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Active biodegradable jellyfish gelatine–chitosan film for preserving sierra fish (*Scomberomorus sierra*) mince at different storage temperatures

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Abstract

This study investigated the applicability of biodegradable film prepared from gelatine extracted from jellyfish *Stomolophus* sp. 2 for preserving sierra fish (*Scomberomorus sierra*) mince at three storage temperatures (0°C, 10°C, and 20°C). Gelatine was obtained from jellyfish collagen extracted under alkaline conditions through conventional hot water extraction. Microbiological quality, pH, and lipid oxidation were evaluated and compared with samples packed with commercial gelatine film (T2), packed with cellophane (C2), and unpackaged controls (C1). Storage temperature and time significantly affected all parameters ($p < 0.05$). Jellyfish gelatine–chitosan film (T1) reduced microbial growth by 2.03–3.38 log colony-forming units (CFU)/g in aerobic mesophiles and >2.31 log CFU/g in *Enterobacteriaceae* at 20°C after 96 h; at 10°C, T1 maintained lower counts, with differences of 0.46–1.48 log CFU/g and >0.68 log CFU/g, respectively, after 288 h. T1 also stabilized pH (<7.0 at 96 h, 20°C) and inhibited lipid oxidation (peroxide values <5 meq O_2/kg at 0°C; and delaying oxidation onset at 10°C). These findings demonstrate that jellyfish gelatine-based film represent a promising biodegradable packaging strategy to enhance shelf life and quality preservation of fish mince.

Keywords: gelatine film; jellyfish; mince; pacific sierra fish; shelf life

Introduction

Fish mince is a versatile and nutritious seafood product; however, its commercial application is often limited by rapid quality deterioration associated with microbial spoilage and lipid oxidation, particularly in pelagic fish species, such as *Scomberomorus sierra*, rich in dark muscle (Frankel, 1991; Maestre *et al.*, 2011). In Mexico, this species represents an important fishery resource and offers potential for value-added processing into fish mince (Comisión Nacional de Acucultura y Pesca [CONAPESCA], 2024). However, mincing disrupts cellular structures and increases exposed surface area, accelerating oxidative reactions and microbial susceptibility, thereby reducing product's shelf life (Ayeloja *et al.*, 2020; Fang *et al.*, 2017). For this reason,

effective preservation strategies are needed to improve the stability and quality of fish mince during storage (Rathod *et al.*, 2023).

In recent years, increasing attention has been directed towards the development of sustainable and environment-friendly strategies to extend the shelf life of perishable products. Among these, active packaging systems have emerged as a promising approach, particularly those based on bio-based materials incorporating functional compounds. Active packaging, including films enriched with antioxidant and antimicrobial agents, plays a key role in delaying food spoilage and preserving food quality (Mohammadzadeh *et al.*, 2024). Consequently, the development of innovative packaging solutions for fish-based products has attracted considerable research interest.

One promising strategy involves the use of biopolymer-based materials, such as gelatine and chitosan (Ranasinghe *et al.*, 2024a). Gelatine is widely recognised as an effective film-forming material because of its excellent film-forming ability as well as its good biocompatibility and biodegradability (Gómez-Guillén *et al.*, 2009). However, gelatine lacks intrinsic antimicrobial activity and is therefore commonly combined with other materials or bioactive compounds to produce composite films with improved functional properties. In contrast, chitosan, a polysaccharide obtained from natural sources, exhibits inherent antimicrobial activity and hence widely applied in food packaging systems (Eranda *et al.*, 2024; Lima *et al.*, 2006). When combined with gelatine, the resulting composite films may exhibit improved biodegradability, film-forming performance, and protective properties (Hosseini *et al.*, 2013). For this reason, gelatine–chitosan blends have attracted growing interest as biodegradable packaging materials.

Within this framework, the combination of jellyfish gelatine and chitosan has emerged as a particularly promising approach for developing innovative bio-based materials. Recent studies have demonstrated that such systems can yield films with enhanced antioxidants and effective moisture barriers (Bhargava *et al.*, 2020; Esparza-Espinoza *et al.*, 2025; Xu *et al.*, 2025). According to our findings, interactions between jellyfish gelatine and chitosan are primarily governed by carboxyl (COOH) and amino groups in proteins, together with polarised heteroatom–hydrogen bonding and other reactive groups, including hydroxyl (OH) and amino (NH_2) groups present in chitosan (Esparza-Espinoza *et al*., 2025). These interactions promoted the formation of jellyfish gelatine–chitosan films exhibiting physical, mechanical, and thermal properties, compared with those of conventional elastic films, thereby highlighting their potential application in food packaging (Esparza-Espinoza *et al*., 2025).

However, information on the use of jellyfish-derived films in food systems remains limited. Although reduced quality loss has been reported in other food matrices packed with jellyfish gelatine-based films (Ranasinghe *et al.*, 2024b), the effect of blue cannonball jellyfish (*Stomolophu*s sp. 2) gelatine–chitosan film for preserving the quality of sierra fish (*Scomberomorus sierra*) mince under different storage temperatures remains unexplored. Therefore, the objective of this study was to evaluate the effects of packaging *Scomberomorus sierra* mince with a jellyfish gelatine–chitosan film on microbial growth, pH variation, and lipid oxidation stability, and storing it at three temperatures (0, 10, and 20°C).

Materials and Methods

Jellyfish sample collection, gelatine extraction, and chemical composition

Fresh samples of blue cannonball jellyfish, *Stomolophus* sp. 2, were collected from the Guasimas Sea of Cortez (27°53'09" N, 110°34'54" W) and rapidly transported on ice to a laboratory. The samples were thoroughly cleaned with water, the gonads were removed, and the mesoglea was extracted.

Gelatine pretreatment was performed in accordance with the method described by Esparza-Espinoza *et al.* (2023). The jellyfish mesoglea was chopped into small fragments and immersed in 0.1-N NaOH at a ratio of 1:5 (w/v) for 24 h. This step was carefully designed to break down jellyfish tissues and facilitate gelatine extraction. The obtained protein extract was filtered through gauze and dialyzed in water at 4°C using a cellulose membrane with a 10 kilodalton (kDa) molecular weight cut-off (MWCO). The collected samples were frozen at −25°C and subsequently freeze-dried. The obtained dried gelatine was stored in air-tight containers. Gelatine type B from bovine skin, 175 blooms (Sigma Chemical Co., St. Louis, MO) was obtained from a local chemical supplier.

Moisture, crude protein, and ash contents of the jellyfish and commercial gelatine were quantified using the methods described in official Association of Analytical Chemists (AOAC, 2000), and the results presented in gravimetric concentration units (g/kg).

The molecular weight distribution in lyophilized jellyfish gelatine was determined by rehydrating the samples (1 mg/mL) with 150-mM sodium phosphate buffer at pH 7.0. The samples were subsequently filtered through a 0.45-µm polytetrafluoroethylene (PTFE) filter and chromatographed using a Varian high-performance liquid chromatography (HPLC) system (Varian™

Pro Star, Agilent, Santa Clara, CA) equipped with a Bio SEC-5™ size-exclusion column (4.6 × 300 mm; Agilent, Santa Clara, CA). The samples were monitored with a diode array detector (Varian™ Pro Star) using the Galaxy™ software (version 1.9.302.952). Elution was performed under isocratic conditions at a flow rate of 0.4 mL/min at room temperature, and the absorbance was monitored at 254 nm. A standard curve was generated using a gel filtration molecular weight marker kit for proteins, and the following five reference molecules were considered: thyroglobulin (MW = 670 kDa), gamma globulin (MW = 158 kDa), ovalbumin (MW = 44 kDa), myoglobin (MW = 17 kDa), and vitamin B_{12} (MW = 1.35 kDa).

Preparation of films with gelatine extracts

Gelatine films were produced by solvent evaporation at 40°C under overnight mechanical stirring (Martínez-Camacho *et al.*, 2010). Gelatine–chitosan–glycerol biofilms were prepared according to the established protocols (Esparza-Espinoza *et al.*, 2025), with minor modifications to improve film detachment and quality. To obtain uniform thickness and appearance, Teflon plates were used as casting supports to facilitate film removal. Separate solutions of jellyfish gelatine (4%, w/v), commercial gelatine (4%, w/v), and chitosan (1%, w/v) were prepared. Lyophilised jellyfish gelatine and commercial gelatine were dissolved in triple-distilled water, while chitosan was dissolved in 0.1-M acetic acid. Gelatine solutions were mixed with chitosan under continuous stirring at 350 rpm and 25°C. Glycerol (1%, w/v) was added as a plasticiser, and stirring was continued for 30 min until a homogeneous mixture was obtained. The final mass ratio of gelatine–chitosan–glycerol was 4:1:2. The film-forming solutions were cast onto Teflon plates and dried at room temperature for 72 h under controlled humidity (approximately 30% relative humidity [RH]) using silica gel.

Preparation of mince blends

Sierra fish (*Scomberomorus sierra*) were sourced from artisanal fishermen in Bahía de Kino, Sonora, Mexico. Specimens were transported to laboratory in polythene bags with flaked ice, with transport time limited to less than 4 h to minimise bacterial growth and maintain freshness. Upon arrival, temperature was confirmed to be less than 4°C to inhibit enzymatic activity and microbial proliferation. Fish weighing 200–300 g were selected to standardise initial biomass and ensure uniform processing. Specimens were washed with chilled potable water, gutted, eviscerated, and headed before filleting. Initial washing at 4°C was considered a critical control step to remove external contaminants while maintaining conditions that prevent spoilage. During filleting, cross-contamination was minimised by using sanitised utensils and surfaces and by following food safety practices. Skinless fillets were separated from the bones, and the muscle was minced using a mechanical mincer (Nikou MX, Mexico) fitted with a 5-mm sieve to obtain uniform mince, facilitating even mixing and consistent texture in subsequent applications. Minced muscle was handled under refrigeration to preserve functional and sensory properties and to limit protein denaturation, with internal temperature regularly monitored to prevent increases above 4°C. All processing steps, including washing, evisceration, filleting, mincing, and stuffing, were performed at 4°C to ensure product quality and minimise microbial and enzymatic deterioration (US Food and Drug Administration [US FDA], 2011). The processing sequence was completed within 20 min to limit exposure to ambient conditions and reduce batch-to-batch variability. Temperature loggers were used throughout to verify maintenance of the cold chain.

Packaging and storage of fresh sierra mince

Fish mince samples were assigned to four groups: unpackaged control (C1), samples packed with commercial cellophane (biaxially oriented polypropylene) (C2), samples packed with jellyfish gelatine–chitosan film (T1), and samples packed with commercial gelatine–chitosan film (T2). Fish mince portions (10 g) were packed using 10 cm × 10 cm films (C2, T1, and T2), ensuring direct contact between the product and its packaging material. Initial analyses were performed immediately after fish mince preparation. Because these initial (time 0) measurements were conducted prior to packaging treatments were applied, the same baseline value was used for all treatments. Following initial analyses, fish mince was distributed among treatments and stored at 0±1°C (ice-point storage), 10±1°C (controlled refrigeration), and 20±1°C (control room temperature), representing optimal preservation, moderate refrigeration, and temperature abuse conditions, respectively. Samples were periodically analysed for pH, microbiological quality, and lipid oxidation. Storage duration and final sampling period were established *a priori* based on previous studies and expected spoilage progression; for consistency, storage at 0°C and 10°C was evaluated up to 288 h. The adequacy of these sampling periods was supported by objective spoilage indicators, including dehydration and colour change in unpackaged control at 0°C, and microbial counts (r) approaching 7 $\log_{10}$ colony-forming units (CFU)/g together with pH variation at 10°C. These observations supported the storage periods presented in Table 1.

Table 1. Sampling time (hours) for fish mince stored under different conditions (C1, C2, T1, and T2) at controlled freezing (0±1°C), refrigerator (10±1°C), and room (20±1°C) temperatures.

Time (h)	Temperature		
	0°C	10°C	20°C
0	S	S	S
6	NS	NS	S
12	NS	NS	S
24	S	S	S
48	S	S	S
72	S	S	S
96	S	S	S
120	S	S	NS
144	S	S	NS
216	S	S	NS
288	S	S	NS

Notes: C1: unpackaged; C2: packaged with commercial cellophane; T1: packaged with jellyfish gelatine–chitosan film; T2: packaged with commercial gelatine-chitosan film. S: sampled; NS: not sampled.

Microbiological analysis

Total aerobes, *Enterobacteriaceae*, and moulds and yeasts were determined in accordance with standards of the International Organization for Standardization (ISO). Total aerobes were enumerated according to ISO 4833-1:2013 by (ISO, 2013) using plate count agar (PCA) and incubated at 30°C for 48 h. *Enterobacteriaceae* were enumerated according to ISO 21528-2:2017 (ISO, 2017a) on violet red bile glucose agar and incubated at 37°C for 48 h. Moulds and yeasts were enumerated according to ISO 21527–1:2008 (ISO, 2008) using Bengal red agar supplemented with dichloran and chloramphenicol and incubated at 25°C for 5 days. The samples were aseptically collected, homogenized, and diluted at a ratio of 1:9 in 0.1% (w/v) peptone water, and serial dilutions were prepared according to ISO 6887-1:2017 (ISO, 2017b). Appropriate dilutions were plated on the corresponding culture media in triplicate by using the spread plate technique with a Drigalski spatula. Colony counts were performed using a digital colony counter (CVP-CM3, CScientific®, Mexico City, Mexico), and the results were expressed as $\log_{10}$ CFU/g fish mince.

Chemical analysis

The pH of fish mince was measured using a digital pH meter (Hanna HI 2211, Mexico) equipped with a puncture electrode, in accordance with the official methods described by the Association of Official Analytical Chemists (AOAC, 2000).

Lipids were extracted using a chloroform–methanol solvent system (1:1, v/v) in accordance with the method described by Bligh and Dyer (1959). Lipid extracts were subsequently evaporated and concentrated using a rotary evaporator (R-100, Büchi, Switzerland). Concentrated lipids were subsequently used to analyse lipid oxidation-related compounds.

Peroxide values (PV) were determined as indicators of lipid oxidation according to the official AOAC method (2000). The results were expressed as milli equivalents of peroxide oxygen per kilogram of sample. Secondary lipid peroxidation products, mainly unsaturated aldehydes (2-alkenals), were evaluated using the para-anisidine value (p-AV), in accordance with the method described by the International Union of Pure and Applied Chemistry (IUPAC, 1992). The p-AV was calculated using Equation (1):

$$p\text{-}anisidine\ value = (25 \times (1.2 \times Abs\ oil\ solution\ after\ reacting\ with\ p - anisidine - Abs\ oil\ solution)) / (sample\ mass\ (g)) \quad (1)$$

The overall oxidation state (TOTOX) was computed according to Equation (2), reported by Pignitter and Somoza (2012):

$$TOTOX = 2\ Peroxide\ value + p - Anisidine\ value \quad (2)$$

Statistical analysis

All numerical values were reported as means and standard deviations (SD) of three independent analytical determinations. A completely randomised factorial design was used, with two fixed factors: packaging treatment (four levels: C1, C2, T1, and T2) and storage temperature (three levels: 0, 10, and 20°C). For each treatment–temperature combination, three independent replicates were prepared, yielding 36 experimental units (4 packaging treatments × 3 storage temperatures × 3 replicates). The effects of these factors on pH, microbial growth, and lipid oxidation were evaluated. Measurements were collected over time, with sampling intervals varying according to temperature; therefore, time was treated as a continuous covariate.

Data was analysed using linear mixed-effect models to account for repeated measurements within each experimental unit. Treatment, temperature, and their interaction were included as fixed effects, while time was modelled as a continuous predictor (linear and quadratic terms).

Replicates nested within treatment and temperature were included as random effects. The significance of fixed effects was assessed using Type III analysis of variance (ANOVA), and Tukey's test was applied for multiple comparisons when significant differences were detected ($p < 0.05$).

Kinetic analyses of changes in quality parameter during storage were performed using linear or non-linear regression models, and rate constants (k) were estimated. Temperature dependence of lipid oxidation rates was evaluated using the Arrhenius equation. Pearson's correlation analysis was conducted to assess relationships among fish quality parameters (microbiological, pH, and lipid oxidation) during storage. All statistical analyses were performed using the R software (R Core Team, 2026; R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

The proximate composition of the jellyfish gelatine obtained in the present study was consistent with the values reported previously for the gelatine extracted from jellyfish species using the same extraction method (Esparza-Espinoza *et al.*, 2023), confirming the reproducibility of extraction procedure. Protein, ash, and moisture contents were 768.3±9.2 g/kg, 54.7±1.5 g/kg, and 107.6±3.7 g/kg, respectively, showing no substantial deviations from previously reported values. Similar composition was observed relative to commercial type B gelatine, although jellyfish gelatine presented higher ash content, potentially related to its marine origin and associated mineral content (Chiarelli *et al.*, 2021).

The molecular weight distribution of jellyfish gelatine was determined by size-exclusion chromatography (Figure 1). The chromatograms revealed a high proportion of high-molecular weight peptides, predominantly in the range of 44–158 kDa, accounting for 59.66% of the total peptide content. This finding was consistent with previous studies on jellyfish collagen, in which high-molecular weight fractions, such as α-chains (~122–140 kDa) and β-components (>250 kDa), are typically predominant (Balikci *et al.*, 2024). In contrast, Leone *et al.* 2015) reported that hydrolysed collagen from Mediterranean jellyfish showed a predominance of low- to intermediate-molecular-weight peptides, mainly up to 50–70 kDa, as determined by SDS-PAGE, with species-specific polypeptides in the range of 15–70 kDa. In the present study, medium-molecular weight (17–44 kDa) and low-molecular weight (1.35–17 kDa) peptides were discovered, representing a total peptide content of 16.3% and 20.34%, respectively (Table 2).

The molecular profile observed could be relevant to the functional properties of the developed films, as the chain length of extracted gelatine is reported to directly affect the film-forming and antioxidant properties of protein-based films (Jridi *et al.*, 2014). Whereas low-molecular weight components may lead to fewer intermolecular interactions and higher molecular mobility (Giménez *et al.*, 2009), high-molecular weight polypeptides have strong film-forming capacity (Zhang *et al.*, 2019). Moreover, previous studies have shown that jellyfish gelatine and chitosan exhibit excellent compatibility, attributed to hydrogen bonding, resulting in flexible and thermostable films with strong free radical-scavenging capacity (Esparza-Espinoza *et al.*, 2025).

Determination of microbiological activity

Data was analysed using mixed-effects models, considering time as a continuous variable, allowing the evaluation of dynamic changes during storage. Microbiological stability was assessed through total aerobic counts and *Enterobacteriaceae* to determine the effects of packaging treatment and storage temperature on microbial development. Total aerobic counts were significantly affected by packaging treatment and interaction between packaging and storage temperature ($p < 0.05$), indicating that the antimicrobial effectiveness of packaging depended on storage conditions. Microbial counts are shown in Figure 2, while detailed mean values and SDs are provided in Supplementary Table S1. In general, C1 exhibited the highest microbial counts, whereas C2 showed moderate reductions only, suggesting a limited barrier effect. In contrast, gelatine-based films, particularly T1 (jellyfish gelatine–chitosan), consistently resulted in lower microbial counts than both control treatments. This effect was most evident under temperature abuse conditions. At 20°C, control treatments reached approximately 6.8–6.9 $\log_{10}$ CFU/g after 72 h and exceeded 7.6 $\log_{10}$ CFU/g after 96 h, whereas T1 remained close to 4.0 $\log_{10}$ CFU/g at 72 h and 4.3 $\log_{10}$ CFU/g at 96 h, corresponding to an approximate reduction of 3.4 log units relative to the controls. Using 6 $\log_{10}$CFU/g as a practical microbiological acceptability limit, the controls reached this threshold at approximately 62–63 h, while T1 did not reach this limit within the 96-h observation period, indicating clear extension of microbiological shelf life. These findings suggest that the active film reduced apparent microbial growth rates and prolonged the lag phase, modifying spoilage kinetics rather than simply reducing microbial counts at individual sampling points.

An apparently inconsistent behaviour was observed for T2, which showed partial inhibition of total aerobic counts at 20°C but higher aerobic development at 10°C. This response suggests that the preservative effect of

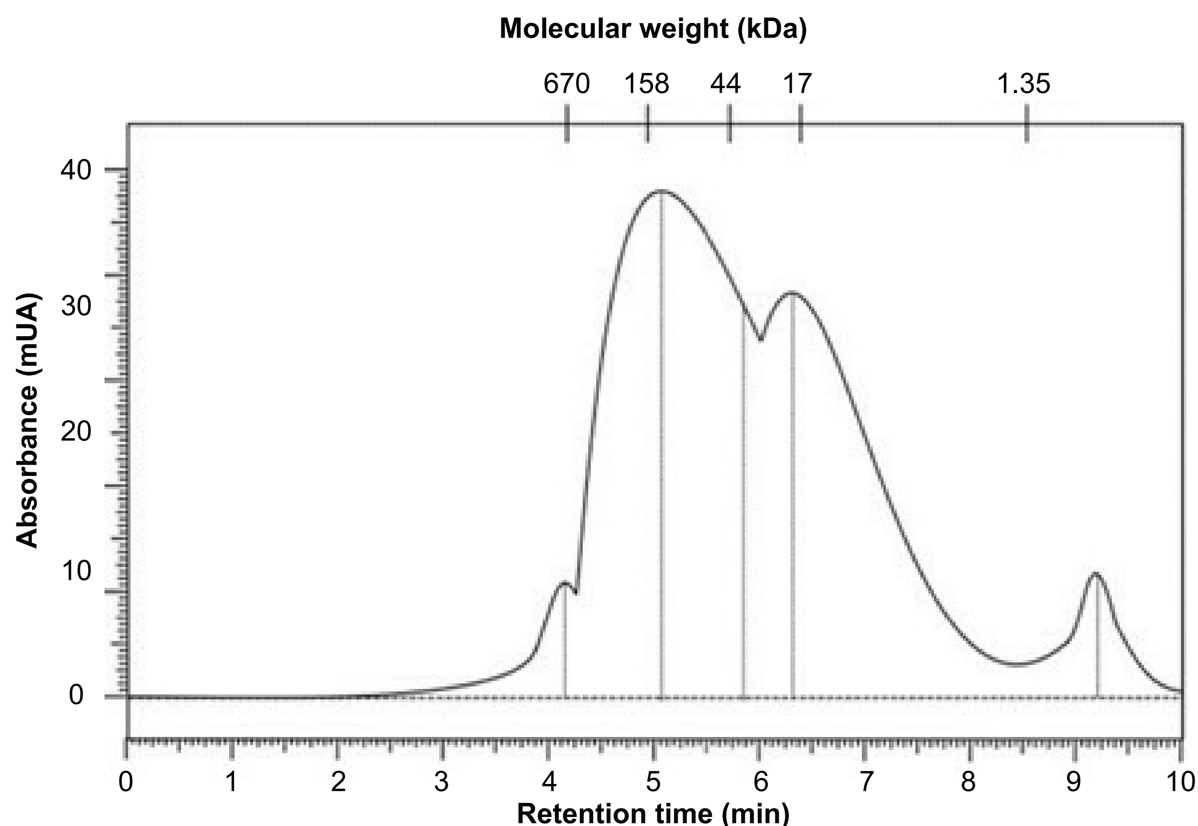

Figure 1. Molecular weight distribution of peptides in jellyfish (*Stomolophus* sp. 2) gelatine determined by size-exclusion chromatography. Chromatographic profile shows the relative abundance of peptide fractions according to molecular size.

Table 2. Chromatographic profile of peptides in cannonball jellyfish (*Stomolophus* sp. 2) gelatine.

Kilodalton (kDa)	Time (min)	Percentage of total peptides
>158	4.64	1.06
158–44	5.09	59.66
44–17	5.82	16.3
17–1.35	6.51	20.34
<1.35	9.6	2.27
Total		**100**

commercial gelatine–chitosan film was temperature-dependent and not equally effective against all microbial groups. At 20°C, T2 delayed increase in total aerobic counts relative to control treatments, whereas at 10°C, it showed the highest aerobic counts during the later stages of storage. This behaviour may indicate that the protective effect of T2 relied mainly on partial barrier properties, which were insufficient to control aerobic spoilage microbiota during prolonged storage at intermediate refrigeration temperature. In contrast, this effect was less evident for *Enterobacteriaceae*, for which T2 remained below the controls (C1) at 10°C during most of the storage period. These findings suggest that T2 generated a less stable preservative response than T1, whose antimicrobial performance was more consistent across temperatures.

A similar trend was observed for *Enterobacteriaceae*. Storage temperature significantly influenced *Enterobacteriaceae* levels ($p < 0.001$), whereas packaging treatment and the interaction between factors were not statistically significant ($p > 0.05$). Counts were considerably lower at 0°C than at 10°C and 20°C, confirming the dominant effect of refrigeration on microbial development. Nevertheless, slightly lower values were generally observed in samples packed with gelatine-based films, particularly T1.

Analysis of moulds and yeasts showed limited fungal development, compared with bacterial growth (Supplementary Table S2). At 20°C, filamentous moulds

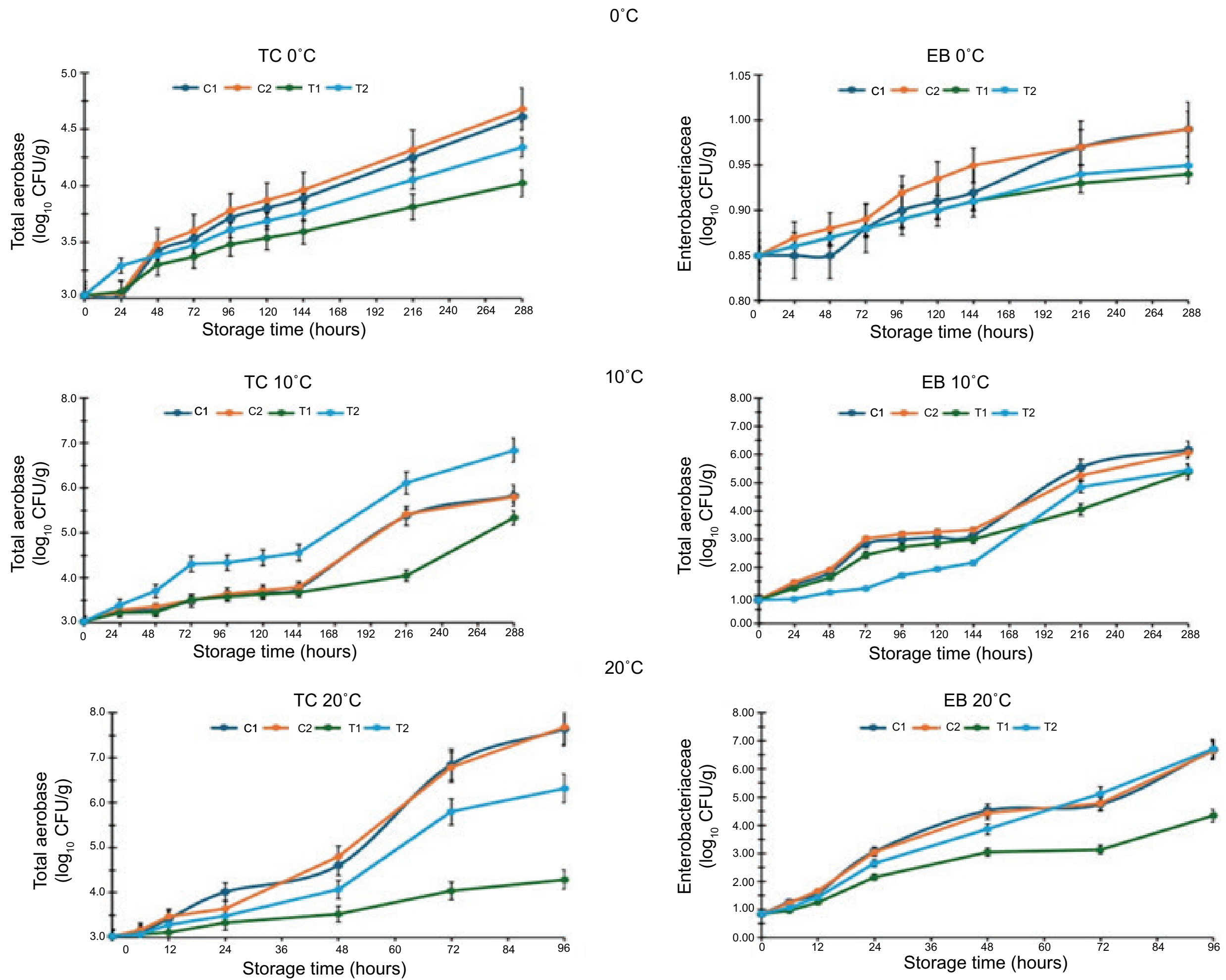

Figure 2. Microbial growth kinetics of total aerobes and *Enterobacteriaceae* in sierra fish (*Scomberomorus sierra*) mince under different packaging treatments during storage at 0, 10, and 20°C. Values are means ± SD (n = 3).

were observed only after 96 h in controls (C1; 6.3 $\log_{10}$ CFU/g) and those packed in cellophane (C2; 1.82 $\log_{10}$ CFU/g). At 10°C, yeasts were observed in all treatments after 288 h, which was consistent with the ability of some yeast to grow in chilled, high-moisture foods (Tournas *et al.*, 2001). Notably, T1 significantly inhibited yeast growth ($p < 0.05$), showing a final increase of 1.42 $\log_{10}$CFU/g, compared with 2.10, 3.37 and 1.94 $\log_{10}$ CFU/g for C1, C2, and T2, respectively. Although filamentous moulds were detected in C1 and C2 at 20°C, their occurrence was limited to temperature abuse conditions and was interpreted as an evidence of advanced spoilage associated with compromised storage control (Tournas *et al.*, 2001). As expected, no moulds or yeasts were detected at 0°C.

The microbiological data indicated that T1 exhibited significant antimicrobial activity during fish mince storage. Although antimicrobial effects in gelatine–chitosan composite films often have been attributed to chitosan (Jridi *et al.*, 2014), the higher effectiveness observed for jellyfish gelatine films, compared with commercial gelatine films, suggests that additional mechanisms are involved. This effect could be associated with low-molecular weight bioactive peptides reported in jellyfish-derived materials (Aziz *et al.*, 2022), which may contribute to microbial inhibition if incorporated into composite films. Furthermore, the reduced oxygen permeability of protein-based films can have limited aerobic spoilage development, contributing to the extension of observed shelf life. Similar effects are reported for active packaging systems applied to refrigerated animal-derived foods (Chen *et al.*, 2020; López *et al.*, 2024; Sánchez-Ortega *et al.*, 2014).

Assessment of pH changes as an indicator of quality deterioration

A progressive increase in pH with storage time ($p < 0.05$) was observed in all fish mince batches under all

temperature conditions, indicating a strong temporal dependence of pH changes (Figure 3). Storage temperature significantly affected pH values ($p < 0.001$), whereas packaging treatment and the interaction between treatment and temperature were not significant ($p > 0.05$), suggesting that temperature was the dominant factor governing pH evolution. Nevertheless, treatment-dependent trends were evident. At 20°C, pH variation in T1 did not exceed 0.5 units from the initial value and remained below 7.0 after 96 h, whereas greater increase was observed in control treatments. At 10°C and 0°C, T1 also showed lower deviations from the initial pH after 288 h. The ranking of pH variation at 20°C and 10°C was C1 > C2 > T2 > T1, while at 0°C it was C1 = C2 > T2 > T1, indicating greater pH stability in active film treatments, particularly T1.

Although the magnitude of pH changes was moderate, these results are technologically relevant, as shifts of 0.5 pH units or greater have been associated with the onset of quality deterioration (Kumaravel *et al.*, 2025). The lower pH drift observed in T1 was consistent with reduced microbial development and agrees with previous studies reporting improved pH stability in fish and meat products packaged with protein-based films (Abeyrathne *et al.*, 2021; Feng *et al.*, 2016).

Increases in pH in fish mince are commonly associated with microbial and enzymatic degradation of nitrogenous compounds, leading to formation of alkaline metabolites such as ammonia and trimethylamine (Dhar *et al.*, 2013). In the present study, pH was positively correlated with microbial counts (r = 0.81–0.97), supporting a close relationship between microbial development and pH evolution; these relationships are further discussed in the 'Correlation Analysis' section. Consistent with microbiological results, T1 showed lower pH variation associated with reduced microbial development, suggesting that the jellyfish gelatine–chitosan film contributed to slowing spoilage-related metabolite accumulation. As discussed above, low-molecular weight peptides in jellyfish gelatine may have contributed to this preservative effect.

Chemical assessment of development of lipid damage

Lipid oxidation was monitored through PV, AV, and TOTOX index to evaluate primary oxidation products, secondary aldehydic compounds, and the overall oxidative stability, respectively (Pignitter and Somoza, 2012) (Figure 4). Packaging treatment significantly affected PV ($p < 0.05$), whereas storage temperature and the interaction between factors were not significant. In general, C1 showed the highest PVs, indicating greater formation of primary oxidation products because of direct exposure to oxygen. Samples packed in cellophane (C2) showed intermediate values, suggesting partial oxidative protection, whereas gelatine-based films, particularly T1, generally exhibited lower PVs, indicating delayed initiation of lipid oxidation. In addition, PVs of control samples tended to increase during early storage and decline at later stages, consistent with the decomposition of hydroperoxides into secondary oxidation products. This behaviour was less pronounced in active film treatments, suggesting greater oxidative stability.

Anisidine value was significantly affected by packaging treatment, storage temperature, and their interaction ($p < 0.05$), indicating that formation of secondary oxidation products was strongly dependent on both storage conditions and packaging system. AVs increased with temperature, with the highest levels generally observed in unpackaged samples stored at 20°C, reflecting progressive accumulation of aldehydic compounds associated with advanced lipid oxidation. In contrast, samples packed with gelatine-based films maintained lower AVs, suggesting reduced decomposition of hydroperoxides and lower formation of secondary oxidation products. This effect was particularly relevant in T1, which consistently showed improved oxidative stability under the most severe storage conditions. The overall oxidative deterioration was further evaluated using the TOTOX index, which integrates both primary and secondary oxidation products. Packaging treatment significantly influenced TOTOX values, while storage temperature and the interaction between factors were not statistically significant. Control samples showed the highest TOTOX values, whereas gelatine-based films generally maintained lower values throughout storage, confirming improved oxidative stability. The lower TOTOX values observed in T1 support the hypothesis that the active film did not merely delay isolated oxidation reactions but also altered oxidation kinetics by slowing both hydroperoxide formation and subsequent aldehyde generation.

The improved oxidative stability observed in samples packed with gelatine-based films can be attributed to the barrier properties of protein-based packaging materials, which reduce oxygen permeability and limit propagation of oxidative reactions. This effect also can be associated with the antioxidant potential of jellyfish-derived components. Previous characterisation studies using Fourier Transform Infrared (FTIR), nuclear magnetic resonance (NMR), and proteomic analyses revealed the presence of compounds in jellyfish collagen associated with antioxidant activity (Villalba-Urquidy *et al.*, 2025), while a prior work demonstrated preservative and antioxidant effects in jellyfish gelatine–chitosan systems (Esparza-Espinoza *et al.*, 2025). These mechanisms may explain the superior performance of T1 relative to the commercial gelatine

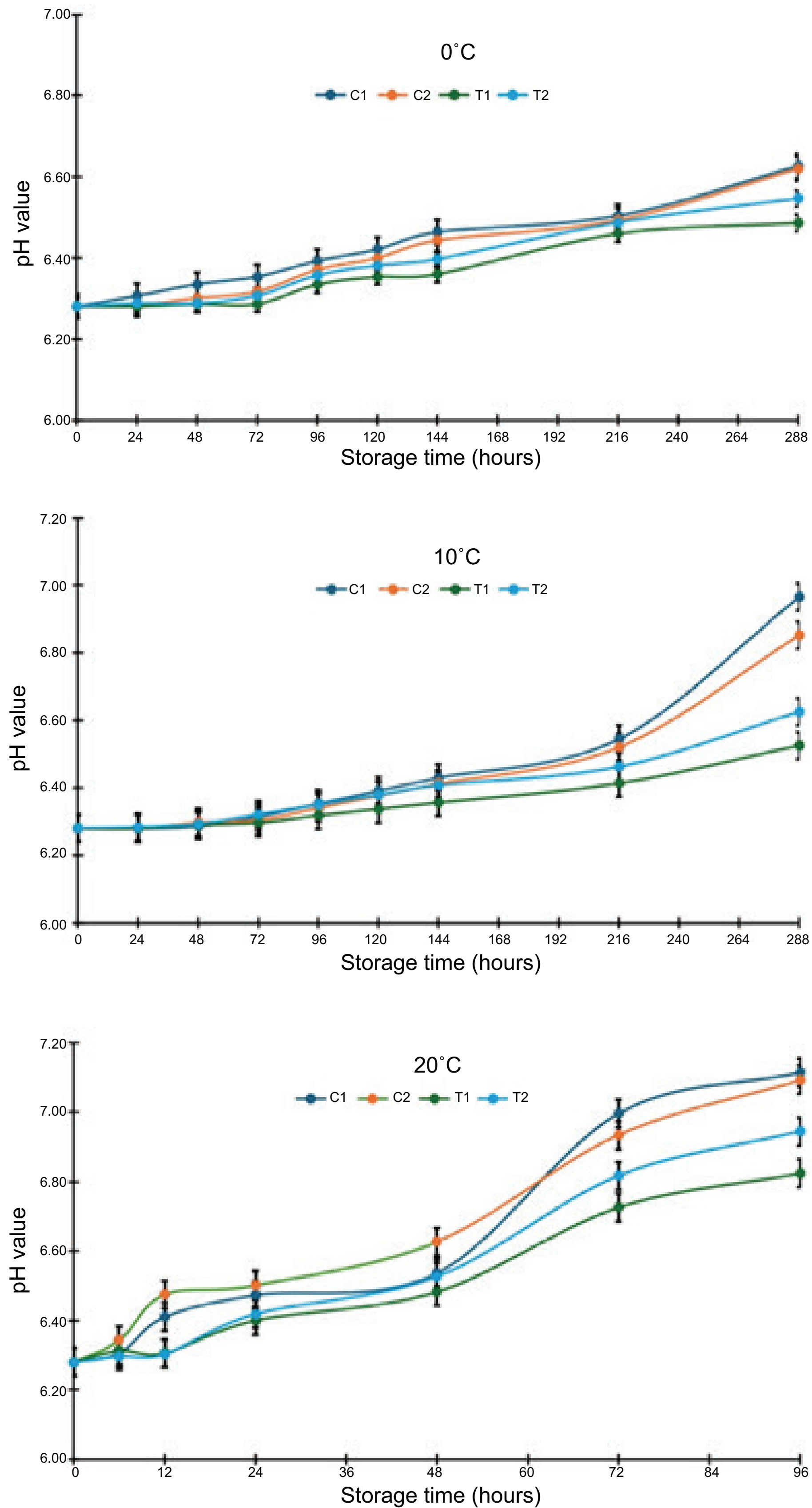

Figure 3. Changes in pH during storage of sierra fish (*Scomberomorus sierra*) mince under different packaging treatments at 0, 10 and 20°C. Values are means ± SD (n = 3).

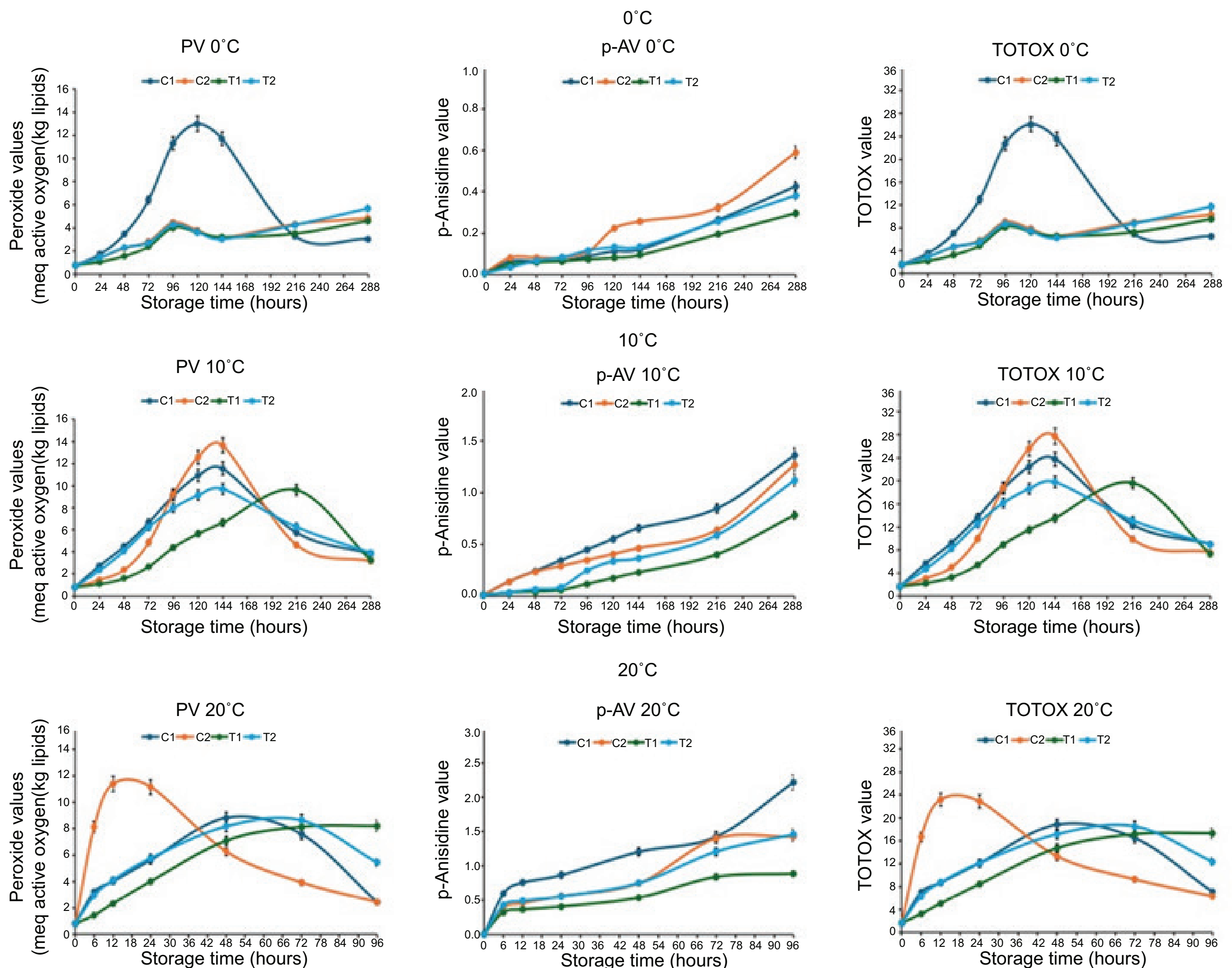

Figure 4. Changes in peroxide, anisidine, and TOTOX values of sierra fish (*Scomberomorus sierra*) mince under different packaging treatments during storage at 0, 10, and 20°C. Values are means ± SD (n = 3).

film. Similar protective effects are reported in seafood products packaged with protein-based films, where reduced peroxide and anisidine formation was associated with delayed oxidative spoilage (Dehghani *et al.*, 2018; Eranda *et al.*, 2024).

Kinetic analysis of lipid oxidation

To further understand the mechanisms governing lipid oxidation during storage, kinetic analysis was applied to selected quality parameters. PV and TOTOX index were used to evaluate oxidation behaviour under different treatments and storage temperatures. TOTOX index exhibited a non-linear evolution during storage, particularly at 10°C and 20°C, characterised by an initial increase, followed by a subsequent decline (Figure 5A). This pattern reflected the formation of primary oxidation products (hydroperoxides) and their subsequent decomposition into secondary compounds. Therefore, TOTOX index was used primarily to describe the overall progression of lipid oxidation, rather than for estimating kinetic rate constants. Compared with the controls (C1), T1 generally showed lower peak TOTOX values, and a less pronounced oxidation trajectory, suggesting delayed oxidative progression.

Peroxide values were used for kinetic analysis because of their more consistent behaviour. Rate constants (k) were estimated from linear trends observed during the initial stages of oxidation. In general, higher storage temperatures increased k values, indicating accelerated oxidation rates. Conversely, samples packed with gelatine-based films, particularly T1, exhibited lower k values, reflecting improved oxidative stability and slower oxidation kinetics.

The temperature dependence of oxidation rates was evaluated using the Arrhenius model. A linear relationship between ln(k) and reciprocal absolute temperature (1/T) was observed (Figure 5B), indicating temperature-dependent oxidation behaviour. Differences in slope among treatments suggested variations in the sensitivity of oxidation rates to temperature, with samples packed with films generally showing lower temperature sensitivity than controls. These results support the protective role of active films in reducing the acceleration of lipid oxidation under increasing storage temperature.

Taken together, these findings demonstrated that T1 not only delayed lipid oxidation but also modified deterioration kinetics, contributing to improved oxidative stability during storage.

Combined preservative effect

Taken together, the microbiological, pH, lipid oxidation, and kinetic results indicated that the jellyfish gelatine–chitosan film acted through a multifunctional preservative mechanism involving microbial growth inhibition, oxidative stabilisation, and delayed spoilage kinetics, ultimately contributing to extension of shelf life. Compared with the control treatments, T1 consistently showed lower microbial counts, improved pH stability, reduced PV, AV, TOTOX values, and lower oxidation rates, indicating superior preservative performance. These findings suggested that the active film did not merely provide passive protection but also influenced development of spoilage through concurrent antimicrobial and antioxidant effects.

The improved performance of T1 can be associated with the combined action of chitosan antimicrobial properties, the oxygen barrier characteristics of the protein matrix, and the potential contribution of bioactive low-molecular weight peptides derived from jellyfish gelatine. In addition to limiting microbial proliferation, reduced oxygen permeability could slow down both hydroperoxide formation and subsequent development of secondary oxidation products. This combined response was particularly evident under temperature abuse conditions, where protective effect of the active film became more pronounced. Overall, the results support the use of jellyfish gelatine–chitosan films as active packaging systems capable of improving both microbiological and oxidative stability during storage.

Relationships among microbiological and oxidative indicators

Pearson's correlation analysis revealed significant relationships among microbial growth, pH changes, and oxidative deterioration, supporting the interconnected nature of spoilage processes during storage. Relationships among variables are shown in

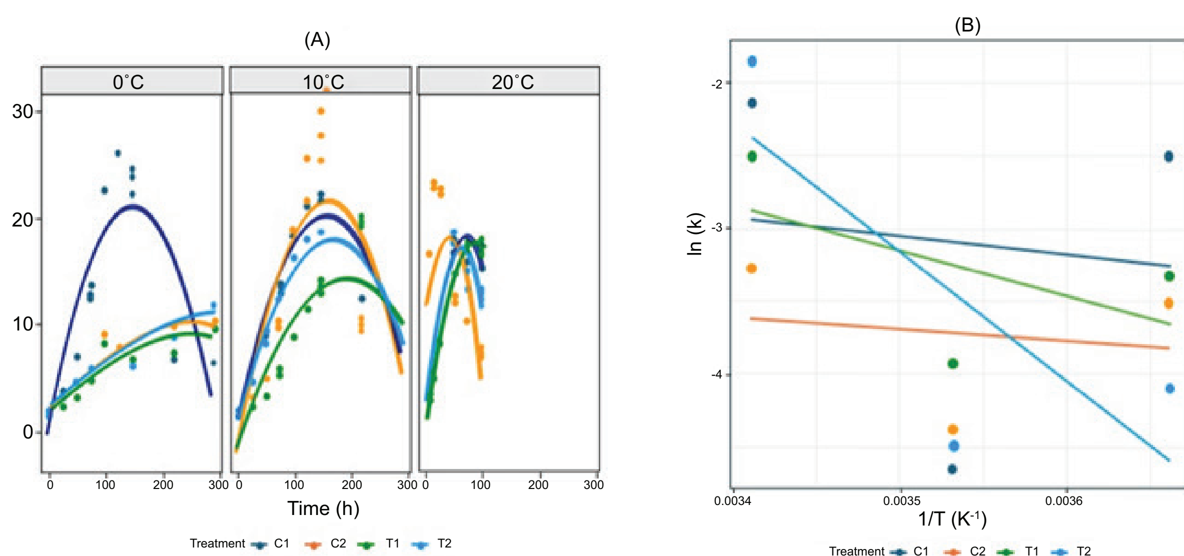

Figure 5. Kinetic analysis of lipid oxidation of sierra fish (*Scomberomorus sierra*) mince packed under different treatments: (A) Evolution of TOTOX values during storage at 0, 10, and 20°C. (B) Arrhenius plots (ln(k) versus 1/T) describing the temperature dependence of peroxide formation rates.

Supplementary Figure S1. Total microbial counts were positively correlated with *Enterobacteriaceae* ($r = 0.808$, $p < 0.001$), confirming a close relationship between the overall microbial development and *Enterobacteriaceae* proliferation. Significant positive correlations were also observed between total microbial counts and pH ($r = 0.865$, $p < 0.001$) as well as AV ($r = 0.844$, $p < 0.001$), suggesting that microbial activity contributed to biochemical changes associated with advanced oxidation. Similarly, *Enterobacteriaceae* showed a strong correlation with AV ($r = 0.872$, $p < 0.001$), reinforcing the relationship between microbial growth and formation of secondary oxidation products.

Moderate correlations were observed between microbial parameters and PV, including total microbial counts ($r = 0.250$, $p < 0.001$) and *Enterobacteriaceae* ($r = 0.359$, $p < 0.001$), indicating that primary oxidation products were less directly associated with microbial activity than secondary oxidation compounds. Overall, these relationships suggest that microbial growth, biochemical changes, and lipid oxidation progressed concurrently during storage and were partially interconnected.

Comparison of jellyfish and commercial gelatine-based packaging

A direct comparison between the two gelatine-based packaging systems showed that both T1 and T2 improved product stability, compared with the control treatments, confirming the effectiveness of protein-based films as biodegradable packaging materials. However, T1 generally showed slightly better performance, particularly in oxidative stability and microbial inhibition, with lower PV and AV, lower microbial counts, and improved pH stability than T2.

Although differences between the two gelatine treatments were not always statistically significant, the results suggest that jellyfish gelatine is a promising alternative raw material for active biodegradable packaging. Its slightly improved performance may be related to differences in molecular structure, permeability, and the presence of bioactive compounds associated with marine-derived gelatine.

Conclusions

The results of this study demonstrated that both packaging treatment and storage temperature significantly influenced the microbiological and oxidative stability of fish mince during storage. Refrigeration effectively limited microbial growth and delayed oxidative deterioration, while active packaging further enhanced product stability. Among the evaluated treatments, T1 (jellyfish gelatine–chitosan) consistently showed the best preservative performance, with lower microbial counts, improved pH stability, and reduced oxidation indicators, compared with the control treatments.

The protective effect of T1 was associated with concurrent antimicrobial and antioxidant actions, suggesting a multifunctional preservative mechanism involving microbial growth inhibition, oxidative stabilisation, and delayed spoilage kinetics. This combined effect contributed to improved stability and extension of shelf life, particularly under conditions of greater spoilage pressure.

Taken together, these findings indicate that jellyfish gelatine–chitosan films represent a promising active packaging alternative for preserving the quality and safety of fish products during storage, while supporting the valorisation of marine-derived resources for sustainable food packaging applications.

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Mandatory AI Declaration

The authors used ChatGPT (OpenAI) solely for language editing and translation support. All AI-assisted content was reviewed and edited by the authors, who take full responsibility for the final version of the manuscript.

Author Contributions

All authors contributed to this work. All authors have read and approved the final version of the manuscript.

Competing Interests

The authors declare no competing interests.

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Supplementary

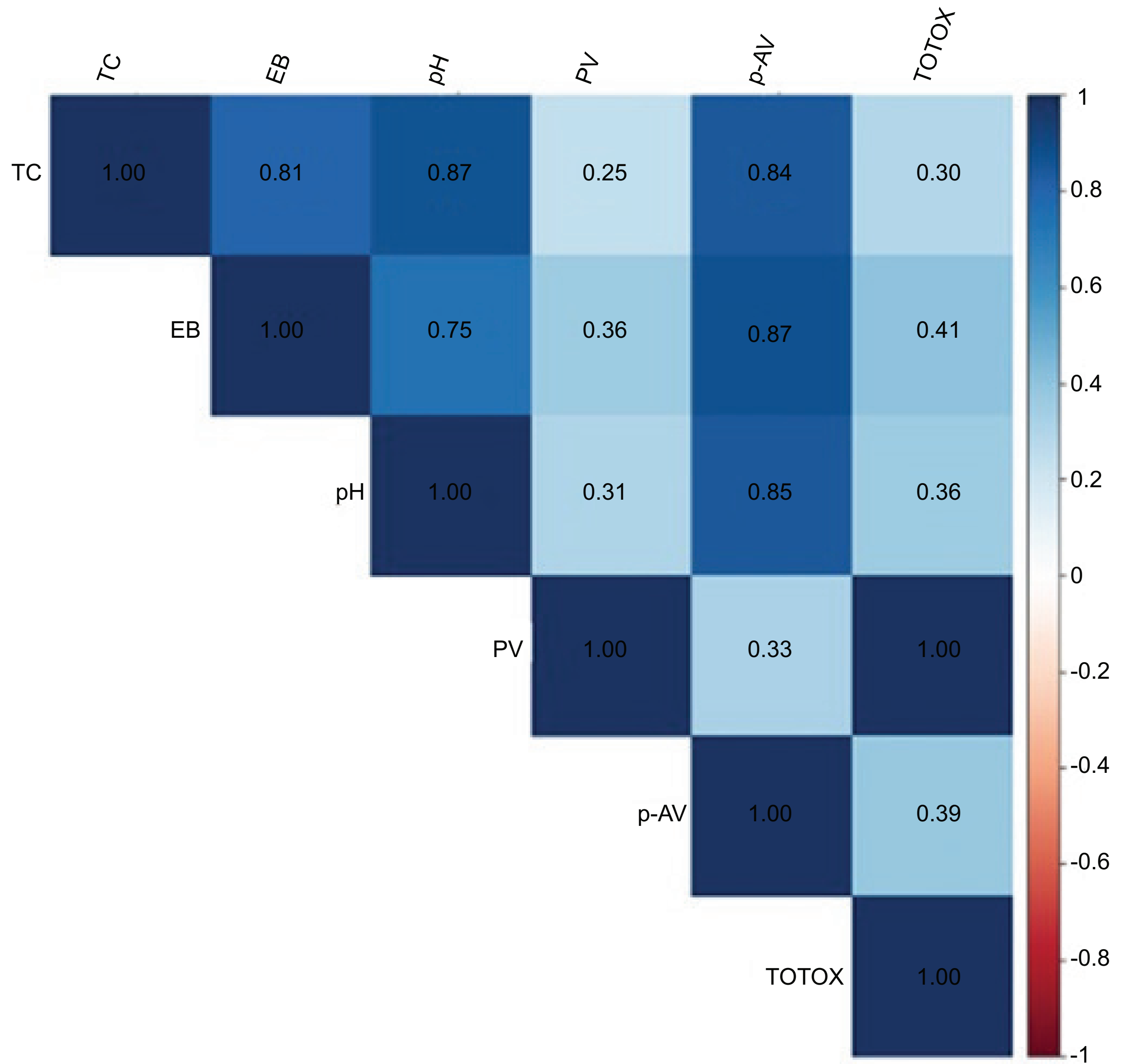

Figure S1. Pearson correlation matrix (r values) among total aerobic counts, Enterobacteriaceae, pH, peroxide value (PV), anisidine value (p-AV) and TOTOX during storage of sierra fish (*Scomberomorus sierra*) mince.

Table S1. Assessment of total aerobes and *Enterobacteriaceae* in fish mince stored under different conditions (C1, C2, T1, and T2) at 0°C, 10°C, and 20°C.

Storage time (hrs)	Total aerobes				*Enterobacteriaceae*			
	C1	C2	T1	T2	C1	C2	T1	T2
			0°C				0°C	
0	3.03 (0.15)	–	–	–	0.85 (0.06)	–	–	–
24	3.13^{a} (0.20)	3.14^{a} (0.15)	3.06^{a} (0.10)	3.29^{a} (0.09)	0.85^{a} (0.04)	0.87^{a} (0.06)	0.86^{a} (0.04)	0.86^{a} (0.05)
48	3.41^{a} (0.04)	3.48^{a} (0.06)	3.30^{a} (0.07)	3.38^{a} (0.14)	0.85^{a} (0.07)	0.88^{a} (0.02)	0.87^{a} (0.05)	0.87^{a} (0.07)
72	3.53^{a} (0.15)	3.60^{a} (0.10)	3.37^{b} (0.05)	3.47^{ab} (0.05)	0.88^{a} (0.11)	0.89^{a} (0.05)	0.88^{a} (0.07)	0.88^{a} (0.04)
96	3.71^{a} (0.04)	3.78^{a} (0.21)	3.48^{a} (0.21)	3.61^{ab} (0.05)	0.90^{a} (0.17)	0.92^{a} (0.05)	0.89^{a} (0.07)	0.89^{a} (0.07)
120	3.80^{a} (0.10)	3.87^{a} (0.08)	3.54^{c} (0.04)	3.69^{b} (0.03)	0.91^{a} (0.15)	0.94^{a} (0.06)	0.90^{a} (0.08)	0.90^{a} (0.08)
144	3.89^{a} (0.05)	3.96^{a} (0.06)	3.59^{c} (0.03)	3.76^{b} (0.02)	0.92^{a} (0.10)	0.95^{a} (0.04)	0.91^{a} (0.10)	0.91^{a} (0.10)
216	4.25^{a} (0.06)	4.32^{a} (0.05)	3.81^{c} (0.06)	4.05^{b} (0.04)	0.97^{a} (0.04)	0.97^{a} (0.06)	0.93^{a} (0.07)	0.94^{a} (0.14)
288	4.61^{a} (0.02)	4.68^{a} (0.03)	4.02^{c} (0.02)	4.34^{b} (0.01)	0.99^{a} (0.15)	0.99^{a} (0.15)	0.94^{a} (0.10)	0.95^{a} (0.09)
			10°C				10°C	
24	3.27^{a} (0.20)	3.29^{a} (0.15)	3.23^{a} (0.10)	3.40^{a} (0.21)	1.38^{ab} (0.04)	1.48^{a} (0.02)	1.26^{bc} (0.07)	0.87^{c} (0.09)
48	3.29^{a} (0.19)	3.36^{a} (0.21)	3.24^{a} (0.21)	3.71^{a} (0.21)	1.83^{ab} (0.07)	1.92^{a} (0.06)	1.63^{b} (0.05)	1.11^{c} (0.04)
72	3.49^{b} (0.10)	3.51^{b} (0.13)	3.52^{b} (0.08)	4.31^{a} (0.14)	2.83^{b} (0.07)	3.02^{a} (0.04)	2.44^{c} (0.04)	1.26^{d} (0.03)
96	3.63^{b} (0.21)	3.64^{b} (0.18)	3.58^{b} (0.03)	4.34^{a} (0.19)	2.99^{b} (0.05)	3.18^{a} (0.03)	2.71^{c} (0.03)	1.71^{d} (0.05)
120	3.69^{b} (0.24)	3.72^{b} (0.17)	3.63^{b} (0.14)	4.45^{a} (0.26)	3.07^{b} (0.03)	3.26^{a} (0.04)	2.84^{c} (0.06)	1.94^{d} (0.06)
144	3.77^{b} (0.27)	3.79^{b} (0.27)	3.68^{b} (0.25)	4.56^{a} (0.33)	3.15^{b} (0.02)	3.33^{a} (0.03)	2.99^{c} (0.02)	2.16^{d} (0.07)
216	5.38^{b} (0.21)	5.42^{b} (0.23)	4.05^{c} (0.31)	6.11^{a} (0.30)	5.55^{a} (0.07)	5.26^{b} (0.03)	4.05^{d} (0.06)	4.85^{c} (0.07)
288	5.83^{b} (0.11)	5.80^{b} (0.03)	5.34^{c} (0.02)	6.84^{a} (0.07)	6.17^{a} (0.07)	6.06^{a} (0.01)	5.38^{b} (0.05)	5.45^{b} (0.09)
			20°C				20°C	
6	3.12^{a} (0.05)	3.17^{a} (0.06)	3.07^{a} (0.01)	3.07^{a} (0.05)	1.28^{a} (0.06)	1.23^{a} (0.07)	0.96^{a} (0.01)	1.07^{a} (0.05)
12	3.42^{a} (0.01)	3.47^{a} (0.02)	3.12^{a} (0.05)	3.29^{a} (0.05)	1.60^{a} (0.06)	1.66^{a} (0.02)	1.25^{b} (0.05)	1.45^{ab} (0.05)
24	4.02^{a} (0.07)	3.64^{b} (0.04)	3.33^{b} (0.04)	3.48^{b} (0.04)	3.07^{a} (0.08)	3.05^{a} (0.1)	2.15^{c} (0.06)	2.65^{b} (0.03)
48	4.61^{a} (0.14)	4.80^{a} (0.06)	3.53^{c} (0.05)	4.07^{b} (0.05)	4.53^{a} (0.16)	4.43^{a} (0.17)	3.04^{c} (0.08)	3.86^{b} (0.05)
72	6.85^{a} (0.16)	6.79^{a} (0.04)	4.04^{c} (0.04)	5.80^{b} (0.02)	4.75^{b} (0.06)	4.77^{b} (0.02)	3.13^{c} (0.05)	5.12^{a} (0.01)
96	7.64^{a} (0.07)	7.68^{a} (0.02)	4.29^{c} (0.04)	6.32^{b} (0.07)	6.69^{a} (0.07)	6.66^{a} (0.03)	4.34^{b} (0.06)	6.72^{a} (0.07)

Mean values represent three independent determinations (n = 3); standard deviations are shown in parentheses. Different lowercase letters within the same row indicate significant differences among packaging treatments (C1, C2, T1 and T2) at a given storage temperature and sampling time (Tukey's test, $p < 0.05$).
At time 0, the same initial value applied to all treatments because measurements were performed before packaging was applied.
The effects of storage temperature, time and their interaction were evaluated using linear mixed-effects models and are reported in the main text.
Comparisons among storage temperatures and temporal effects were assessed through mixed-effects modelling rather than pairwise lettering, owing to the repeated-measures factorial design.

Table S2. Mould and yeast counts detected under storage conditions where fungal growth was observed (log_{10} CFU/g)

Microorganism	Storage condition	Treatments			
		C1	C2	T1	T2
Moulds	20°C, 96 h	6.30 ± 0.76	1.82 ± 0.057	ND	ND
Yeasts	10°C, 288 h	2.10 ± 0.94	3.37 ± 0.62	1.42 ± 0.22	1.94 ± 0.19

Values are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3). ND, not detected.