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Formulation Optimization of Chitosan-Based Edible Films Using Organic Acid Solvents, Fatty Acids, and Sodium Benzoate for Sustainable Food Packaging Applications

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Abstract

The environmental impact of conventional plastic food packaging has driven the development of biodegradable and edible materials from renewable resources. Chitosan, a biopolymer derived from chitin, is a promising candidate due to its biodegradability, film-forming ability, and antimicrobial activity. However, pure chitosan-based edible films are limited by low mechanical strength, high water vapor permeability, and variable antimicrobial performance. This study evaluated the combined effects of solvent type, fatty acid incorporation, and sodium benzoate addition on the physical, mechanical, barrier, microstructural, and antimicrobial properties of chitosan-based edible films. Chitosan edible films were prepared by solution casting using acetic acid and lactic acid as solvents. Stearic acid and oleic acid were incorporated at 2% and 5% (w/w of chitosan), with sodium benzoate added at 0 and 0.03%. Film properties were analyzed in terms of pH, water activity, optical properties, thickness, tensile strength, elongation at break, WVTR, microstructure (SEM), and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Acetic acid–based films showed higher tensile strength and lower WVTR, whereas lactic acid–based films exhibited greater elasticity. Stearic acid improved moisture-barrier performance and microstructural homogeneity, while sodium benzoate enhanced antimicrobial activity, particularly against *S. aureus*. It can be concluded that Chitosan dissolved in 1% acetic acid with 5% stearic acid and sodium benzoate provided the most balanced mechanical, barrier, and antimicrobial performance, supporting its potential as sustainable food packaging.

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Introduction

The increasing global concern over environmental sustainability and public health has intensified research into alternative food-packaging materials that can replace conventional synthetic plastics. Plastic-based food packaging is predominantly nonbiodegradable, leading to persistent environmental pollution, accumulation of microplastics in ecosystems, and potential health risks through food-chain contamination (Zhang et al., 2023). Beyond waste accumulation, plastic packaging contributes to marine pollution, greenhouse gas emissions during production, and the depletion of nonrenewable fossil resources, further exacerbating environmental degradation (Barrino et al., 2023). These challenges have driven a paradigm shift toward biodegradable and edible packaging systems derived from renewable natural polymers, such as polysaccharides and proteins, which align with circular economy principles and sustainability goals (Bose et al, 2023).

Among natural polymers, chitosan has emerged as a particularly promising candidate for edible and biodegradable food-packaging applications. Chitosan is obtained through the deacetylation of chitin and exhibits intrinsic biodegradability, which directly addresses waste-management issues associated with synthetic plastics (Wrońska et al., 2023). In addition, chitosan demonstrates excellent film-forming ability, enabling the production of cohesive matrices that function as barriers to moisture and gases, thereby supporting food preservation (Bose et al., 2023). Importantly, chitosan possesses inherent antimicrobial activity that can inhibit the growth of spoilage and pathogenic microorganisms, contributing to shelf-life extension and food safety (Fu et al., 2024). Collectively, these functional attributes position chitosan as a key biopolymer in the development

of sustainable packaging solutions for the food industry.

Despite these advantages, the practical application of pure chitosan-based edible films remains limited by several critical performance constraints. Mechanical properties such as tensile strength and elongation at break are highly sensitive to the hydration state of chitosan, often resulting in brittle films with insufficient flexibility when compared to conventional plastic packaging materials (Venkatachalam et al., 2023). In addition, although chitosan films generally exhibit satisfactory moisture-barrier behavior, their gas-barrier performance is frequently inadequate, reducing their effectiveness in preventing oxidative spoilage and quality deterioration (Gürler, 2022). These limitations indicate that the inherent properties of chitosan alone are insufficient for demanding food-packaging applications, and that formulation strategies involving plasticizers, lipids, or composite systems are necessary to enhance both mechanical integrity and barrier performance (Madihalli et al., 2025).

From a sustainability perspective, the utilization of chitosan also offers significant advantages through the valorization of crustacean-processing by-products, such as shrimp and crab shells. These by-products represent an abundant and renewable resource, particularly in coastal and maritime regions, where their conversion into chitosan can reduce environmental waste while providing an economically viable raw material for biodegradable films (Abdullah et al., 2023). Such approaches support circular economy models by transforming industrial waste into value-added materials and simultaneously promoting environmental protection and local economic development (Ma et al., 2021).

Beyond serving as passive barriers, edible films based on chitosan play an increasingly important role in active and antimicrobial food-packaging systems. These films can function as carriers for bioactive compounds, including essential oils and antimicrobial agents, which actively inhibit microbial growth and oxidative processes responsible for food spoilage (Lopes et al., 2025). Several studies have demonstrated that incorporating bioactive substances into chitosan films enhances antimicrobial and antioxidant performance without negatively affecting sensory quality (Lopes et al., 2025). Furthermore, the ability of chitosan-based films to regulate moisture and gas exchange contributes to maintaining product quality and extending the shelf life of perishable foods (Deol, 2025).

Developing multi-functional chitosan films requires overcoming the persistent trade-off between moisture-barrier efficiency and mechanical resilience. Previous strategies often fell short; for instance, incorporating antimicrobial organic salts by Dong et al. (2022) accelerated moisture transmission due to salt hydrophilicity, while embedding hydrophobic fatty acids to block water compromised tensile strength and caused uneven preservative dispersion (Ahmadi et al., 2020). Additionally, while Yang et al. (2021) examined binary interactions between chitosan and sodium benzoate, the structural and functional interplay within a complex ternary matrix involving diverse acid solvents, fatty acids, and aromatic preservatives remains unexplored.

To resolve these limitations, this study evaluates the concurrent impacts of solvent type (acetic vs. lactic acid), fatty acid structures (stearic vs. oleic acid), and sodium benzoate on film performance. Sodium benzoate was intentionally chosen over volatile essential oils or alternative salts due to its superior cost-efficiency, processing stability, and targeted functionality. In the acidic casting solution, it converts into uncharged benzoic acid, easily penetrating microbial membranes to disrupt pathogen metabolism (Dong et al., 2022). Mechanistically, its ionic character avoids the phase separation and opacity typical of plant extracts (Ahmadi et al., 2020), instead driving strong electrostatic and hydrogen bonding with protonated chitosan amine groups and fatty acid carboxyl units. This unique molecular affinity yields a highly integrated, flawless biopolymer matrix (Yang et al., 2021) that satisfies the study's goal: engineering a robust, sustainable active packaging film with predictable antimicrobial release.

To bridge this critical research gap, this study introduces a novel approach by systematically evaluating a ternary composite system composed of different organic acid solvents (acetic vs. lactic acid), varying saturation levels of fatty acids (stearic vs. oleic acid), and sodium benzoate. The core novelty lies in uncovering how the solvent type governs the electrostatic integration of both lipophilic barriers and ionic preservatives within the protonated chitosan network, moving beyond generic blending to deliver a highly compatible, engineered active packaging matrix. Consequently, this research aims to determine an optimized chitosan-based formulation that yields a balanced combination of mechanical strength, moisture-barrier efficiency, and antimicrobial potency. Ultimately, these findings provide critical scientific insights into structural formulation strategies, offering a highly viable blueprint to expand the application of eco-friendly, high-performance biopolymer films in sustainable food packaging systems.

Materials and Methods

This study employed an experimental, laboratory-based design using a factorial approach to systematically evaluate the effects of formulation variables on the performance of chitosan-based edible films. The independent variables consisted of: (i) solvent type (1% acetic acid and 2% lactic acid), (ii) fatty acid type and concentration (stearic acid and oleic acid at 2% and 5% w/w of chitosan), and (iii) sodium benzoate concentration (0 and 0.03% w/w). The dependent variables included physical properties (pH, water activity, color brightness, and thickness), mechanical properties (tensile strength and elongation at break), barrier properties (water vapor transmission rate), microstructural characteristics, and antimicrobial activity.

Chitosan was used as the primary film-forming biopolymer. Distilled water, glycerol (as a plasticizer), acetic acid, lactic acid, stearic acid, oleic acid, and sodium benzoate were employed in film formulation. Ethanol (96%) was used for mold cleaning. Microbiological analyses utilized *Staphylococcus aureus* and *Escherichia coli* cultures, Nutrient Broth (NB), and Nutrient Agar (NA). Desiccants ($CaCl_2$), paraffin paper, and saturated salt solutions were used for humidity control.

The principal equipment included an

oven/incubator, homogenizer, magnetic stirrer with hot plate, analytical balance, shaker, desiccator, pH meter, water activity meter (Shibaura WA-360), Chromameter Minolta CR-200, micrometer, tensile strength and elongation tester (Comten Industries), WVTR testing cups, and a Scanning Electron Microscope (SEM JEOL 5200).

Solution casting was selected as the film-preparation technique due to its simplicity, reproducibility, and widespread use in chitosan edible-film research (Pavlátková et al., 2022). Chitosan (3 g) was dissolved in 300 mL of either 1% (v/v) acetic acid or 2% (v/v) lactic acid under continuous stirring at 50 °C for 60 min to ensure complete solubilization. Glycerol (15% w/w of chitosan) was added as a plasticizer to improve flexibility. Fatty acids (stearic acid or oleic acid at 2% or 5% w/w of chitosan) were incorporated as hydrophobic modifiers to reduce water-vapor permeability and modify mechanical behavior. Sodium benzoate (0 or 0.03% w/w) was added as a preservative component. The mixture was homogenized to obtain a uniform film-forming solution.

The resulting solution was poured into ethanol-cleaned glass molds (20 cm diameter). Initial drying of the edible films was carried out at room temperature for 24 h. Subsequently, controlled oven-drying was executed, adjusting the temperature to 45 °C for acetic acid-based films and 55 °C for lactic acid-based variants. Drying conditions are known to influence film thickness, uniformity, and mechanical properties through their effects on solvent evaporation rate and polymer-chain arrangement (Pavlátková et al., 2022). After drying, films were carefully peeled from the molds, wrapped in aluminum foil, and stored in sealed plastic bags prior to analysis.

Standardized analytical methods were employed to ensure comparability and reproducibility of results, as recommended in edible-film research (Kaczmarek et al., 2022). Film pH was measured using a calibrated pH meter. Water activity (aw) was determined using a Shibaura WA-360 aw-meter. Color parameters (L*, a*, b*) were measured using a Chromameter Minolta CR-200, with brightness (L*) used as the primary optical indicator. Film thickness was measured at five random positions using a micrometer with 0.001 mm precision, and the average value was reported.

Tensile strength and elongation at break were measured using a tensile testing machine (Comten Industries) following ASTM D882 procedures, which are widely used for evaluating mechanical properties of edible films (Kaczmarek et al., 2022). Prior to testing, films were conditioned at 25 °C and 75% relative humidity for 24 h to standardize moisture content. Water Vapor Transmission Rate (WVTR) was determined using the gravimetric method according to ASTM E96. Film samples were sealed over WVTR cups containing $CaCl_2$ desiccant and placed in a controlled-humidity desiccator. Weight gain was recorded daily, and WVTR values were calculated from the slope of mass increase versus time, providing a measure of moisture-barrier performance (Kaczmarek et al., 2022).

Microstructural characteristics of the films were examined using Scanning Electron Microscopy (SEM) to systematically evaluate both surface morphology and cross-sectional structures. To ensure clear visualization of the polymer network, film samples were subjected to a hexane pretreatment specifically aimed at defatting the matrix surface and removing unbound fatty acid residues that could otherwise cause artifacts or burning under the electron beam. Following this cleaning step, the samples were fractured in liquid nitrogen for cross-sectional analysis, sputter-coated with gold to impart conductivity, and observed at magnifications of 500× and 2000×. This systematic dual-plane SEM analysis is commonly applied to visualize surface topography and internal cross-sectional frameworks, enabling accurate interpretation of additive dispersion, phase separation, porosity, and polymer-network organization (Pavlátková et al., 2022).

Pure cultures of *Staphylococcus aureus* and *Escherichia coli* were activated in Nutrient Broth and incubated at 37 °C for 12–24 h to obtain standardized suspensions (10^5 CFU/mL). Edible film discs (1 cm diameter) were placed on NA plates inoculated with the test microorganisms and incubated at 37 °C for 24 h. Antimicrobial activity was expressed as the diameter of the inhibition zone, calculated as the difference between the clear zone diameter and the film-disc diameter.

To guarantee data reliability, all instruments were calibrated prior to analysis, and film formulations were fabricated from scratch in two independent experimental batches (duplicate experimental replicates). Subsequent characterizations of physical and barrier properties (thickness, moisture content, and WVTR) and mechanical attributes (tensile strength and elongation at break) were performed using two analytical replicates randomly sampled per batch. Optical and antimicrobial assays were likewise carried out in duplicate for each treatment. All quantitative data are reported as mean ± standard deviation and were subjected to an analysis of variance (ANOVA), with mean separations determined using Duncan's Multiple Range Test at a 5% significance level ($p < 0.05$). Statistically significant differences are denoted in tables and figures by distinct superscript letters. This rigorous framework of standardized methods, strict replication, and statistical validation ensures high reproducibility and permits direct comparison with existing literature on chitosan-based edible films.

Results and Discussion

Physical Properties of Chitosan Edible Films

pH of Film-Forming Solutions

Across all formulations, the film-forming solutions remained acidic, with measured pH values spanning 3.37–4.97 (Table 1). Films prepared using acetic acid exhibited statistically higher pH values (3.70–4.97) than those prepared using lactic acid (3.37–3.93) ($p < 0.05$), a trend directly governed by the higher dissociation constant (K_a) and stronger acidity of lactic acid within the biopolymer matrix. This variance in acidity significantly influenced the film's water activity (a_w) and optical properties ($p < 0.05$). Specifically, lactic acid-based films demonstrated significantly lower a_w values, which correlates with the enhanced hygroscopic nature and water-binding capacity of the lactic acid-chitosan complex. Furthermore, the statistical analysis confirmed a significant reduction in brightness (L*) for films

prepared with lactic acid and incorporated with fatty acids compared to their acetic acid counterparts ($p < 0.05$). This loss of brightness is statistically significant and can be attributed to the altered refractive index and tighter internal polymer-network organization, which limits light transmittance through the multi-component matrix.

Although both solvents protonate chitosan amino groups, the observed pH ranges indicate that solvent choice and additive inclusion can shift ionic conditions in complex ways, which is relevant because solution acidity governs chitosan solubility and the cationic character that underpins functional performance (Xu et al., 2022). The present pattern also aligns with reports that solvent systems influence film chemistry and microbial stability indirectly through acid–polymer interactions (Kaur et al., 2023).

Water Activity (aw)

Water activity values ranged from 0.577 to 0.636 (Table 1). In general, incorporating fatty acids reduced aw, consistent with the formation of more hydrophobic domains that limit free water in the matrix. Sodium benzoate addition further decreased aw in several formulations, indicating reduced availability of unbound water. This outcome is consistent with literature emphasizing that solvent conditions and formulation choices influence aw, which is a key indicator of microbial stability (Xu et al., 2022). Notably, all measured aw values were below levels typically required for robust microbial proliferation, supporting the feasibility of these films as active packaging materials (Kaur et al., 2023).

Optical Properties (Brightness, L*)

Brightness (L*) ranged from 55.24 to 79.49 (Table 1). Films containing sodium benzoate, particularly in lactic-acid systems, showed comparatively high brightness (e.g., C:A:O:B (2%) at 79.49 ± 1.15), indicating acceptable visual appearance and potential consumer-facing suitability. Variations in brightness are consistent with prior findings that hydrophobic modifiers can increase opacity and reduce transparency depending on concentration and dispersion. Microstructural changes during standard solvent evaporation can alter light scattering and reduce transparency, which contributes to the brightness variability among treatments (Kumari et al., 2021). Rather than an effect of drying temperature as an independent variable, this visual variation is driven by the distinct thermal sensitivity and structural compaction of the different acid-solvent matrices under their respective curing conditions. Lactic acid-based films, requiring a higher baseline evaporation temperature (55 °C) due to lower volatility, undergo denser polymer network organization and potential mild Maillard-like browning interactions, which statistically reduces film brightness compared to the more volatile acetic acid system cured at 45 °C. Additionally, differences in crystallinity and microstructure, as described for acid-modified chitosan systems, can affect optical behavior and are consistent with earlier observations for chitosan films modified by organic acids (Liberto & Dintcheva, 2024).

Mechanical and Barrier Properties

Film Thickness

Film thickness varied substantially by solvent system, ranging from 0.093 to 0.423 mm (Table 2). Acetic acid–based films were thinner (0.093–0.188 mm) than lactic acid–based films (0.199–0.423 mm). Oleic acid inclusion tended to increase thickness in several treatments, consistent with phase separation and layered structures that can increase effective cross-sectional distance. The systematic variation in film thickness observed in this study is directly driven by the architectural arrangement of the ternary components within the matrix. Specifically, the incorporation of structured fatty acids led to a significant increase in thickness for both acetic and lactic acid-based films, with stearic acid inducing a more pronounced expansion compared to oleic acid. This outcome is attributed to the steric hindrance and lower structural packing efficiency of the saturated solid crystalline network of stearic acid, which forces the protonated chitosan chains further apart. In contrast, the kinked cis-double bond structure of oleic acid allowed for a more compliant, less disruptive dispersion within the polymer network. This distinct internal phase behavior and varying molecular dispersion profiles align with recent findings by Fitriyanti et al., (2023) and Dong et al. (2022), confirming that the physical dimensions of active biopolymers are heavily dictated by the spatial accommodation of lipid additives within the continuous matrix phase.

Elongation at Break

Elongation at break exhibited an exceptionally wide variation, ranging from 23.96% to 747.77% (Table 2). This substantial divergence is directly driven by a synergistic plasticization anomaly occurring specifically within the lactic acid-solvent systems. Unlike the volatile acetic acid, residual lactic acid molecules remain entrapped within the matrix, acting as a powerful co-plasticizer alongside glycerol. This dual-plasticizer environment drastically disrupts the intermolecular hydrogen bonding of chitosan, expanding the free volume and increasing the amorphous fraction of the film, which significantly enhances chain mobility and reduces matrix stiffness (Gürler, 2022). Furthermore, the interaction within this flexible network explains why stearic acid consistently supported higher elongation than oleic acid in comparable solvent systems. The solid crystalline domains of stearic acid likely formed discrete, lubricated interstitial phases that facilitated smooth macromolecular gliding under tensile stress, whereas the kinked structure of oleic acid induced localized phase separation that prematurely disrupted film integrity. This highly formulation-dependent dispersion behavior underscores how specific lipid-additive structures dictate the mechanical thresholds of composite chitosan films (Fitriyanti et al., 2023).

Tensile Strength

Tensile strength showed an inverse relationship with elongation, reflecting a classic mechanical trade-off characteristic of biopolymer systems. Acetic acid-based films achieved high mechanical resilience, reaching tensile strength values up to 26.88 MPa (Table 2). In contrast, lactic acid-based films consistently fell below

the instrument's reliable detection threshold (<0.0078 MPa). This extreme reduction in tensile strength reflects a true material behavior driven by a localized plasticization overload rather than a mechanical flaw in measurement sensitivity. Because lactic acid is highly hygroscopic and non-volatile, its molecules remain heavily entrapped within the chitosan network. During ambient equilibration, this excess acid absorbs substantial atmospheric moisture, transforming the rigid polymer matrix into a highly hydrated, hydrogel-like state. This liquid-like state drastically diminishes intermolecular cohesion and hinders any effective stress transfer across the continuous network. This profound structural disruption aligns with reports that the specific choice of acid solvent can fundamentally modify the baseline film structure and destroy mechanical integrity by shifting the material from a cohesive matrix to a highly amorphous, decoupled chain organization (Gürler, 2022).

Water Vapor Transmission Rate (WVTR)

WVTR values ranged from 81.47 to 277.63 g/m²/day (Table 2). Acetic acid–based films tended to display lower WVTR than lactic acid–based films, suggesting better moisture-barrier performance. Incorporation of fatty acids and sodium benzoate reduced WVTR in multiple treatments, supporting the role of hydrophobic and functional additives in improving moisture resistance. These findings are consistent with reports that lipid or fatty-acid additives create more hydrophobic pathways and reduce moisture permeability in chitosan films (Pulvirenti et al., 2025). The observed WVTR reductions in lipid-containing systems align with prior evidence that lipid additives such as stearic acid can significantly improve moisture retention and extend shelf-life potential (Fitriyanti et al., 2023). Moreover, antimicrobial agents and functional additives may contribute synergistically to barrier performance when integrated into the polymer matrix (Woźniak et al., 2024).

Microstructural Characteristics (SEM)

Surface Morphology

SEM observations indicated notable differences in additive dispersion. Stearic acid appeared more homogeneously distributed within the chitosan matrix, while oleic acid was associated with layered structures and nonuniform features that reflect phase separation tendencies. This pattern is consistent with literature explaining that incompatibility between hydrophobic additives and polysaccharide matrices can yield visible phase separation, producing roughness and heterogeneity in SEM images (Wang et al., 2025). Conversely, better additive compatibility supports more uniform microstructure and improved interfacial adhesion, contributing to stronger mechanical and barrier performance (Buitrago-Arias et al., 2025).

Cross-Sectional Structure

Cross-sectional micrographs showed that acetic-acid films were more compact and uniform, while lactic-acid films were thicker and exhibited layered/elastic structures. Such differences are consistent with the reported link between microstructure and mechanical/barrier outcomes: rougher or more heterogeneous structures may compromise tensile behavior due to irregular stress distribution (Gürler, 2022), while smoother or denser structures frequently correlate with improved tensile strength and lower WVTR (Fitriyanti et al, 2023). In addition, denser microstructures increase tortuosity and reduce diffusion pathways for water vapor , supporting improved moisture

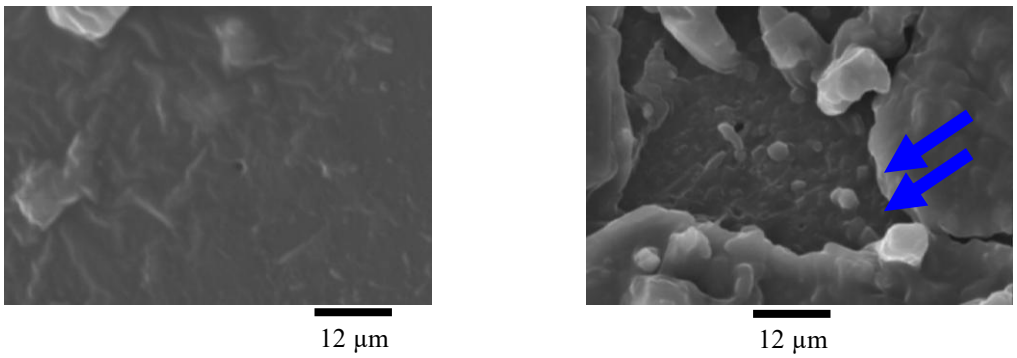


Figure 1. Surface microstructure of chitosan edible films: (a) acetic acid solvent + 5% stearic acid; (b) acetic acid solvent + 5% oleic acid (SEM, 2000×).

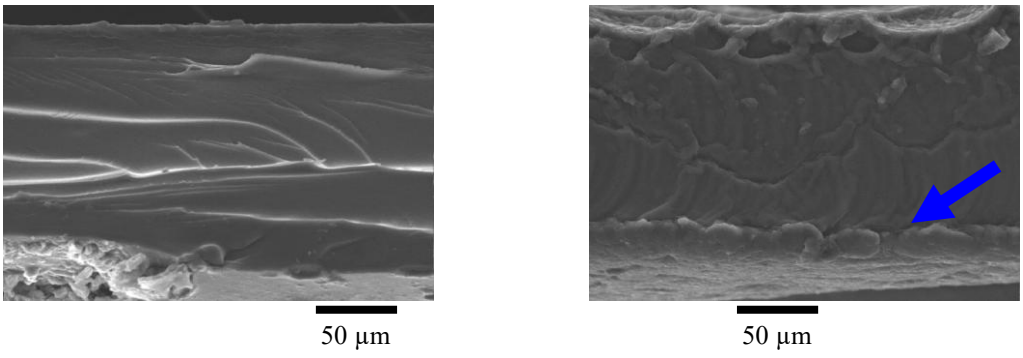


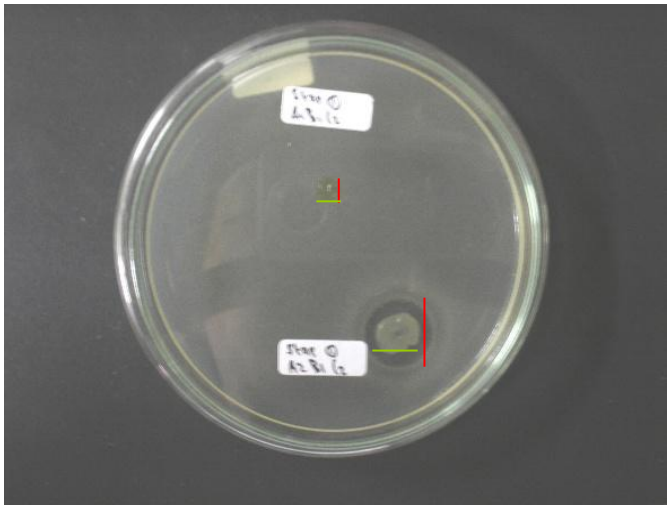
Figure 2. Cross-sectional structure of chitosan edible films: (a) acetic acid solvent + oleic acid; (b) lactic acid solvent + oleic acid (SEM, 500×)

barrier properties (Madihalli et al., 2025). Microstructural and compactness changes induced by these additives significantly influence both tensile and barrier performance. Based on our SEM observations, the formation of a highly ordered, dense, and homogeneous polymer network with fewer micro-voids directly restricts water vapor diffusion pathways while enhancing resistance to mechanical deformation. This structural compactness and molecular organization mimic the physical performance typically associated with high-crystallinity regions in biopolymers, where a more aligned matrix leads to superior tensile strength and better moisture resistance (Fitriyanti et al., 2023). Therefore, the variations in film properties are heavily governed by the physical integration and dispersion state of the additives within the amorphous-crystalline framework of the chitosan matrix, as evidenced by the dense cross-sectional morphology.

Antimicrobial Activity

Inhibition of *Staphylococcus aureus*

Inhibition zones against *Staphylococcus aureus* ranged from 0.00 to 11.00 mm. Lactic-acid formulations generally exhibited larger inhibition zones, while sodium benzoate enhanced inhibition particularly in selected acetic-acid treatments. These outcomes are consistent with the mechanistic understanding that chitosan's cationic character enables binding to negatively charged components of bacterial cell envelopes, disrupting membrane permeability and causing leakage of intracellular material (Ke et al., 2021). Additional antimicrobial actions include interference with nucleic acid synthesis and protein production (Borandeh et al., 2023). Because chitosan solubility and charge density are pH-dependent, acidic conditions can strengthen antimicrobial effects by enhancing protonation and solubilization, whereas higher pH can reduce activity by lowering solubility (Aranaz et al., 2021). Solvent choice can therefore modulate antibacterial performance by influencing protonation dynamics and film chemistry (Borandeh et al., 2023).

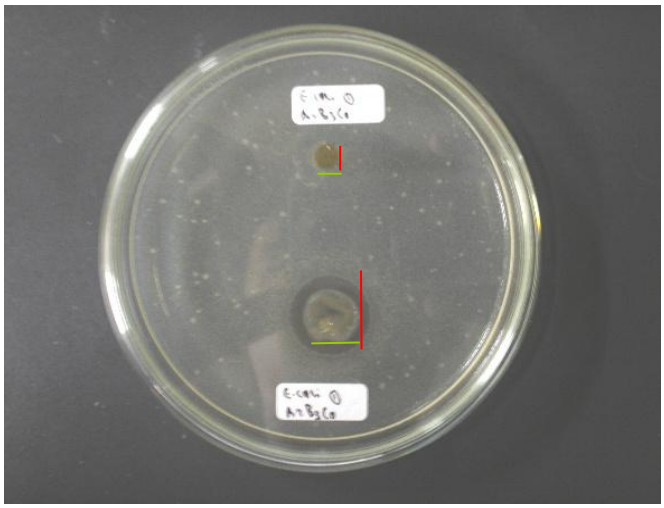


Note —— : edible film diameter (df)
—— : diameter of the clear area (inhibition zone) (da)

Figure 3. Inhibition Diameter of Edible Chitosan Films with Different Solvents. Top: Acetic Acid+2% Stearic Acid+Benzoate, Bottom: Lactic Acid+2% Stearic Acid+Benzoate on the Growth of Gram-Positive Bacteria (*Staphylococcus aureus*)

Inhibition of *Escherichia coli*

Inhibition zones against *Escherichia coli* ranged from 0.00 to 11.00 mm and were generally lower than those against *S. aureus*. This pattern is consistent with reports that Gram-negative bacteria possess an outer membrane that provides additional protection and reduces susceptibility to chitosan-based systems (Pulvirenti et al., 2025). Gram-positive bacteria are often more susceptible because chitosan can interact more readily with their cell wall components, yielding larger inhibition zones (Pulvirenti et al., 2025). For Gram-negative bacteria, literature indicates that higher chitosan concentrations or the inclusion of permeability-modifying additives (e.g., fatty acids) may be required to improve antimicrobial action (Ardean et al., 2021). The present results, in which sodium benzoate showed limited or antagonistic effects in some combinations against *E. coli*, further suggest that preservative synergy is strongly dependent on formulation chemistry and target organism.



Note —— : edible film diameter (df)
—— : diameter of the clear area (inhibition zone) (da)

Figure 4. Inhibition Diameter of Edible Chitosan Films with Different Solvents. Top: Acetic Acid+2% Oleic Acid, Bottom: Lactic Acid+2% Oleic Acid on the Growth of Gram-Negative Bacteria (*Escherichia coli*).

The results confirm that solvent selection is a primary determinant of chitosan edible-film performance, affecting not only solubilization and protonation of chitosan but also the mechanical and barrier outcomes of the resulting matrix. Acetic acid–based films in this study exhibited substantially higher tensile strength and generally lower WVTR than lactic acid–based films, while lactic acid systems showed markedly higher

elongation. This pattern is consistent with the reported role of solvent systems in enabling coherent chitosan network formation and influencing film integrity (Aranaz

Table 1. pH, Water Activity (aw), and Brightness (L*) of Chitosan Edible Films

Formula	pH	aw	L*
C:A (1:100)	4.70 ± 0.00	0.636 ± 0.001	76.64 ± 0.49
C:A:S (2%)	3.75 ± 0.00	0.612 ± 0.001	69.53 ± 0.16
C:A:S (5%)	3.96 ± 0.02	0.614 ± 0.004	60.89 ± 0.04
C:A:O (2%)	4.66 ± 0.03	0.599 ± 0.000	68.37 ± 0.69
C:A:O (5%)	4.58 ± 0.01	0.600 ± 0.000	55.24 ± 1.18
C:A:B	3.70 ± 0.03	0.592 ± 0.000	68.00 ± 0.88
C:A:S:B (2%)	4.97 ± 0.01	0.587 ± 0.001	69.06 ± 0.64
C:A:S:B (5%)	4.87 ± 0.01	0.590 ± 0.001	75.26 ± 0.61
C:A:O:B (2%)	4.00 ± 0.05	0.592 ± 0.001	79.49 ± 1.15
C:A:O:B (5%)	4.70 ± 0.01	0.599 ± 0.002	74.01 ± 1.56
C:L (1:100)	3.45 ± 0.01	0.611 ± 0.008	59.45 ± 0.28
C:L:S (2%)	3.83 ± 0.03	0.604 ± 0.004	67.49 ± 0.00
C:L:S (5%)	3.86 ± 0.01	0.610 ± 0.000	61.62 ± 0.95
C:L:O (2%)	3.93 ± 0.03	0.597 ± 0.000	66.13 ± 1.63
C:L:O (5%)	3.86 ± 0.04	0.611 ± 0.002	67.39 ± 0.07
C:L:B	3.54 ± 0.00	0.598 ± 0.001	73.61 ± 1.94
C:L:S:B (2%)	3.57 ± 0.02	0.577 ± 0.000	76.32 ± 0.13
C:L:S:B (5%)	3.50 ± 0.00	0.591 ± 0.001	76.14 ± 0.10
C:L:O:B (2%)	3.37 ± 0.00	0.579 ± 0.003	73.13 ± 1.44
C:L:O:B (5%)	3.43 ± 0.01	0.589 ± 0.001	72.09 ± 1.16

Note: C = chitosan; A = acetic acid; L = lactic acid; S = stearic acid; O = oleic acid; B = sodium benzoate.

Table 2. Mechanical and Barrier Properties of Chitosan Edible Films

Formula	Thickness (mm)	Elongation (%)	Tensile Strength (MPa)	WVTR (g/m²/day)
C:A	0.125 ± 0.032	23.96 ± 6.52	10.99 ± 1.54	198.71 ± 31.37
C:A:S (2%)	0.151 ± 0.002	53.13 ± 4.42	19.17 ± 4.87	170.09 ± 56.38
C:A:S (5%)	0.188 ± 0.001	25.88 ± 6.61	7.94 ± 0.21	163.90 ± 46.35
C:A:O (2%)	0.126 ± 0.002	39.63 ± 1.29	26.88 ± 1.95	148.32 ± 34.28
C:A:O (5%)	0.134 ± 0.001	48.46 ± 7.92	4.09 ± 0.65	133.28 ± 6.71
C:A:B	0.141 ± 0.002	34.74 ± 19.14	11.18 ± 6.21	195.53 ± 64.66
C:A:S:B (2%)	0.184 ± 0.000	35.42 ± 2.95	8.42 ± 0.87	81.47 ± 13.47
C:A:S:B (5%)	0.185 ± 0.000	55.35 ± 11.72	16.62 ± 0.47	109.01 ± 21.87
C:A:O:B (2%)	0.093 ± 0.013	57.27 ± 2.97	13.04 ± 8.25	141.83 ± 6.68
C:A:O:B (5%)	0.106 ± 0.018	36.67 ± 4.71	4.52 ± 2.53	97.09 ± 8.01
C:L	0.423 ± 0.047	270.21 ± 42.13	<0.0078	277.63 ± 27.15
C:L:S (2%)	0.307 ± 0.001	357.86 ± 11.11	<0.0078	272.31 ± 31.02
C:L:S (5%)	0.300 ± 0.017	374.17 ± 30.25	<0.0078	256.88 ± 41.95
C:L:O (2%)	0.228 ± 0.005	267.32 ± 54.79	<0.0078	256.37 ± 23.72
C:L:O (5%)	0.279 ± 0.005	277.24 ± 3.17	<0.0078	253.04 ± 15.37
C:L:B	0.266 ± 0.011	575.00 ± 58.93	<0.0078	275.53 ± 46.77
C:L:S:B (2%)	0.199 ± 0.004	747.77 ± 47.35	<0.0078	237.81 ± 11.32
C:L:S:B (5%)	0.286 ± 0.021	677.27 ± 79.28	<0.0078	216.05 ± 38.64
C:L:O:B (2%)	0.254 ± 0.028	600.04 ± 22.87	<0.0078	228.61 ± 51.33
C:L:O:B (5%)	0.239 ± 0.030	273.60 ± 58.13	<0.0078	200.01 ± 3.82

Note: C = chitosan; A = acetic acid; L = lactic acid; S = stearic acid; O = oleic acid; B = sodium benzoate.

et al., 2021). Mechanistically, improved chitosan dissolution and stronger intermolecular cohesion can promote denser networks that resist deformation and reduce vapor transport, whereas solvents and co-formulants that enhance chain mobility can increase flexibility at the expense of tensile strength.

A pronounced inverse relationship between tensile strength and elongation was observed, reflecting a classic polymer trade-off wherein strengthening intermolecular interactions improves resistance to fracture but reduces extensibility. Chitosan films form network structures stabilized by hydrogen bonding, which contributes to mechanical strength; however, the inclusion of plasticizers and acid systems that increase chain mobility can reduce effective hydrogen bonding and promote elasticity. This behavior aligns with polymer-network theory describing how plasticizers weaken intermolecular bonding and increase flexibility, often diminishing tensile strength (Li et al., 2025). In the present work, lactic acid formulations exhibited extreme elongation but tensile strength below the detection limit, indicating that the network became highly deformable and insufficiently cohesive for load-bearing performance. These results emphasize that optimizing chitosan films requires balancing cohesion (for strength) and mobility (for flexibility), rather than maximizing a single mechanical parameter.

The trade-off also extends to barrier performance. Denser polymer matrices typically provide more tortuous diffusion paths for water vapor and thereby reduce WVTR, while highly plasticized or heterogeneous structures may facilitate transport through increased free volume. Microstructural organization, therefore, mediates the mechanical–barrier coupling and represents a key target of formulation design.

SEM observations provide structural evidence supporting the measured mechanical and barrier behavior. Stearic-acid systems displayed more homogeneous dispersion, whereas oleic-acid systems more frequently exhibited layered or heterogeneous features consistent with partial phase separation. This pattern is strongly aligned with literature emphasizing that compatibility between chitosan and additives yields denser and more uniform microstructures, improving barrier properties and overall film integrity. Conversely, incompatibility-driven phase separation creates weak points in the film matrix and can compromise mechanical and barrier performance (Gürler, 2022).

The distinct behavior of stearic versus oleic acid can be interpreted through fatty-acid structure. Saturated fatty acids are reported to form more rigid and less permeable structures when incorporated into polysaccharide films, thereby enhancing moisture-barrier behavior (Luo et al., 2021). In contrast, unsaturated fatty acids may create more mobile molecular arrangements that improve flexibility but can weaken mechanical cohesion and promote heterogeneity when dispersion is limited (Li et al., 2025). The present findings are consistent with this framework: stearic acid supported a more balanced performance profile, while oleic acid was more associated with increased thickness and layered morphology, which can reduce uniform stress distribution and alter diffusion pathways.

Chitosan's antimicrobial function is enhanced under acidic conditions because protonation increases its cationic character, strengthening interactions with negatively charged bacterial surfaces. Incorporating sodium benzoate into chitosan films can further improve antimicrobial performance because an acidic environment favors the undissociated form of benzoic acid, which is more effective in penetrating microbial membranes. Prior studies report that sodium benzoate can reduce pathogen populations at low pH, including *Escherichia coli* and *Salmonella*, supporting its role as a preservative component in active packaging systems (Kaur et al., 2023). In addition, chitosan's cationic behavior under acidic conditions can synergize with benzoate systems, strengthening microbial inhibition while also supporting film functionality through maintained structural integrity and barrier properties (Venkatachalam et al., 2023).

Recent literature further suggests that chitosan–preservative combinations broaden antimicrobial effectiveness across conditions, although the magnitude of inhibition and optimal conditions can differ by target organism and formulation. The present results—showing stronger inhibition against Gram-positive bacteria and more variable effects against Gram-negative bacteria—are compatible with the broader trend in which preservatives help mitigate chitosan's limitations against Gram-negative organisms (Chang et al., 2021). Complementary findings also indicate that sodium benzoate–containing films can effectively reduce foodborne pathogen populations under acidic conditions, reinforcing the practical relevance of the preservative synergy observed here (Kaur et al., 2023).

The findings strengthen the evidence base for chitosan edible films as sustainable alternatives to synthetic packaging and demonstrate that multi-factor formulation strategies can resolve key functional limitations. Integrating solvent selection with hydrophobic modifiers and preservatives makes it possible to tune moisture resistance, mechanical behavior, and antimicrobial activity simultaneously. In this study, the formulation of chitosan dissolved in 1% acetic acid with 5% stearic acid and sodium benzoate emerged as a promising balance point, combining improved tensile strength, reduced WVTR, favorable microstructural uniformity, and enhanced antimicrobial performance.

These results align with the broader direction of sustainable packaging research emphasizing that functionality can be expanded through combined systems of natural polymers and preservatives. Such combined systems may improve food safety and shelf life while supporting biodegradability and circular economy objectives (Zhang et al., 2023).

Although the optimized formulation showed promising multi-response performance, further work is required to validate applicability in real food systems. Future studies should evaluate long-term storage stability, performance under fluctuating humidity and temperature, and potential sensory impacts when applied to foods. In addition, scale-up feasibility and processing robustness should be assessed to support industrial implementation. Finally, exploring alternative preservative systems and complementary natural

antimicrobials may further enhance chitosan-film functionality and broaden application scope, consistent with ongoing research into next-generation sustainable edible packaging designs (Zhang et al., 2023).

The formulation synthesized with 1% acetic acid, 5% stearic acid, and sodium benzoate exhibited the most balanced mechanical, barrier, and antimicrobial profiles. The volatile nature of acetic acid preserved a highly cohesive chitosan backbone, yielding superior tensile strength. Simultaneously, the 5% stearic acid integrated uniformly within this matrix, establishing a dense hydrophobic network that significantly improved moisture-barrier performance (lower WVTR) and microstructural homogeneity. This matrix successfully incorporated sodium benzoate, which provided potent antimicrobial activity, particularly against *S. aureus*.

Conversely, formulations utilizing lactic acid combined with oleic acid performed poorly across almost all physical baselines. The non-volatile, highly hygroscopic profile of residual lactic acid caused a severe plasticization overload. When paired with oleic acid, this interaction disrupted polymer cohesion and induced phase separation, resulting in a catastrophic drop in tensile strength (<0.0078MPa) and compromised vapor resistance, despite showing higher elasticity.

Conclusion

This study demonstrated that the performance of chitosan-based edible films is strongly dependent on formulation variables, particularly solvent type, fatty acid incorporation, and sodium benzoate addition. The results confirmed that acetic acid and lactic acid solvents produce films with distinctly different structural and functional characteristics, with acetic acid favoring mechanical strength and barrier performance, and lactic acid promoting high elasticity. The incorporation of fatty acids effectively reduced water vapor permeability by introducing hydrophobic domains into the chitosan matrix, while the fatty-acid structure governed microstructural homogeneity and mechanical behavior. Sodium benzoate contributed to enhanced antimicrobial activity, especially against Gram-positive bacteria, through synergistic interactions under acidic conditions.

Importantly, the findings indicate that no single formulation parameter alone can optimize all desirable properties of chitosan edible films. Instead, a balanced, multi-factor formulation approach is required to resolve trade-offs between mechanical strength, flexibility, moisture resistance, and antimicrobial effectiveness. The optimized formulation identified in this study provides a practical foundation for the development of environmentally friendly, functional edible films and supports the broader adoption of chitosan-based materials in sustainable food-packaging systems. Future research should focus on long-term storage stability, sensory impacts on packaged foods, and scale-up feasibility to facilitate industrial implementation.

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