

Research Article

CHITOSAN FILM WITH THYME NANOEMULSION (*THYMUS VULGARIS* L.): A SUSTAINABLE APPROACH FOR MEAT PRESERVATION

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Abstract: Recent incidents of meat spoilage and outbreaks of meat-borne pathogens have increased, primarily attributable to breaches in the cold chain and insufficient preservation techniques throughout the supply chain. To address these issues, the current study focused on developing a natural preservation method using a chitosan film. Here, a nanoemulsion of thyme (*Thymus vulgaris* L.) essential oil was prepared, and its morphology was studied by transmission electron microscopy (TEM). Additionally, thyme oil nanoemulsions at concentrations of 0.5%, 1%, and 2% (w/v) were incorporated into various chitosan films for bioactive packaging, and the morphological characteristics of the films were analysed using scanning electron microscopy (SEM). The elongation at break (EAB) and water vapour permeability (WVP) of the chitosan film could be improved by adding the nanoemulsion in a dose-dependent manner. The antimicrobial activity was assessed *in vitro* against *Salmonella* Typhimurium (ATCC 14028) and *Listeria monocytogenes* (ATCC 19111), and the film containing 2% thyme nanoemulsion (T3) exhibited the highest efficacy with the least bacterial counts (2.89 ± 0.10 log CFU/cm² and 2.00 ± 0.00 log CFU/cm², respectively). Similarly, the highest radical-scavenging activity ($45.37 \pm 1.68\%$) was observed for T3. Owing to their significant effectiveness as bioactive films, 1% and 2% formulations were applied as aerobic active packaging on chicken drumsticks. The pH, microbiological characteristics, and sensory attributes of the control and different treatment groups were analysed over 15 days of refrigerated storage ($4 \pm 1^\circ\text{C}$) to assess the overall meat quality. This study demonstrated that a chitosan film incorporating 2% thyme nanoemulsion significantly extended the shelf life of chicken drumsticks to 15 days during refrigerated storage, without noticeable sensory degradation. This bioactive film serves as an innovative green preservation method for extending the shelf life of uncooked chicken stored at $4 \pm 1^\circ\text{C}$.

Keywords: Bioactive, Nanoemulsion, Antimicrobial, Chitosan, Quality, Shelf-life

INTRODUCTION

Ensuring food safety, especially in meat and meat products, is an important public health issue that must be addressed worldwide. Foodborne illnesses caused by pathogens like *Salmonella* spp., *E. coli*, *Staphylococcus*

aureus, and *Listeria monocytogenes* affect millions annually, resulting in thousands of hospitalisations and many deaths [1]. Addressing these threats with effective measures to safeguard public health is essential. In lower- and middle-income countries, foodborne illnesses

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incur high costs yearly due to lost productivity, medical expenses, and long-term economic ripple effects on businesses and communities [2, 3]. The meat sector grapples with inadequate cold-chain infrastructure, leading to spoilage and post-harvest losses. These challenges put extra pressure on the economy. The food industry suffers losses due to product recalls, damage to its brand reputation, and lost sales. Meanwhile, healthcare systems and restaurants face high medical and operational costs. For restaurants, a single outbreak can wipe out millions in revenue through lawsuits, lost consumer trust, and legal penalties, highlighting how food spoilage and safety lapses endanger public health and drag down economic vitality across sectors. Hence, meat producers have compulsively implied the abrupt usage of chemical preservatives in the meat food system [4]. These chemical preservatives maintain the meat's quality and sensory attributes but silently damage cellular integrity, have carcinogenic and mutagenic effects, and lead to various health issues over time. Attempts has also been taken to exploit the efficacy of nano-materials to be used for improving the shelf life of meat and meat products [5]. As a result, natural preservation strategies have gained prominence across the industry, government, and research sectors, serving as a sustainable approach in the production and processing of meat and meat products. Bioactive packaging stands out as a leading natural preservation system for extending the shelf life of meat and minimising plastic pollution. Meat producers and sellers use petroleum-derived non-degradable plastic packaging, and it accumulates in nature as a heap of poison that destroys the ecological balance and has a toxic effect on animal and human health [6]. Above all, these cost-effective plastics cannot significantly hasten the shelf life of meat and meat products.

Therefore, introducing biodegradable bioactive packaging in the meat industry has emerged as a means to address plastic pollution and food waste while extending the storage life of muscle foods. Chitosan is an essential source of cationic biopolymers (due to its free amino groups: $-NH_2$), and it is a biodegradable, biocompatible polysaccharide derived from chitin, derived from the exoskeleton of insects, crustaceans, etc. [7]. Chitosan has antimicrobial activity due to its positively charged amino groups ($-NH_3^+$), which interact with the negatively charged membranes of microbial cells, leading to leakage of intracellular contents and ultimately cell death [8]. Nevertheless, their antimicrobial efficiency is insufficient to inhibit or kill pathogens and spoilage organisms in the food

matrix [9]. Hence, using essential oils from different plant parts enhances their antimicrobial efficacy. Thyme (*Thymus vulgaris*) is a notable culinary and medicinal herb of the Lamiaceae (mint) family. Essential oils extracted from thyme leaf have potential antimicrobial activity against Gram-positive and Gram-negative microorganisms. Thyme essential oil is a rich source of phenolic components and terpenoids, including carvacrol and thymol, which contribute to its effective free radical scavenging activity, aiding in preserving muscle foods [10, 11]. Several studies have been conducted to enhance the bioefficacy of thyme essential oil in a chitosan film matrix to preserve meat and meat products under refrigeration [12, 13]. However, the strong flavour and intense aroma of free thyme oil can sometimes hinder its acceptance in food preservation. Free essential oil within a chitosan film matrix releases quickly into the food matrix, thereby reducing the long-term protective effect and ensuring only surface protection. The emerging encapsulation technology, specifically nanoemulsions with biopolymers, can address this issue, thereby extending the storage period of meat [14].

The present study developed a chitosan-based bioactive biodegradable film incorporating thyme oil nanoemulsion and assessed its antimicrobial and antioxidant properties. This bioactive, biodegradable packaging can address environmental pollution caused by non-biodegradable plastics, as identified in the existing literature, and sets a future goal for bio-preservation methods for meat. The developed bioactive film was applied to raw chicken drumsticks, which were then stored at $4\pm 1^\circ C$ for 15 days, and a comprehensive quality assessment was conducted at every 3-day intervals.

MATERIALS AND METHODS

Chemicals and Bacterial strains

The essential oil of *Thymus vulgaris* L. (thyme, 100% purity) was purchased from Sigma-Aldrich, India. Chitosan (75% deacetylated), glycerol, Polysorbate 80 (Tween 80), and other chemicals were procured from Sisco Research Laboratories Pvt. Ltd. (SRL), India. Enrichment broth, selective media and phosphate-buffered saline (PBS) were purchased from Hi-media Laboratories Pvt. Ltd., India. Foodborne pathogens, such as *Salmonella* Typhimurium (ATCC 14028) and *Listeria monocytogenes* (ATCC 19111), were obtained from the Department of Veterinary Public Health and Epidemiology Laboratory at the Faculty of Veterinary and Animal Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, India, for the antibacterial

assay. Chicken drumsticks (lower part of leg) were procured from the Instructional Livestock Farm Complex (ILFC), Faculty of Veterinary and Animal Sciences, Banaras Hindu University, India.

Preparation of nanoemulsion

The nanoemulsion of thyme essential oil was prepared as described by [8, 15] with slight modifications. A coarse emulsion was made by mixing thyme oil with Tween 80 (in a ratio of 1:2) in deionised water at 8000 rpm (5 minutes) using a T18 digital ULTRA-TURRAX® (0003720000) homogeniser, IKA, Germany. Next, the homogenised microemulsion was sonicated (Branson Sonifier SFX250, USA) at 20 kHz, 50% amplitude, and 250 W using a 10 mm probe for 10 minutes to prepare the nanoemulsion. The formulation was stored in the dark at 4°C until used for film formation.

Morphology of nanoemulsion by Transmission Electron Microscopy (TEM)

A transmission electron microscope (TECNAI 20G2, THERMO FISHER, United States, operating at 200 kV) was used to observe the structure, distribution pattern, and size of the nanoemulsion. The emulsion was diluted with added ultra-pure water, and a 5 µl drop of sample was kept onto a carbon-coated copper grid (200 mesh). It was then allowed to dry completely at room temperature. The nano capsule's morphology and size were subsequently analysed under the microscope [16].

Preparation of bioactive chitosan film

Chitosan films incorporating thyme nanoemulsion were prepared using a modified laboratory-based casting method [8, 17, 18] (Fig. 1). 1.5 g chitosan (w/v) was dissolved into 1% acetic acid solution. This mixture was maintained under constant magnetic stirring at 1000 rpm and 30°C for 6 hours for complete dissolution of chitosan. Following this, 0.75% (w/v) glycerol was added to the mixture 30 minutes prior to casting. The thyme nanoemulsion was then blended with the filmogenic suspension at a 1:1 ratio, and the resulting mixture was maintained at concentrations of 0.5%, 1% and 2% (w/v). The active suspension was homogenised using an IKA T18 homogeniser (Germany) at 6000 rpm for 2-3 minutes and subsequently cast into sterile glass petri dishes, each with an 8-inch diameter. For comparison, a control chitosan film was prepared without any nanoemulsion. The suspension was dried in a hot air oven (BST/HAO-1123, Bionics Scientific

Technologies, India) at 40°C for 36 hours. Dried films were then carefully collected from plates in a sterile manner and placed in a vacuum chamber for conditioning for a minimum of 72 hours before characterisation.

Characterisation of bioactive film

Surface morphology of film by Scanning Electron Microscopy (SEM)

The surface structure of each film was examined using a scanning electron microscope (ZEISS EV010, ZEISS, Germany). 2-3 mm sized dried films were cut and affixed to copper stubs with double-sided carbon tape. The samples were subsequently treated with gold and examined under an accelerating voltage of 10 kV [7].

Mechanical properties of film

Tensile strength (TS) and elongation ability are crucial measures for assessing mechanical durability. The key properties were evaluated using a texture analyser (ESM 303, MARK-10, USA) in accordance with ASTM D882-91. Each rectangular film specimen, measuring 10 mm by 55 mm, was stretched at a rate of 0.5 mm/s until it broke. The initial distance between the two ends was established at 30 mm. Six samples from each film were tested, and their average results were computed [8, 19].

Water vapour permeability (WVP)

A circular film segment was securely positioned around a 30 ml glass beaker filled with 15 ml of distilled water [8, 20]. The beaker for the experiment was placed inside a desiccator with silica gel to control humidity and stored at 25±1°C for 24 hours. The beaker's weight variation was recorded, and the water vapour permeability was determined using the provided formula.

$$WVP = \frac{(\Delta W \times T)}{(\Delta t \times A \times \Delta P)}$$

The variance in weight is indicated as ΔW (g), with T symbolising the mean thickness of the film (mm), Δt representing the overall experimental duration (24 h), A denoting the exposed area of film (mm²), and, ΔP (kPa) illustrating the difference in water vapour pressure across both sides of the experimental film.

Antimicrobial property

The antibacterial properties of the films were assessed against *Salmonella* Typhimurium ATCC14028 and *Listeria monocytogenes* ATCC19111 using a growth inhibition test, similar to the methods of Ferreira and

colleagues [21, 22], with appropriate modifications. In 5 ml of Mueller-Hinton broth, a 1 cm² UV-treated sterile film sample was placed, and an overnight-grown fresh culture (10 µl, approximately 1×10⁶ CFU/ml) of each organism was added. The tubes were then kept at 37 ± 1 °C in a shaker incubator overnight. Serial dilutions were prepared in PBS broth from each tube, and their growth inhibition ability was checked in Hektoen enteric agar (HEA) for *S. Typhimurium* and PALCOM agar medium for *L. monocytogenes* after incubation for 24 hours at 37±1°C. Colony-forming units were counted from a plate with 30 to 300 colonies, and CFU/cm² was estimated by multiplying with the dilution factor.

Radical scavenging activity (RSA)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay successfully assessed the antioxidant potential of the chitosan film, offering important insights into its effectiveness [8, 23]. Each chitosan film, weighing 100 mg, was immersed in deionised water and stirred continuously in the shaker incubator for 4 hours. Afterwards, 1 mL of this extract was mixed with 0.5 mL methanolic DPPH solution (0.1 mM). The mixed solution was then kept in the dark for another 30 minutes before measuring its absorbance at 517 nm with a UV–Vis spectrophotometer (Spectra Max® M5, Molecular Devices, San Jose, CA, USA). The RSA% of each chitosan film was calculated based on these measurements.

Radical scavenging activity (RSA%) of chitosan film

$$= \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

Here, *Abs* represents the absorbance values measured with a UV–Vis spectrophotometer for the control and treatments.

Application of bioactive packaging on raw chicken meat

The bioactive films were wrapped around raw chicken drumsticks to evaluate their shelf life under refrigeration conditions (4±1°C). The chicken drumsticks (nearly 200g) were cleaned with sterile deionised water, wrapped with UV-treated bioactive films and the samples were named as CS: meat packed with control chitosan film, TNEF1: meat packed with chitosan film and 1% thyme nanoemulsion, TNEF2: meat sample wrapped with 2% thyme nanoemulsion added chitosan film, and kept in a secondary low-density polyethylene (LDPE) package under aseptic conditions. Here, LDPE packaging, as a secondary

packaging material, ensures the mechanical strength and water vapour resistance necessary for product safety. The control meat (C) was also examined, which was packed in a sterile LDPE bag and stored at 4±1°C for a total experimental time of 15 days. The pH, microbiological quality, and sensory attributes of the drumsticks were evaluated every three days to monitor their freshness and determine shelf-life.

pH

The meat sample was mixed with deionised water using an ultra-turrax tissue homogeniser (IKAT 18, Germany) for 2 minutes, and its pH was estimated with a digital pH meter (TLPH-3, Trulab, India) [24].

Microbiological assessment of chicken drumsticks

A 25 g sample of the refrigerated drumstick was aseptically blended with 225 mL of sterile peptone water (0.1%) for 2-3 minutes. From the initial dilution (10⁻¹), 1 mL was transferred into a test tube containing 9 mL of sterile 0.1% peptone water and vortexed for 1 minute (10⁻²). Serial dilutions were subsequently performed carefully. Suitable dilutions were plated on sterile agar media, including plate count agar for measuring total plate count (kept at 37±1 °C for 24 hours), psychrophilic bacteria (incubated for 7 days at 4±1 °C), PALCAM agar for *L. monocytogenes* (stored at 37 °C for 24 hours), and Hektoen enteric agar (HEA) for *S. Typhimurium* (incubated for 24 hours at 37 °C). Colonies from plates with 30 to 300 were counted, and the average was multiplied by the dilution factor to find the log CFU/g of the sample [8, 24]. Each experiment was conducted in triplicate.

Sensory analysis of meat

A group of trained sensory evaluators (n=30), including assistant professors and postgraduate students from the Faculty of Veterinary and Animal Sciences, BHU, assessed the product's sensory attributes. The sensory evaluations were conducted consistently after 3:30 pm. The raw chicken meat (100 g) was evaluated based on colour and odour in a hedonic scale of 9 points, where an extremely preferable sample was scored with 9, and an extremely undesirable sample was scored with 1 [24].

Statistical analysis

Experiments were conducted thrice in duplicate (n=6), except for sensory analysis (n=30), and the data were analysed using SPSS version 20 (IBM, USA). Two-way ANOVA and one-way ANOVA were used to analyse different experimental parameters. Tukey's HSD multiple-range post hoc test was applied to compare and analyse mean ± standard error (SE) values across

treatments. Statistical significance was assessed at the 95% confidence level ($p < 0.05$). Graphs were created using GraphPad Prism (Version 9.0; GraphPad Software, Boston, MA, USA).

RESULTS AND DISCUSSION

Transmission electron microscopy (TEM) of nanoemulsion

Micrographs of nanoemulsions obtained by transmission electron microscopy at different concentrations showed significant morphological features (Fig. 2). The micrograph indicated the presence of irregular, spherical nanosized droplets measuring 19-40 nm; all measurements were within 100 nm. Encapsulations of oil droplets with Tween 80 were confirmed by the presence of dark, dense droplets with a clear outline in the contrast light field [16, 25].

The higher pressure of ultrasonication successfully broke down the coarse emulsion into nanoparticles across all treatments. These reductions in particle size improve stability, surface-to-volume ratio, and potential drug delivery properties compared to free oil [26]. At the same time, noticeable flocculation and clumping occurred in T3 with the higher concentration of (2%) oil. Drying the solvent and flattening the nanoparticles with the grid during experimental processing may influence the shape and size of nanoemulsions [16]. Several studies in the existing literature have confirmed thyme nanoemulsion droplet sizes (<100 nm) using transmission electron microscopy, with Tween 80 used as a solvent [8, 26, 2476]. In a study, thyme nanoemulsion droplets were prepared and characterised by TEM, revealing spherical oil droplets with a size range of 20-55.2 nm [25].

Table 1: Physical, mechanical, antioxidant and antimicrobial properties of bioactive films.

Treatments	TS (MPa)*	EAB (%)*	WVP (g mm m ⁻² day ⁻¹ kPa ⁻¹)*	RSA (%)*	<i>Listeria monocytogenes</i> (log CFU/cm ²)*	<i>Salmonella Typhimurium</i> (log CFU/cm ²)*
C	12.78±0.39 ^d	40.56±1.18 ^{ab}	2.32±0.02 ^c	5.61±0.69 ^a	3.67±0.02 ^c	4.80±0.02 ^c
T1	9.84±0.70 ^c	39.92±1.78 ^a	2.35±0.05 ^c	17.13±0.68 ^b	3.59±0.01 ^c	4.59±0.01 ^{bc}
T2	7.15±0.50 ^b	47.26±1.44 ^b	1.92±0.02 ^b	31.11±1.02 ^c	2.69±0.05 ^b	4.44±0.04 ^b
T3	4.70±0.37 ^a	57.56±1.58 ^c	1.64±0.05 ^a	45.37±1.68 ^d	2.00±0.00 ^a	2.89±0.10 ^a

*Data are represented as mean ± standard error (n = 6); and Superscripts with lowercase letters (a, b, c) show significant differences ($p < 0.05$) in the columns; TS= Tensile strength; EAB= Elongation at break; WVP= water vapour permeability; RSA= Radical scavenging activity; C= control chitosan film; T1= chitosan film infused with thyme nanoemulsion (0.5%); T2= chitosan film added with thyme nanoemulsion (1%); T3= chitosan film added with thyme nanoemulsion (2%).

Table 2: Sensory evaluation (colour and odour scores) of raw chicken drumsticks under refrigeration for 15 days.

Treatments	0D	3D	6D	9D	12D	15D
Colour*						
C ₀	8.80±0.05 ^{Ae}	8.48±0.04 ^{Be}	6.47±0.06 ^{Ad}	4.88±0.16 ^{Ac}	3.61±0.08 ^{Ab}	2.81±0.12 ^{Aa}
CS	8.53±0.01 ^{Ad}	8.31±0.01 ^{Abd}	6.75±0.10 ^{Ac}	5.34±0.17 ^{ABb}	3.81±0.12 ^{Aa}	3.43±0.09 ^{Aa}
TNEF1	8.82±0.06 ^{Ae}	8.22±0.06 ^{Ae}	7.53±0.01 ^{Bd}	6.03±0.28 ^{BCc}	4.41±0.08 ^{Bb}	3.62±0.06 ^{Aa}
TNEF2	8.81±0.13 ^{Ad}	8.44±0.06 ^{Abcd}	7.70±0.14 ^{BCc}	6.52±0.01 ^{Cb}	5.8±0.10 ^{Cb}	4.86±0.31 ^{Ba}
Odour*						
C ₀	8.41±0.09 ^{Af}	7.71±0.02 ^{Ae}	6.56±0.06 ^{Ad}	3.88±0.07 ^{Ab}	3.33±0.03 ^{Ab}	2.6±0.06 ^{Aa}
CS	8.56±0.12 ^{ABCe}	8.04±0.15 ^{Abe}	7.18±0.06 ^{Bd}	5.91±0.19 ^{Bc}	4.73±0.13 ^{Bb}	3.34±0.05 ^{Ba}
TNEF1	8.83±0.03 ^{Be}	8.27±0.13 ^{Abde}	7.7±0.10 ^{Bd}	6.3±0.16 ^{Bc}	5.03±0.28 ^{BCb}	3.66±0.22 ^{Ba}
TNEF2	8.90±0.05 ^{Bf}	8.41±0.06 ^{Abe}	7.55±0.07 ^{Bd}	6.47±0.07 ^{Bc}	5.56±0.00 ^{Cb}	4.7±0.15 ^{Ca}

*Data are represented as mean ± standard error (n = 30); and Superscripts with lower cases (a, b, c, d) show significant differences ($p < 0.05$) in a row whereas superscripts with other letters (A, B, C, D) shows significant variances ($p < 0.05$) in a column; C₀=control meat; CS= meat wrapped with chitosan film; TNEF1= meat wrapped with 1% thyme nanoemulsion incorporated chitosan film; TNEF2= meat wrapped with 2% thyme nanoemulsion incorporated chitosan film; D= storage days.

Characterisation of chitosan film

Scanning electron microscopy (SEM)

Surface morphology of each film sample from SEM analysis is shown in Fig. 3a to 3d. This experiment presents microstructural observations of the bioactive films, interaction patterns between two phases, and modifications to the nanoemulsion after drying within a chitosan matrix. In the control chitosan film surface (Fig. 3a), noticeable cracks and folds were observed due to improper crystallisation of the chitosan film matrix for its degree of deacetylation with the additive used and the influence of the drying environment [8, 28]. As the nanoemulsion was incorporated, the folds disappeared, and multiple micropores emerged in a non-homogeneous pattern. These micropores ensured the incorporation of oil nanodroplets into the chitosan matrix during drying, followed by evaporative loss. As the concentration was increased to 1% and 2% (Fig. 3c to 3d), a few large clumps and flocculations were noticed on the surface, but higher concentrations improved the surface roughness and compactness of the film [29, 30].

The cross-sectional study (Fig. 3e-3h) showed that adding essential oil nanoemulsion improved the

smoothness of control chitosan films. With higher concentrations, the films became more compact and denser. This study demonstrates a compatible integration of thyme essential oil nanoemulsion within the chitosan film matrix [31]. The amalgamation depends on the type of essential oil used and on the film properties. This structural compactness improves the film's moisture- and gas-barrier properties. Still, limitations in tensile strength may arise from perforated microstructures and flocculation at higher oil concentrations in bioactive films [32]. Similar structural arrangements of chitosan films with essential oil nanoemulsions have been reported in various studies [16, 23, 33].

Mechanical properties of chitosan film

Measuring the mechanical properties of the film, especially Tensile Strength (TS) and Elongation at Break (EAB), is essential for assessing its suitability in food applications. The study found that the TS of the chitosan film was 12.78 ± 0.39 MPa, but it dropped after adding the essential oil nanoemulsion, reaching 4.70 ± 0.37 MPa in T3 (see Table 1). Tensile strength indicates the maximum stress a biopolymer can withstand before breaking or fracturing. This property is

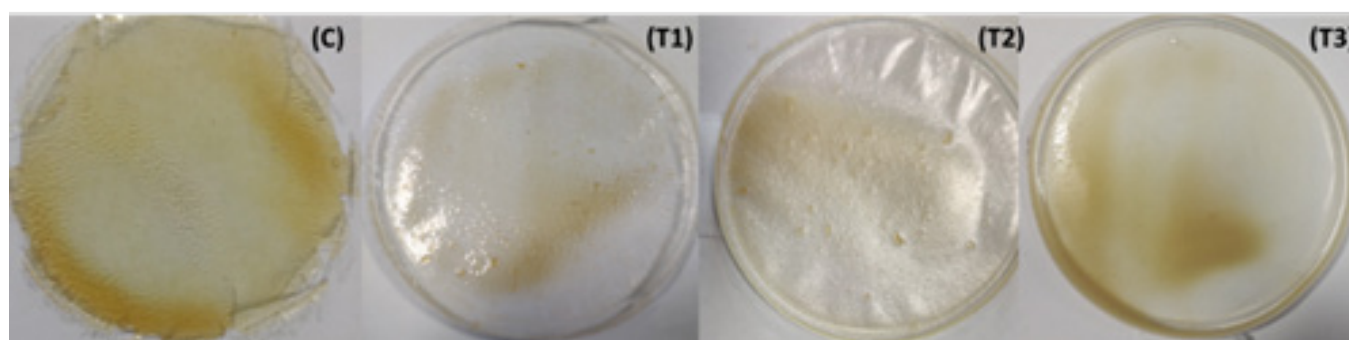


Fig. 1. Prepared bioactive films; C = Chitosan film, T1= Chitosan film and thyme nanoemulsion (0.5%), T2= Chitosan film and thyme nanoemulsion (1%), T3 = Chitosan film and thyme nanoemulsion (2%).

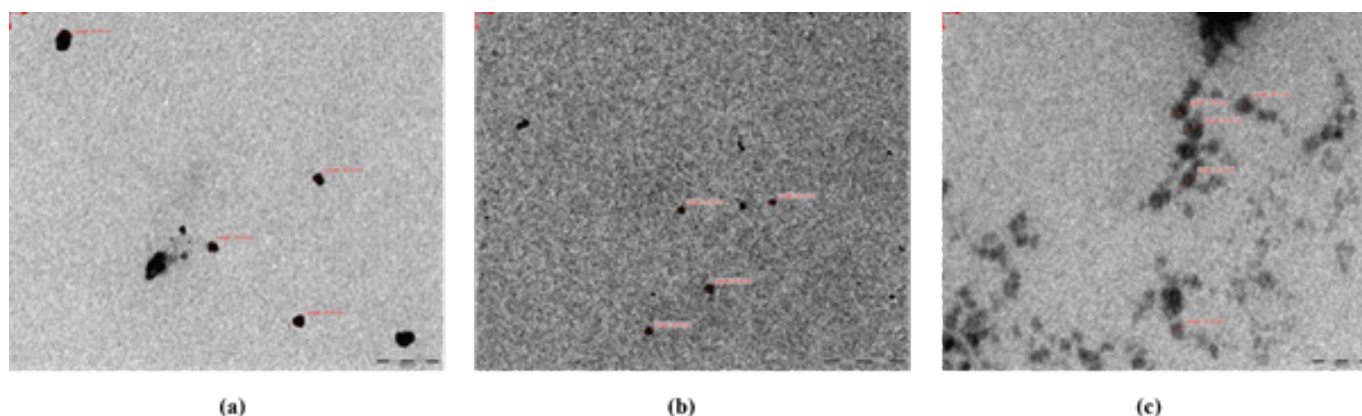


Fig. 2. Morphology of nanoemulsions by Transmission electron microscopy (TEM);(a) image of T1 (b) image of T2 (c) image of T3.

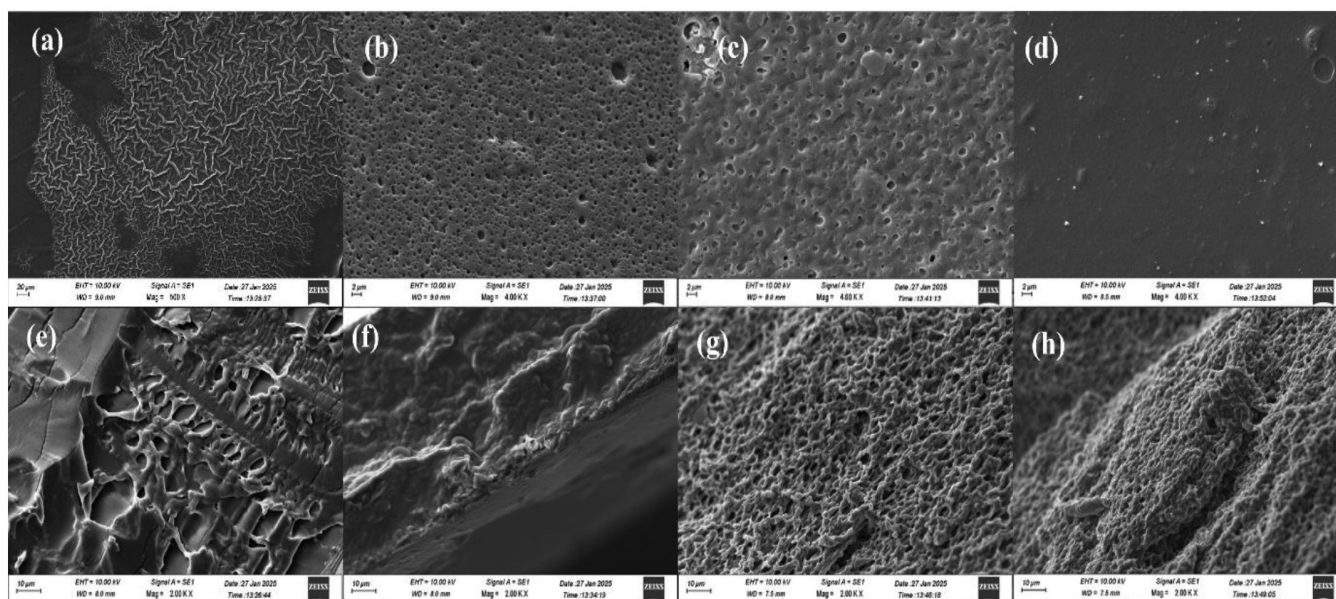


Fig. 3. Surface morphology (from left to right a-d) and cross-section (from left to right e-h) study of chitosan biopolymer with different concentrations of thyme nanoemulsion; SEM images were taken at 10 kv and 500 X, 2000 X and 4000X magnifications; (surface morphology: a = C, b = T1, c = T2, d = T3; Cross section: e = C, Cross section f = T1, g = T2, h = T3).

essential for understanding the limits of a biopolymer's mechanical performance. TS has decreased during treatment due to weak intermolecular interactions between the nanoemulsion and the chitosan matrix. Additionally, evaporative damage to oil nanodroplets during film drying, with multiple holes and pores, weakens the biopolymer's internal cohesive properties. Similar findings were analysed in different literature where TS was compromised after the addition of essential oil nanoemulsion [33, 34].

Conversely, the elongation at break (EAB) increased (Table 1) by adding the essential oil nanoemulsion into the control film. Elongation at break (EAB) is a critical measure representing a film's ability to stretch or deform before it ultimately fractures under tensile stress. This property reflects the material's flexibility and resilience, indicating how much strain it can tolerate before reaching its breaking point. The highest EAB was measured in T3 at $57.56 \pm 1.5\%$, which differed significantly ($p < 0.05$) from the others. C and T1 showed no significant difference ($p > 0.05$), exhibiting minimal variation in values. The increase in the bioactive film's stretchability is likely attributed to hydroxyl groups in thyme essential oil nanoemulsions, which could improve the film's plasticising properties [35]. Various studies have reported an increase in EAB of bioactive films, mainly influenced by essential oils and their nanoemulsions [7, 36].

Water vapour permeability (WVP)

Water vapour barrier properties are crucial for food packaging, particularly for muscle foods. In this study, T1 exhibited a higher water vapour permeability (WVP) compared to the control film, although the difference was not statistically significant ($p > 0.05$) (Table 1). This observation can be explained by the presence of multiple heterogeneous pores in T1, which can facilitate increased water vapour transmission. The water vapour permeability (WVP) decreased significantly ($p < 0.05$) in T2 and T3, suggesting that higher nanoemulsion concentrations in films are more effective. A similar analysis showed that a chitosan biopolymer with a higher concentration of thyme oil exhibited improved physical cohesion due to intermolecular chain interactions, thereby enhancing its water-vapour barrier properties.

Radical scavenging activity assay (RSA)

Estimation of the antioxidant property of bioactive films is important to justify their bio-preservative nature. The DPPH (2,2-diphenyl-2-picrylhydrazyl) assay is an important method for assessing the free radical scavenging activity (RSA) of a film and is acknowledged as one of the most reliable techniques for determining antioxidant capacity. In this study, the chitosan film infused with a 2% thyme oil nanoemulsion exhibited greater electron-donating capability and free radical scavenging activity ($45.37 \pm 1.68\%$) than the other samples (Table 1). In contrast, the control chitosan

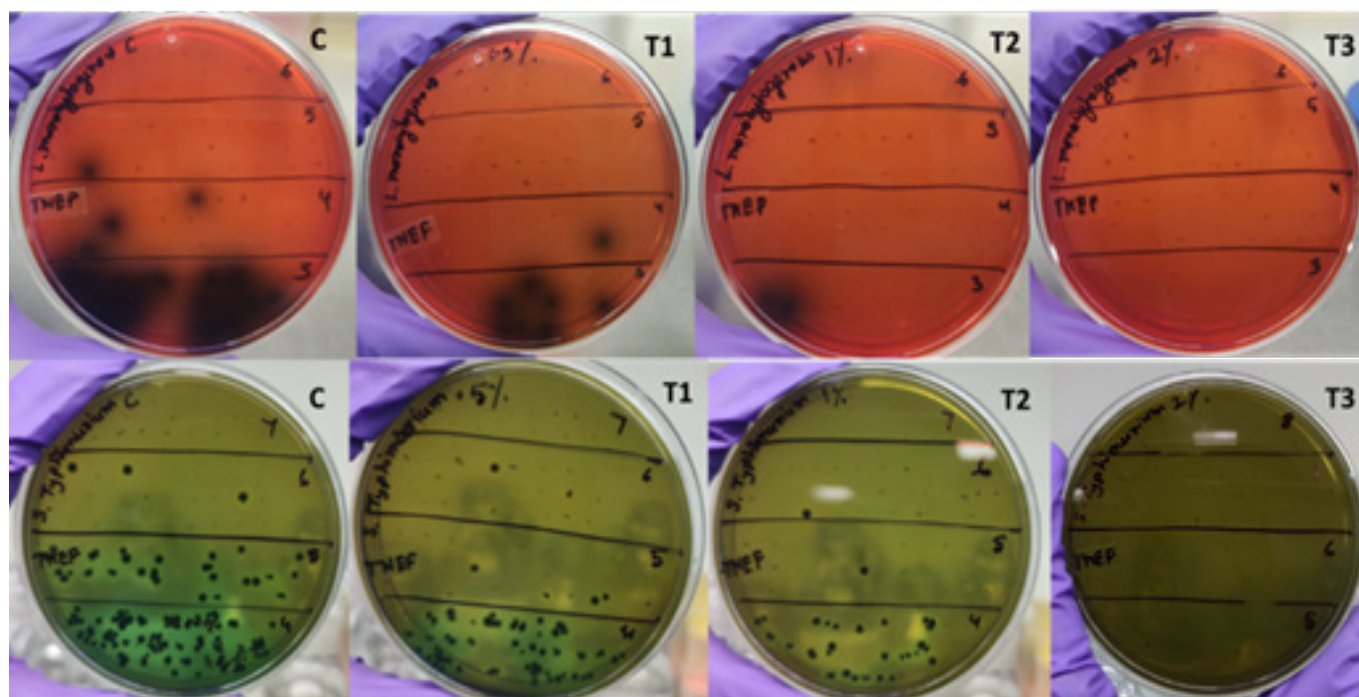


Fig. 4. Antimicrobial activity of thyme nanoemulsion film (TNEF); (C= chitosan film, T1= TNEF with thyme nanoemulsion (0.5 %), T2= TNEF with thyme nanoemulsion (1 %), T3= TNEF with thyme nanoemulsion (2 %) against *Salmonella* Typhimurium (ATCC 14028) and *Listeria monocytogenes* (ATCC 19111).

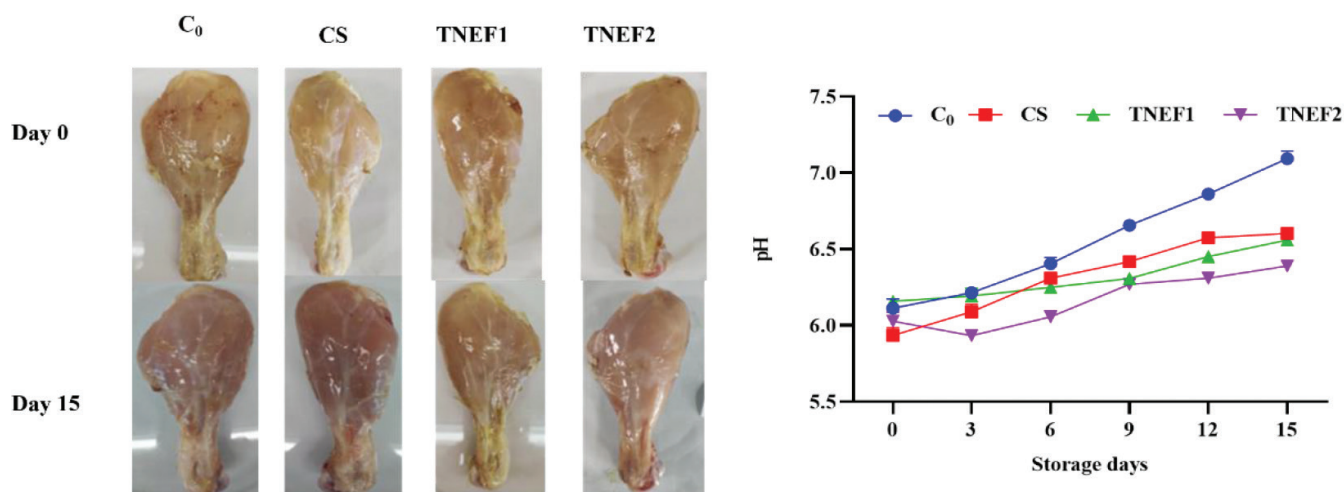


Fig. 5. (a) Biopreservation of raw chicken drumsticks with bioactive films with different concentrations of thyme nanoemulsion under refrigerated Storage for 15 days; (b) pH estimation of meat samples stored at 4±1°C for 15 days; C₀ = control sample without bioactive film, CS= sample packed with chitosan control film, TNEF1= meat wrapped with chitosan film infused with 1% thyme nanoemulsion, TNEF2= meat sample wrapped with chitosan film and 2% thyme nanoemulsion.

film exhibited limited antioxidant properties, likely due to the presence of amine and hydroxyl groups in its fundamental structural unit and a significant degree of deacetylation [9]. In contrast, the thyme nanoemulsion is rich in thymol and carvacrol, key phenolic compounds that enhance the antioxidant efficacy of the chitosan film [8, 11]. Several studies support the current

findings regarding the emerging free radical scavenging properties of chitosan film after the inclusion of thyme nanoemulsion [37, 39].

Antimicrobial property

The antimicrobial properties of bioactive biopolymers represent a significant biofunctional

characteristic that may demonstrate their efficacy in bio-preservation applications. The antimicrobial activity of bioactive films is shown in Table 1 and Fig. 4. It was observed that the Chitosan film with 1% and 2% thyme nanoemulsions exhibited significantly higher ($p < 0.05$) antimicrobial efficacy than the control chitosan film. However, the T1 and control films did not differ significantly ($p > 0.05$) for Gram-negative and Gram-positive organisms. Thymol and carvacrol in thyme nanoemulsion contribute to the antimicrobial effectiveness observed in bioactive films, while chitosan films exhibit antimicrobial properties due to their polycationic nature. It was noted that increasing the nanoemulsion concentration within the biopolymer enhanced antimicrobial properties. Similar antimicrobial activity of thyme essential oil was depicted in different literature [26, 40]. Additionally, bioactive films exhibited greater sensitivity to Gram-positive bacteria than to Gram-negative bacteria. This phenomenon can be explained by the outer membrane of Gram-negative bacteria, which acts as a strong protective barrier made up of Lipopolysaccharide (LPS). Due to their hydrophobic nature, essential oils find it difficult to pass through the LPS barrier of Gram-negative bacteria, leading to reduced effectiveness against Gram-negative organisms compared to Gram-positive ones. The principal bioactive components in thyme essential oil disrupt the cell structure integrity of microorganisms, leading to leakage of ions and intracellular components,

disruption of proton motive force, alteration of DNA, and sometimes chelation with microbial enzymes, interfering with cellular metabolism and resulting in cellular death [25, 41].

Application of biopolymer on chicken drumsticks preservation

pH

Evaluating pH levels is essential for assessing the degradation of meat quality during storage investigations. In this research, four sample groups were refrigerated and examined: one group included chicken drumsticks without any preliminary wrapping (C_0), another group utilised a control film, and the rest two groups were covered in chitosan films infused with 1% and 2% thyme nanoemulsion, respectively (Fig. 5a). It was observed that the pH of each meat sample increased over time due to changes in meat quality. In C_0 , the pH rose notably ($p < 0.05$), from 6.11 ± 0.06 on day 0 to 7.09 ± 0.05 at the endpoint (see Fig. 5b). In TNEF2, the pH level started at 6.02 ± 0.06 on day 0 and rose to 6.39 ± 0.00 by day 15 ($p < 0.05$), which remained below the control level. It was further observed that in C_0 and CS, starting from day 6, pH values increased significantly ($p < 0.05$). In the treatment groups, this significant enhancement was maintained during later storage stages. When alkaline metabolites accumulate in the meat matrix, including ammonia, non-nitrogenous substances, etc., pH increases, indicating meat quality deterioration by microbial spoilage and enzymatic

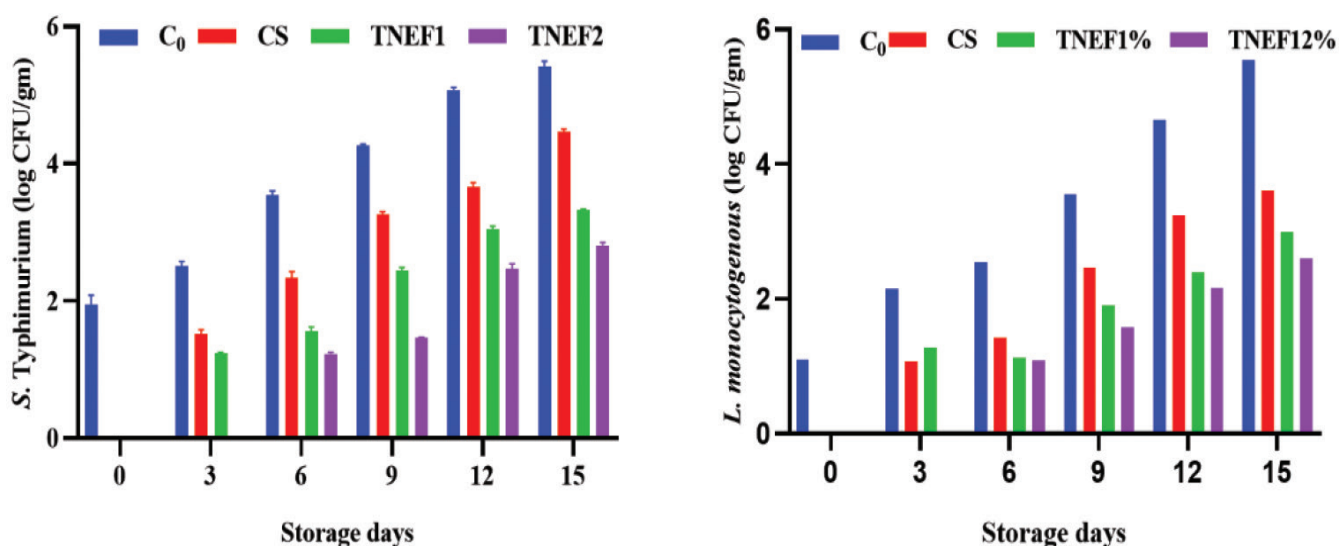


Fig. 6. Microbiological analysis of chicken drumsticks under refrigeration storage for 15 days (a) Total plate count (TPC); (b) Psychrophilic bacterial count (PBC); C_0 = chicken drumstick without any wrapping; CS= chicken drumstick wrapped with chitosan film; TNEF1= chicken drumstick wrapped with chitosan film with 1% thyme nanoemulsion; TNEF2= chicken drumstick wrapped with chitosan film with 2% thyme nanoemulsion.

decline [39]. In the treatment samples, the prolonged release of thyme nanoemulsion from the bioactive film slows down the microbial spoilage of meat because of the effective polyphenolic antimicrobial compounds, namely carvacrol and thymol [8, 42].

Microbiology of the meat sample

In the current experiment, total plate count (TPC) increased from 4.95 ± 0.03 log CFU/g on day 0 to 7.63 ± 0.02 log CFU/g on the 15th day of storage in the control meat sample ($p < 0.05$), indicating that microbial spoilage could not be prevented under refrigeration for up to 15 days (Fig. 7a). Meanwhile, chicken drumsticks wrapped in chitosan films failed to preserve meat quality after 6 days of storage due to the chitosan film's insufficient antimicrobial activity. TNEF1 and TNEF2 initially reduced the microbial count, followed by a gradual increase in bacterial numbers up to the 15th day. The TNEF2 started with an initial microbial count of 4.44 ± 0.11 log CFU/g on day 0, which rose to 5.39 ± 0.09 log CFU/g on day 15 (not exceeding the acceptable limit of 5 log CFU/g as per the Food Safety Standards Authority of India). In TNEF1, microbial loads were significantly higher ($p < 0.05$) than in TNEF2 because of a lower concentration of thyme essential oil nanoemulsion in the biopolymer. The bioactive compounds embedded within the biofilm diffuse out effectively, inhibiting bacterial growth by disrupting cellular membranes and interfering with enzymatic systems. This disruption leads to a loss of ions and fluids, thereby adversely affecting the bacteria [7, 43]. [44] reported a similar outcome, showing that garlic nanoliposomes embedded in a chitosan film could prolong the storage of chicken meat for up to 14 days at $4 \pm 1^\circ\text{C}$. In this experiment, 2% garlic essential oil nanoliposomes exhibited the most effective antimicrobial activity against chicken fillets. A 1.5-log reduction in aerobic counts was observed in the 2% nanoliposome sample compared to the control sample.

Similar findings were observed in the psychrophilic bacterial counts (PBC) (Fig. 6b). Here, the control sample exhibited an exponential increase ($p < 0.05$) in microbial counts, reaching 7.30 ± 0.04 log CFU/g on the 15th day from 4.44 ± 0.05 log CFU/g loads on day 0. A decreasing pattern of microbial counts was observed in the treatment groups (CS and TNEF1) up to the 3rd day, and in TNEF2 up to the 6th day, followed by sluggish growth towards the end of storage. In TNEF2, PBC counts increased from 3.24 ± 0.03 log CFU/g on the 0th day to 4.88 ± 0.08 log CFU/g at the end of the meat storage. Gram-negative psychrophiles are the key

organisms responsible for aerobic spoilage of muscle food under refrigeration. Importantly, they are less sensitive to essential oils due to their lipopolysaccharide-based cell wall structure [44, 45]. Thyme essential oil nanodroplets effectively prevented microbial spoilage compared to control samples without any protective bioactive films. The current study aligns with [44], which noted that applications of whey protein isolate- and cinnamon essential oil-based coatings suppressed the growth of psychrophiles in chicken breasts. In a separate study, the nanoemulsion of *Eryngium campestre* essential oil at a concentration of 2.5% effectively reduced the total psychotropic counts in ostrich meat to 1.37 ± 0.06 log CFU/g. This was in contrast to the control group, which showed 7.31 ± 0.55 log CFU/g after 12 days of storage period at $4 \pm 1^\circ\text{C}$ [46].

Compared with gram-negative organisms (*S. Typhimurium*), the thyme essential oil nanoemulsion demonstrated potential antimicrobial activity against gram-positive microorganisms (*L. monocytogenes*) in a meat model system, which aligns with the in vitro antimicrobial activity of the bioactive biopolymer (Fig. 8a and 8b). In this study, chicken drumsticks packaged with chitosan films containing a 2% thyme essential oil nanoemulsion exhibited superior antimicrobial effects against both microorganisms compared with other treatments. In the case of *S. Typhimurium* and *L. monocytogenes*, microbial growth was absent until the 3rd day of refrigerated storage, at which point the bacteria were treated with TNEF2. The microbial load increased to 2.81 ± 0.04 log CFU/g (*S. Typhimurium*) and 2.60 ± 0.04 log CFU/g (*L. monocytogenes*) by day 15, from initial counts of 1.23 ± 0.02 log CFU/g (*S. Typhimurium*) and 1.09 ± 0.03 log CFU/g (*L. monocytogenes*) in TNEF2. Moreover, TNEF1 yielded comparable results in extending the shelf life of raw chicken drumsticks. Thymol and carvacrol are the active antimicrobial components in the bioactive film with a 2% thyme nanoemulsion, which reduces the numbers of spoilage and pathogenic microbes in meat [47–49].

Sensory evaluation of meat samples

Sensory attributes are a vital factor influencing consumer acceptance or rejection of food. In this study, trained evaluators were assembled to assess the sensory aspects of raw chicken drumsticks (Table 2). Colour is an important parameter in fresh meat preservation studies, as it indicates freshness. In this current study, colour scores decreased as days passed. Up to day 3, higher scores were observed ($p > 0.05$) across all samples, followed by a decline. This deterioration was observed

more slowly ($p > 0.05$) in treatment samples, especially in TNEF2. It was also noted that TNEF1 and TNEF2 did not differ significantly ($p > 0.05$) until day 9 of storage. Colour differences were noticeable between treatments and the control ($p < 0.05$), indicating that essential oil nano droplets significantly help maintain meat freshness. Similar observations were reported in the literature, where essential oil nanoemulsion delayed microbial spoilage as well as oxidative depletion in meat myoglobin and extended storage life [50, 51]. Sensory scores correlated with microbiological analysis and pH studies. Decreased color scores can be attributed to chemical changes in myoglobin pigment and the production of metmyoglobin during storage due to unavoidable oxidation [8, 52].

The odour of raw chicken drumsticks was assessed throughout the storage period by sensory panelists (Table 2). It was observed that odour scores decreased over time, with foul odour becoming more prominent in the later stages of storage. The shift from meaty to foul odour was caused by proteolytic degradation and the release of volatile nitrogenous and non-nitrogenous metabolites. Microbial growth and enzymatic activity are key factors behind these odours [53]. The odour score dropped from 8.41 ± 0.09 on day 0 in the control sample (C_0) to 2.56 ± 0.06 at the termination of the storage period ($p < 0.05$). Conversely, treatment samples suppressed the foul smell during storage due to the mild aromatic fragrances of essential oil, which consumer panelists appreciated. Additionally, these bioactive films inhibited microbial growth and chemical oxidation in the meat samples. In the treatment groups, odour scores also decreased with storage time but remained higher than those in the control group, reflecting consumer preference. The highest score was recorded for TNEF2, starting at 8.90 ± 0.05 and decreasing to 4.7 ± 0.15 . The odour scores below 4 on the hedonic scale were deemed objectionable. The control sample was discarded after day 6 based on odour scores; the CS sample exhibited a major objectionable odour by day 12, and the TNEF1 sample was rejected at day 15 due to off-odours. These rising off-odour scores indicate significant microbial spoilage. A pleasant aromatic fragrance, masking the meaty smell of raw chicken that sometimes consumers dislike, was preferred over all other treatments in TNEF1 and TNEF2, owing to their consistent release of EO nanoemulsion onto the chicken drumsticks [50]. Different authors have reported similar works in their literature, where it was stated that the application of micro or nano emulsions of essential oils in meat preservation systems consistently achieved higher

acceptance compared to control samples in terms of odour and other sensory attributes [50, 54–56]. In another study, it was found that the odour and colour scores of meat emulsion treated with thyme and oregano essential oil nanoparticles (500 ppm each) remained satisfactory for up to 9 days of refrigerated storage, with scores exceeding 3.5. In contrast, the control meat samples, which were packaged aerobically without any treatments, only remained satisfactory for 3 days [57].

CONCLUSION

The present study successfully developed chitosan-based bioactive films infused with thyme essential oil nanoemulsions at concentrations of 0.5%, 1% and 2% (v/w). The structural characteristics and mechanical properties of the developed films were analysed. Chitosan film with 2% thyme nanoemulsion (T3) exhibited potent radical-scavenging and antimicrobial activities, especially against *S. Typhimurium* and *L. monocytogenes*. Notably, the T3 film formulation, containing a 2% thyme essential oil nanoemulsion, demonstrated significant improvements in elongation at break and water-vapour barrier performance, indicating strong potential for use in bioactive packaging. Scanning electron microscopy (SEM) images revealed greater compactness and structural stability in the T3 formulation than in the other films. Further, during *in situ* application to raw meat, the bioactive film containing 2% thyme nanoemulsion (TNEF2) extended the shelf life of refrigerated ($4 \pm 1^\circ\text{C}$) drumsticks to 15 days, compared with 6 days for the control meat without any biopolymer. Furthermore, the chitosan film containing the 1% thyme nanoemulsion produced comparable microbiological results. Therefore, based on these findings, a chitosan film containing a 2% thyme oil nanoemulsion can be considered an excellent biopreservation strategy, particularly for the meat packaging industry, as it may improve meat quality and prolong shelf life during refrigerated storage.

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REFERENCES

1. Bintsis T. Foodborne pathogens. *AIMS Microbiol.* 2017;3(3):529–63.
2. Lianou A, Panagou EZ, and Nychas G-JE. Meat Safety—I Foodborne Pathogens and Other Biological Issues. *Lawrie's Meat Science*. Elsevier; 2017.

3. Das A, Bhattacharya D, Nanda PK, Nath S, and Das AK. *Biopreservation in Meat and Meat Products*. 2024.
4. Guerra AF. Update on the use and abuse of preservatives in meat products. *Food Control*. 2024;166:110775.
5. Mahato A, Chatterjee PN, Sarkar S, Sen AR, Pal A, Roy S, Patra AK. Effects of Chemically and Green Synthesized Zinc Oxide Nanoparticles on Shelf Life and Sensory Quality of Minced Fish (*Pangasius hypophthalmus*). *Foods*. 2024; 13(17):2810.
6. S. Bhuvaneshwari*, D.Sruthi*, V. Sivasubramanian*, and Niranjana kalyani** and J. Sugunabai **. Development and characterization of chitosan film. *Int J Eng Res Appl*. 2011;1(2):292–9.
7. Elshamy S, Khadizatul K, Uemura K, Nakajima M, and Neves MA. Chitosan-based film incorporated with essential oil nanoemulsion foreseeing enhanced antimicrobial effect. *J Food Sci Technol*. 2021;58(9):3314–27.
8. Bhattacharya D, Kumar D, Pavan Kumar P, Das AK, Nanda PK, Das A, Karunamay S, Satyaprakash K, Singh Parmar A, and Kumar A. Development and characterization of chitosan–thyme essential oil nanoemulsion films for active packaging of raw chicken drumsticks. *AIMS Agric Food*. 2025;10(4):1004–38.
9. López-Mata MA, Ruiz-Cruz S, Silva-Beltrán NP, Ornelas-Paz J de J, Ocaño-Higuera VM, Rodríguez-Félix F, Cira-Chávez LA, Del-Toro-Sánchez CL, and Shirai K. Physicochemical and Antioxidant Properties of Chitosan Films Incorporated with Cinnamon Oil. *Int J Polym Sci*. 2015;2015:1–8.
10. Loizzo MR, Tundis R, Menichini F, and Duthie G. Anti-rancidity effect of essential oils, application in the lipid stability of cooked turkey meat patties and potential implications for health. *Int J Food Sci Nutr*. 2015;66(1):50–7.
11. Tohidi B, Rahimmalek M, and Arzani A. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chem*. 2017;220:153–61.
12. Satora P, Michalczyk M, and Banaś J. Impact of Thyme Essential Oil on the Aroma Profile and Shelf Life of Vacuum-Packed Minced Turkey Meat. *Molecules*. 2024;29(15):3524.
13. Marzlan AA, Hussin ASM, Bourke P, Chaple S, Barroug S, and Muhialdin BJ. Combination of Green Extraction Techniques and Essential Oils to Develop Active Packaging for Improving the Quality and Shelf Life for Chicken Meat. *Food Rev Int*. 2023;39(7):3783–805.
14. Das S, Singh VK, Dwivedy AK, Chaudhari AK, Upadhyay N, Singh P, Sharma S, and Dubey NK. Encapsulation in chitosan-based nanomatrix as an efficient green technology to boost the antimicrobial, antioxidant and in situ efficacy of *Coriandrum sativum* essential oil. *Int J Biol Macromol*. 2019;133:294–305.
15. Amiri E, Aminzare M, Azar HH, and Mehrasbi MR. Combined antioxidant and sensory effects of corn starch films with nanoemulsion of *Zataria multiflora* essential oil fortified with cinnamaldehyde on fresh ground beef patties. *Meat Sci*. 2019;153:66–74.
16. Xavier LO, Sganzerla WG, Rosa GB, da Rosa CG, Agostinetto L, Veeck AP de L, Bretanha LC, Micke GA, Dalla Costa M, Bertodi FC, *et al*. Chitosan packaging functionalized with *Cinnamodendron dinisii* essential oil loaded zein: A proposal for meat conservation. *Int J Biol Macromol*. 2021;169:183–93.
17. Gómez-Estaca J, López de Lacey A, López-Caballero ME, Gómez-Guillén MC, and Montero P. Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiol*. 2010;27(7):889–96.
18. Bhattacharya D and G. K. Selection of biopolymers to develop a biodegradable and edible film for packaging of luncheon chicken meat slices. *Asian J Dairy Food Res*. 2017;36(01).
19. Fallah M, Rouhi M, Soltani M, Mohammadifar MA, Bahrami R, Davachi SM, Abbaspourrad A, and Mohammadi R. Physico-mechanical, Antimicrobial, and Antioxidant Properties of Gelatin Edible Films Incorporated with *Olibanum* Essential Oil and Sodium Hexametaphosphate on the Rainbow Trout Fillet Under Refrigerated Conditions. *J Polym Environ*. 2021;29(7):2174–84.
20. Haghju S, Beigzadeh S, Almasi H, and Hamishehkar H. Chitosan films incorporated with nettle (*Urtica dioica* L.) extract-loaded nanoliposomes: I. Physicochemical characterisation and antimicrobial properties. *J Microencapsul*. 2016;33(5):438–48.
21. Ferreira RR, Souza AG, and Rosa DS. Essential oil-loaded nanocapsules and their application on PBAT biodegradable films. *J Mol Liq*. 2021;337.
22. Santos M, Fonseca A, Mendonça P, Branco R, Serra A, Morais P, and Coelho J. Recent Developments in Antimicrobial Polymers: A Review. *Materials (Basel)*. 2016;9(7):599.
23. Almasi H, Azizi S, and Amjadi S. Development and characterization of pectin films activated by nanoemulsion and Pickering emulsion stabilized marjoram (*Origanum majorana* L.) essential oil. *Food Hydrocoll*. 2020;99.

24. Das A, Biswas S, Satyaprakash K, Bhattacharya D, Nanda PK, Patra G, Moirangthem S, Nath S, Dhar P, Verma AK, *et al.* Ratanjot (*Alkanna tinctoria* L.) Root Extract, Rich in Antioxidants, Exhibits Strong Antimicrobial Activity against Foodborne Pathogens and Is a Potential Food Preservative. *Foods*. 2024;13(14):2254.
25. Doghish AS, Shehabeldine AM, El-Mahdy HA, Hassanin MMH, Al-Askar AA, Marey SA, AbdElgawad H, and Hashem AH. *Thymus Vulgaris* Oil Nanoemulsion: Synthesis, Characterization, Antimicrobial and Anticancer Activities. *Molecules*. 2023;28(19):6910.
26. Gupta P, Preet S, Ananya, and Singh N. Preparation of *Thymus vulgaris* (L.) essential oil nanoemulsion and its chitosan encapsulation for controlling mosquito vectors. *Sci Rep*. 2022;12(1):4335.
27. El-Sayed SM and El-Sayed HS. Antimicrobial nanoemulsion formulation based on thyme (*Thymus vulgaris*) essential oil for UF labneh preservation. *J Mater Res Technol*. 2021;10:1029–41.
28. Muñoz-Núñez C, Hevilla V, Zágora J, Plachá D, Muñoz-Bonilla A, and Fernández-García M. Functionalization of Chitosan-Chitin Nanowhiskers Films By Impregnation With Essential Oils Via Supercritical CO₂. *J Polym Environ*. 2025;33(1):96–111.
29. Guo S, Li T, Chen M, Wu C, Ge X, Fan G, Li X, Zhou D, Mi L, Zhao X, *et al.* Sustainable and effective Chitosan-based edible films incorporated with OEO nanoemulsion against apricots' black spot. *Food Control*. 2022;138:108965.
30. Sayadi M, Abedi E, and Oliyaei N. Effect of Persian gum-gelatin based-pickering emulsion film loaded with Thyme essential oil on the storage quality of Barred mackerel (*Scomberomorus commerrson*) fillet. *LWT*. 2025;215:117241.
31. Norcino LB, Mendes JF, Natarelli CVL, Manrich A, Oliveira JE, and Mattoso LHC. Pectin films loaded with copaiba oil nanoemulsions for potential use as bio-based active packaging. *Food Hydrocoll*. 2020;106:105862.
32. Gharibi S, Tabatabaei BES, and Saeidi G. Comparison of Essential Oil Composition, Flavonoid Content and Antioxidant Activity in Eight *Achillea* Species. *J Essent Oil Bear Plants*. 2015;18(6):1382–94.
33. Shen Z and Kamdem DP. Development and characterization of biodegradable chitosan films containing two essential oils. *Int J Biol Macromol*. 2015;74:289–96.
34. Çoruhlu P, Rayman Ergün A, and Baysal T. Characterization of chitosan films incorporating thyme oil and its effect on black olives. *Ital J Food Sci*. 2025;37(1):307–18.
35. Ferreira RR, Souza AG, and Rosa DS. Essential oil-loaded nanocapsules and their application on PBAT biodegradable films. *J Mol Liq*. 2021;337:116488.
36. Al-Ali RM, Al-Hilifi SA, and Rashed MMA. Fabrication, characterization, and anti-free radical performance of edible packaging-chitosan film synthesized from shrimp shell incorporated with ginger essential oil. *J Food Meas Charact*. 2021;15(4):2951–62.
37. Lian H, Shi J, Zhang X, and Peng Y. Effect of the added polysaccharide on the release of thyme essential oil and structure properties of chitosan based film. *Food Packag Shelf Life*. 2020;23:100467.
38. Almasi L, Radi M, Amiri S, and McClements DJ. Fabrication and characterization of antimicrobial biopolymer films containing essential oil-loaded microemulsions or nanoemulsions. *Food Hydrocoll*. 2021;117.
39. Ghani S, Barzegar H, Noshad M, and Hojjati M. The preparation, characterization and in vitro application evaluation of soluble soybean polysaccharide films incorporated with cinnamon essential oil nanoemulsions. *Int J Biol Macromol*. 2018;112:197–202.
40. Zehra A, Wani SM, Jan N, Bhat TA, Rather SA, Malik AR, and Hussain SZ. Development of chitosan-based biodegradable films enriched with thyme essential oil and additives for potential applications in packaging of fresh collard greens. *Sci Rep*. 2022;12(1):16923.
41. Kanatt SR. Development of active/intelligent food packaging film containing Amaranthus leaf extract for shelf life extension of chicken/fish during chilled storage. *Food Packag Shelf Life*. 2020;24:100506.
42. Amiri H. Essential oils composition and antioxidant properties of three thymus species. *Evidence-based Complement Altern Med*. 2012; 2012.
43. Elsherief MF, Devecioglu D, Saleh MN, Karbancioglu-Guler F, and Capanoglu E. Chitosan/alginate/pectin biopolymer-based Nanoemulsions for improving the shelf life of refrigerated chicken breast. *Int J Biol Macromol*. 2024;264:130213.
44. Kamkar A, Molaei-aghache E, Khanjari A, Akhondzadeh-basti A, Noudoost B, Shariatifar N, Alizadeh Sani M, and Soleimani M. Nanocomposite active packaging based on chitosan biopolymer loaded with nano-liposomal essential oil: Its characterizations and effects on microbial, and chemical properties of refrigerated chicken breast fillet. *Int J Food Microbiol*. 2021;342:109071.
45. Sheerzad S, Khorrami R, Khanjari A, Gandomi H, Basti AA, and Khansavar F. Improving chicken meat shelf-life: Coating with whey protein isolate, nanochitosan,

- bacterial nanocellulose, and cinnamon essential oil. LWT. 2024;197:115912.
46. Moadab FR and Azizkhani M. The Effect of Chitosan Edible Coating Containing Nanoemulsion of *Eryngium campestre* Essential Oil on the Quality of Ostrich Meat During Storage in Refrigerator. Food Sci Nutr. 2025;13(5).
47. Karam L, Roustom R, Abiad MG, El-Obeid T, and Savvaidis IN. Combined effects of thymol, carvacrol and packaging on the shelf-life of marinated chicken. Int J Food Microbiol. 2019;291:42–7.
48. Kiprotich S, Mendonça A, Dickson J, Shaw A, Thomas-Popo E, White S, Moutiq R, and Ibrahim SA. Thyme Oil Enhances the Inactivation of *Salmonella enterica* on Raw Chicken Breast Meat During Marination in Lemon Juice With Added *Yucca schidigera* Extract. Front Nutr. 2021;7.
49. Gholami-Ahangaran M, Ahmadi-Dastgerdi A, Azizi S, Basiratpour A, Zokaei M, and Derakhshan M. Thymol and carvacrol supplementation in poultry health and performance. Vet Med Sci. 2022;8(1):267–88.
50. Wang W, Zhao D, Xiang Q, Li K, Wang B, and Bai Y. Effect of cinnamon essential oil nanoemulsions on microbiological safety and quality properties of chicken breast fillets during refrigerated storage. LWT. 2021;152:112376.
51. Noori S, Zeynali F, and Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food Control. 2018;84:312–20.
52. Wang X, Wang Z, Zhuang H, Nasiru MM, Yuan Y, Zhang J, and Yan W. Changes in color, myoglobin, and lipid oxidation in beef patties treated by dielectric barrier discharge cold plasma during storage. Meat Sci. 2021;176:108456.
53. Bleicher J, Ebner EE, and Bak KH. Formation and Analysis of Volatile and Odor Compounds in Meat—A Review. Molecules. 2022;27(19):6703.
54. Y. Youssef D. Assessment of the Impact of Rosemary Chitosan Microemulsion Effect on *Escherichia coli* and *Listeria monocytogenes* Dipping in Chicken Meat Stored at 4°C. Int J Agric Biol. 2022;27(01):69–76.
55. Khorshidi S, Mehdizadeh T, and Ghorbani M. The effect of chitosan coatings enriched with the extracts and essential oils of *Elettaria Cardamomum* on the shelf-life of chicken drumsticks vacuum-packaged at 4 °C. J Food Sci Technol. 2021;58(8):2924–35.
56. Xiang H, Liao J, Tang Y-T, Wen P, and Wang H. Development of cinnamon essential oil-loaded core-shell nano film for the preservation of chilled flesh foods. Food Packag Shelf Life. 2024;45:101310.
57. Hussain SA, Wani SA, Rafeh S, Adil S, Sofi AH, Ghamry HI, and Wani M. Enhancing Meat Emulsion Quality and Storage Stability During Refrigeration Using Thyme and Oregano Essential Oil Nanoparticles. Foods. 2025;14(6):1076.

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