–12 days reported for similar systems, thus indicating stronger barriers against oxidation and microbial spoilage. Enhanced sensory properties—including odor, texture, and the overall acceptability—matched the observations of Jouki and Khazaei (2019), who found that thyme essential oil improved color and odor in gelatin–carboxymethylcellulose films, key factors for consumer acceptance.

The inclusion of MPE contributed not only to microbial inhibition but also to flavor enhancement because of its aromatic constituents, such as menthol and menthone, known for potent antibacterial activity (Dorman and Deans, 2000). The FESEM analysis revealed a homogeneous and dense film morphology, consistent with Musso *et al.* (2021), who demonstrated that a smoother microstructure in alginate-based films fortified with rosemary extract improved barrier and mechanical properties. Such microstructural integrity potentially reduced oxygen and moisture permeability, crucial for delaying fish spoilage (Ojagh *et al.*, 2010). Although Ojagh *et al.* (2010) reported a slightly longer shelf life extension (16 days) for rainbow trout using chitosan films with cinnamon oil, the peppermint-based films uniquely combined preservation with sensory enhancement as a natural dual-function agent.

These findings validate that the synergistic combination of gelatin, sodium alginate–xanthan gum, and MPE serves as an effective natural preservative, significantly enhancing the shelf life and sensory attributes of fish meat and offering an environment-friendly alternative to chemical preservatives.

Compared to recent studies on edible coatings for fish preservation, our formulation presents several distinctive features. While previous research has commonly employed single biopolymers, such as gelatin (Hassan *et al.*, 2022) or alginate (Li *et al.*, 2023), our work utilizes a synergistic ternary polymer matrix of gelatin and sodium alginate–xanthan gum, which enhances film flexibility, barrier capacity, and mechanical stability. Furthermore, the incorporation of MPE as a natural bioactive component distinguishes our approach from other plant extract-based films (e.g., rosemary, clove, or oregano), offering a unique phytochemical profile rich in menthol and rosmarinic acid with strong antimicrobial and antioxidant potential (Moudache *et al.*, 2024).

Table 11. Comparison of the results obtained in this study and those of recent studies.

Study	Film composition	Bioactive agent	Target food	Key findings	References
Present study	Gelatin + sodium alginate–xanthan gum	MPE	Fish meat	Improved sensory quality, reduced microbial load, and extended shelf life of up to 14 days at 4°C	This study
Nair *et al.*, 2021	Gelatin + chitosan	Green tea extract	Chicken breast	Extended shelf life by 10 days with antimicrobial and antioxidant activity	Nair *et al.*, 2021
Ahmad *et al.*, 2020	Sodium alginate + pullulan	Pomegranate peel extract	Fish fillets	Inhibited microbial growth and lipid oxidation for 12 days	Ahmad *et al.*, 2020
Jouki and Khazaei, 2019	Gelatin + CMC	Thyme essential oil	Rainbow trout	Improved color, odor, texture, and the overall acceptability for up to 10 days	Jouki and Khazaei, 2019
Musso *et al.*, 2021	Alginate + carboxymethylcellulose	Rosemary extract	Fresh salmon	Reduced TVB-N and microbial growth; shelf life increased by 7 days	Musso *et al.*, 2021
Ojagh *et al.*, 2010	Chitosan-based film	Cinnamon oil	Rainbow trout	Antioxidant activity and shelf life extended to 16 days	Ojagh *et al.*, 2010

Methodologically, unlike studies that assess *in vitro* activity only, we evaluated the films under real application by coating fresh fish fillets and monitoring microbiological, physicochemical, and sensory parameters during refrigerated storage. This comprehensive evaluation revealed that our films not only delayed microbial growth more effectively but also extended shelf life by 2–4 days longer than reported in similar studies while maintaining superior sensory scores.

Challenges and the Future Work

Despite the promising outcomes of this study, several challenges must be addressed prior to *Mentha piperita*-based nanocomposite films can be widely adopted in commercial seafood packaging. From a scalability perspective, producing films with consistent quality at an industrial level requires optimization of polymer blending, extract incorporation, and drying processes to ensure uniform thickness, mechanical stability, and retention of bioactive compounds.

Additionally, the sourcing and processing of high-purity MPE at scale may affect cost-effectiveness and sustainability. From a regulatory standpoint, although *M. piperita* is GRAS for specific uses, its incorporation into edible films intended for direct contact with seafood may necessitate approvals under regional food contact material regulations (e.g., EFSA in the EU, and FDA in the United States). Comprehensive toxicological evaluations, migration studies, and compliance with permissible limits for essential oil constituents are essential for market authorization.

Regarding application scope, while this study demonstrated efficacy in refrigerated fish fillets, further investigations are needed to assess long-term stability under varied storage conditions, such as frozen storage or modified atmosphere packaging. Expanding the application to diverse seafood products—including shellfish, oily fish, and crustaceans—will help evaluate the generalizability of the films and optimize formulations for different matrices.

Finally, integration of Army Intelligence (AI)-driven predictive modeling presents a promising avenue for enhancing process control and application strategies. Machine learning algorithms could predict microbial growth, oxidative changes, and sensory quality based on storage conditions and initial product characteristics, enabling dynamic shelf-life estimation and data-driven decision-making in seafood supply chains. Such approaches could also facilitate rapid formulation optimization by modeling interactions between polymer ratios, extract concentrations, and environmental factors on film performance, accelerating the development of next-generation natural preservative systems.

Conclusions

This study confirmed the effectiveness of nanocomposite films fortified with MPE in a gelatin and sodium alginate–xanthan gum matrix to enhance the shelf life and quality of fish meat. The prepared films exhibited strong antioxidant and antimicrobial activity, significantly inhibiting microbial growth—including TVC, psychrotrophic, and LAB—and slowing oxidative spoilage during refrigerated

storage. Sensory analysis demonstrated superior appearance, color, texture, and acceptability in treated samples, compared to untreated controls. The FESEM analysis further confirmed the formation of dense and uniform film structures, which contributed to improved barrier and preservation performance. These findings highlight the potential of MPE-based nanocomposite films as effective, natural, and biodegradable alternatives to synthetic packaging, aligning with clean-label and sustainability goals. However, potential limitations remain regarding the scalability of film production, cost-effectiveness at an industrial level, and regulatory approval processes for edible film application in diverse markets. Addressing these challenges in the future studies is crucial to advancing the practical adoption of such bioactive films in the food industry.

Disclosure of AI Assistance

During manuscript preparation, OpenAI's ChatGPT (GPT-5) was used solely to improve grammar and clarity. All research design, data analysis, interpretation, and conclusions were entirely the author's work, and the author takes full responsibility for the final content.

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Data Availability

Data are made available on request.

Conflicts of Interest

None.

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