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Characterization of chitosan-based edible coatings enriched with oil palm empty fruit bunch liquid smoke and neem extract

Muhammad Faisal[1,2], Siswanda[3], Mukhlisien[1], Hanifah Rosyidah[1], Sufi Khadarsih[1], Hera Desvita[4*]

[1] Department of Chemical Engineering, Faculty of Engineering, Universitas Syiah Kuala, Banda Aceh, Indonesia
[2] Climate Change Research Center, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[3] Doctoral Program, School of Engineering, Graduated School, Universitas Syiah Kuala, Darussalam, Banda Aceh, 23111, Indonesia
[4] Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN), Gunungkidul, Yogyakarta 55861, Indonesia
* Corresponding author's e-mail: hera005@brin.go.id

ABSTRACT

This study developed a chitosan-based edible coating enriched with liquid smoke derived from the pyrolysis of oil palm empty fruit bunches (OPEFB) and neem (*Azadirachta indica*) leaf extract as a sustainable alternative to synthetic packaging. The coating was prepared using liquid smoke (1–3% v/v), gelatin (1% w/w), chitosan (1% w/w), and neem extract (2%). Liquid smoke was obtained through OPEFB pyrolysis at 300–380 °C. The coatings were characterized using Fourier transform infrared spectroscopy (FTIR) and X-ray fluorescence (XRF), and evaluated for total flavonoid content, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and antimicrobial activity against Gram-positive and Gram-negative bacteria using the Kirby-Bauer disk diffusion method. FTIR spectra confirmed the presence of hydroxyl, amine, and phenolic groups responsible for structural stability and antimicrobial functionality, while mineral components such as P_2O_5, MgO, and CaO enhanced the film matrix. The formulation containing 1% liquid smoke derived at 340°C showed the highest flavonoid content and strongest antimicrobial effect against *Escherichia coli*, *Salmonella Typhimurium*, *Staphylococcus aureus*, and *Bacillus subtilis*. Inhibition zones were classified as strong against *E. coli* (14.87–17.57 mm) and *S. Typhimurium* (11.77–12.09 mm), and moderate against *S. aureus* (7.25–8.44 mm) and *B. subtilis* (9.79–10.39 mm). These results indicate that antimicrobial activity was influenced not only by flavonoid concentration but also by synergistic effects of other bioactive compounds. The developed coating shows potential as a natural food preservative while supporting circular economy practices through agricultural waste utilization.

Keywords: edible coating, chitosan, OPEFB liquid smoke, neem extract, antimicrobial activity.

INTRODUCTION

Plastic waste has become a major contributor to environmental pollution, affecting terrestrial and aquatic ecosystems. Its pervasive dispersal into the oceans has resulted in serious impacts on human health and the global economy. Worldwide plastic production exceeds 400 million tons annually, equivalent to approximately 11 tons per second or about 68 kg per person per year (Mohajan, 2025). Unfortunately, most plastics are poorly managed due to low recycling rates and unsustainable disposal practices. The food packaging industry is one of the largest contributors to this problem, accounting for nearly 36% of global plastic pollution (Das *et al*., 2025). This issue stems largely from the dominance of petroleum-based plastics, which are non-biodegradable and persist in the environment for extended periods, posing significant challenges to environmental preservation and the transition toward sustainable production systems.

In response, food packaging technology is evolving, with growing emphasis on natural and environmentally friendly materials as alternatives

to synthetic plastics. One promising innovation is the use of edible coatings – thin, consumable films that act as natural preservatives. These coatings protect food products from physical damage, oxidation, and microbial contamination while enhancing their nutritional and functional properties (Pandev *et al*., 2025; Pellegrino et al., 2024). As biodegradable and non-toxic materials, edible coatings offer an innovative and sustainable approach to extending food shelf life and reducing dependence on single-use plastic packaging.

Edible coatings are typically formulated using polysaccharide-based materials such as chitosan, alginate, pectin, carrageenan, and gum (Rostamabadi *et al*., 2024). However, polysaccharide-based coatings alone often exhibit limited effectiveness in preventing chemical and microbiological spoilage (Nunes et al., 2023). Among these materials, chitosan has emerged as a particularly promising biopolymer for edible coatings. Chitosan is a natural polysaccharide derived from the deacetylation of chitin, the primary structural component of crustacean shells such as shrimp, crabs, and other marine organisms (Ali *et al*., 2024). It possesses good film-forming ability, biodegradability, and intrinsic antimicrobial properties, making it a common choice for edible coating formulations (Pan *et al*., 2024). Despite these advantages, the antimicrobial activity of pure chitosan remains modest (Bajer *et al*., 2020). Therefore, enhancing chitosan-based coatings by incorporating additional bioactive agents with antimicrobial and antioxidant functions has become a major research focus. One promising strategy involves the utilization of agricultural by-products as sources of such active compounds.

Indonesia, one of the world's largest palm oil producers, generates substantial amounts of agricultural waste, particularly oil palm empty fruit bunches (OPEFB). Converting this residue into value-added product like liquid smoke supports waste utilization and contributes to achieving Sustainable Development Goals (SDGs). Liquid smoke derived from agricultural biomass – such as durian rinds (Faisal *et al*., 2025), cocoa shells (Desvita *et al*., 2022), and rice husks (Arundina *et al*., 2024) – has shown great potential as an additive in edible coatings to enhance antimicrobial and antioxidant properties. It is typically produced through pyrolysis, a thermal decomposition process that yields a liquid rich in phenolic compounds and organic acids, which are known for their strong antimicrobial and antioxidant activities (Brustolin *et al*., 2024).

In addition to liquid smoke, neem (*Azadirachta indica*) leaf extract has long been recognized for its antimicrobial and antifungal potential, attributed to bioactive compounds such as azadirachtin and nimbin (Acharya, 2025; Singh *et al*., 2024). Neem extract offers a natural, safe, and renewable source of antimicrobial agents that can act synergistically to enhance the biological activity of edible coatings.

Previous studies have reported the development of edible coatings based on various biopolymer systems, including polysaccharides (Fu *et al*., 2025), proteins (Lieu *et al*., 2025), polyphenols (Kaniyamparambil *et al*., 2025), and lipids (Yazicioglu *et al*., 2025). However, to date, no studies have focused on the formulation of chitosan-based edible coatings enriched with liquid smoke and neem extract. Combining chitosan with these natural bioactive ingredients could yield coatings that are not only environmentally friendly but also highly effective in prolonging the shelf life of food products. This combination is expected to improve the functional properties, particularly its ability to inhibit the growth of spoilage-causing microorganisms.

To ensure the feasibility of such a system for food industry applications, a comprehensive understanding of its structural and functional characteristics is essential. Therefore, this study aimed to develop and characterize a chitosan-based edible coating enriched with OPEFB liquid smoke and neem leaf extract. The evaluation included analyses of the coating's physicochemical and antimicrobial properties using Fourier transform infrared spectroscopy (FTIR), X-ray fluorescence (XRF), total flavonoid content (TFC), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), as well as antibacterial assays against Gram-negative (*Escherichia coli, Salmonella typhimurium*) and Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) bacteria. Through this approach, the study seeks to provide an innovative and sustainable solution for food preservation that not only enhances product safety and shelf stability but also contributes to reducing plastic waste and promoting the utilization of agricultural residues.

METHODOLOGY

Pyrolysis for liquid smoke production

Oil palm empty fruit bunches (OPEFB) were dried and cut into small pieces before being subjected to pyrolysis at 300 °C, 340 °C, and 380 °C, following the preparation method of Faisal *et al*. (2025b). The process yielded crude liquid smoke (grade 3), which was subsequently purified by distillation at approximately 190 °C to remove tar components, producing grade 1 liquid smoke suitable for edible coating formulation.

Formulation of the edible coatings

The edible coating was prepared based on the method of Faisal *et al*. (2025) with slight modifications. The formulation consisted of liquid smoke (1%, 2%, and 3% v/v), gelatin (1% w/w), chitosan (1% w/w), and neem leaf extract (2% v/v) dissolved in distilled water to a total volume of 100%. The mixture was homogenized and heated to 70 °C on a hot plate. Neem extract, gelatin, and chitosan were then added gradually with continuous stirring using a magnetic stirrer until the mixture was homogeneous. The resulting solution was stored under sterile conditions until use.

Edible coating evaluation

Characterization

Functional groups in the coatings were identified using Fourier Transform Infrared Spectroscopy (FTIR) (Thermo Scientific Nicolet iS-10). The elemental composition was analyzed using X-Ray Fluorescence (XRF) (Bruker S2 PUMA). Total flavonoid content (TFC) was determined with a UV–Vis spectrophotometer (Shimadzu UV-1800) and expressed as µg of quercetin equivalent (QE) per gram of extract.

Antibacterial activity assay

Antibacterial activity was evaluated using the Kirby-Bauer disk diffusion method, following Faisal *et al*. (2025a) with modifications. The tested microorganisms included the Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Salmonella enterica serovar Typhimurium* ATCC 14028, and the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633. Bacterial suspensions were adjusted to the 0.5 McFarland standard, spread on nutrient agar plates, and overlaid with sterile paper discs containing the edible coating solutions. Positive controls consisted of standard antibiotics: vancomycin (30 µg) for *S. aureus*, gentamicin (10 µg) for *E. coli* and *S. Typhimurium*, and chloramphenicol (30 µg) for *B. subtilis*. Distilled water served as the negative control. After incubation, inhibition zones were measured (in mm) as indicators of antibacterial effectiveness. Data were analyzed using one-way ANOVA, and significant differences among treatments were determined by LSD tests, employing SPSS version 22.

Determination of MIC and MBC

Minimum inhibitory concentration and minimum bactericidal concentration were determined against *S. Typhimurium* using a broth serial dilution method. Gentamicin (10 mg) and distilled water served as positive and negative control, respectively. The MIC was defined as the lowest concentration that completely inhibited visible bacterial growth, and the MBC as the lowest concentration that completely killed bacterial cells, confirmed through total plate count (TPC) analysis.

RESULTS AND DISCUSSION

FTIR analysis

Fourier transform infrared spectroscopy is a widely used analytical technique for identifying functional groups in both pure compounds and complex mixtures. FTIR operates based on the interaction between infrared radiation and chemical bonds within molecules, generating characteristic absorption spectra that reflect the molecular structure and chemical composition of the analyzed sample (Siddique, 2024). In this study, FTIR analysis was performed to evaluate the functional group characteristics of the edible coatings from liquid smoke formulated at different pyrolysis temperatures (300, 340, and 380 °C) and concentrations (1%, 2%, and 3%), as illustrated in Figure 1.

The spectra show that all coatings exhibited broad absorption bands between 3346 and 3362 cm^{-1}, corresponding to –OH stretching vibrations from alcohols and phenols.

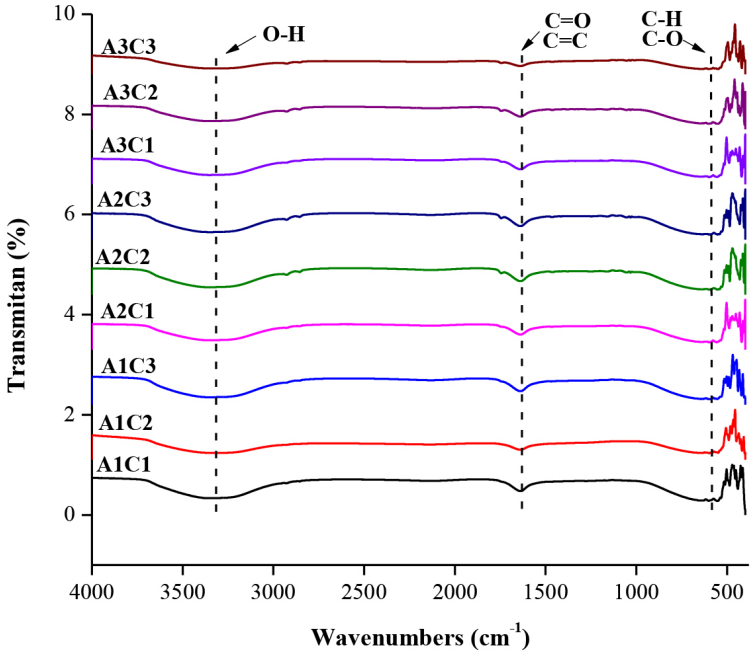

Figure 1. FTIR spectra of edible coatings formulated with liquid smoke at various pyrolysis temperatures and concentrations

This indicates the formation of intermolecular hydrogen bonds among the coating components, which contribute to the film's structural stability. This finding aligns with Shanbhag *et al.* (2023), who reported that absorption in the 3000–3500 cm^{-1} range is characteristic of hydrogen bonding in films composed of hydrophilic polymers such as starch and pectin. Similarly, Paul *et al*. (2018) observed comparable peaks around 3360 cm^{-1} in chitosan-based edible coatings, attributed to –OH (hydroxyl) and –NH (amine) stretching vibrations.

Strong absorption bands were also detected between 1637 and 1640 cm^{-1}, representing the stretching vibrations of amide carbonyl groups (C=O) in gelatin, along with potential contributions from aromatic C=C bonds associated with phenolic compounds in the neem extract. An additional absorption around 598–602 cm^{-1} indicates aromatic C–H out-of-plane bending vibrations. Compared to the spectra reported by Paul *et al.*, (2018), the peaks in this study exhibited higher intensity and spectral complexity, suggesting successful incorporation of bioactive compounds from neem extract and gelatin. These compounds, containing aromatic phenolic and nitrogen-bearing amine groups, are likely responsible for enhancing the antimicrobial and antioxidant properties of the developed edible coating (Patil *et al.*, 2024).

XRF analysis

X-ray fluorescence analysis was conducted to determine the elemental composition of the edible coating formulations. XRF spectrometry detects characteristic X-rays emitted from elements within a sample upon excitation by high-energy X-rays (Vanhoof *et al.*, 2025), allowing qualitative and quantitative identification of chemical elements. The XRF results are presented in Table 1.

As shown in Table 1, twelve elements were identified across the edible coating formulations, with no significant difference among them. The most abundant component was phosphorus pentoxide (P_2O_5) at 30.2%, while potassium (K) had the lowest concentration at 1.3%. Faisal *et al*. (2025b) reported similar findings in chitosan–starch-based coatings derived from durian rind liquid smoke, which contained elements such as chlorine (Cl), calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), silicon (Si), and sulfur (S), with chlorine observed as the element with the highest concentration and potassium as the element with the lowest concentration. The presence of components such as P_2O_5, MgO, and CaO suggests a role in forming cross-links between chitosan polymer chains and phenolic compounds from the neem extract, potentially improving the coating's mechanical strength and structural stability

Table 1. Elemental composition of edible coatings as determined by X-ray fluorescence analysis

Element/ concentration (wt%)	A1C1	A1C2	A1C3	A2C1	A2C2	A2C3	A3C1	A3C2	A3C3
CaO	7.4	8.4	7.3	7.9	8	7.6	7.4	7.7	7.7
Cl	12.4	11.9	12.3	13.2	12.8	12.9	10.7	11.8	11.6
K_2O	2.1	3	1.6	2.2	2.2	2. 2	2.3	2.6	2.6
MgO	23.2	23.3	24.7	24.6	23	23.6	22.7	24.6	23.7
P_2O_5	29.8	29.8	29.6	29.6	30.2	29.6	30.2	29.6	29.8
SiO_2	9.3	9.5	9.4	9.6	9.6	9.1	9.3	9.7	9.4
Ca	5.3	6	5.2	5.6	5.7	5.5	5.3	5.5	5.5
K	1.7	2.5	1.3	1.8	1.8	1.8	1.9	2.2	2.1
Mg	14	14	14.9	14.8	13.9	14.2	13.7	14.9	14.3
P	13	13	12.9	12.9	13.2	12.9	12.9	13.1	13
S	6.3	5.7	6	5.2	5.7	6	7	5.5	6.1
Si	4.3	4.4	4.4	4.5	4.5	4.3	4.4	4.5	4.4

(Wang *et al*., 2020). Meanwhile, ions like Cl and K may contribute to antimicrobial activity by disrupting osmotic balance and cellular membrane permeability in microorganisms. These findings were in agreement with Milinović *et al*. (2022), who characterized the macro mineral composition of macroalgal biomass using ED-XRF analysis. They demonstrated that macronutrient elements such as P, Mg, Ca, and K in macroalgal biomass contribute to both structural reinforcement and biological protection against microbial pathogens.

Total flavonoid content

Flavonoids are plant-derived bioactive compounds with potent antioxidant and antimicrobial properties, capable of scavenging free radicals by donating electrons and thereby stabilizing reactive species. In particular, flavonoids in liquid smoke have been reported to inhibit the growth of microorganisms in meat and meat-based processed products (Yan *et al.*, 2024). The TFC results for the coating formulations are presented in Table 2.

As shown in Table 2, flavonoids were detected in all samples, with concentrations ranging from 16.05 to 146.25 µg QE/g extract. The highest TFC was observed in formulation A2C1 (pyrolysis temperature 340 °C, 1% liquid smoke, 2% neem extract), yielding 146.25 µg QE/g extract, while the lowest was in A1C3 (300 °C, 3%) at 16.05 µg QE/g extract. Overall, the 340 °C pyrolysis condition yielded the highest flavonoid content, particularly at lower liquid smoke concentrations. This suggests that 340 °C optimizes the release of flavonoids from the biomass without excessive thermal degradation, whereas lower temperatures (300 °C) are insufficient for complete release and higher temperatures (380°C) promote degradation due to thermal instability (Lin and Xiao, 2024).

The flavonoid levels in these formulations were lower than those reported for pure neem extract, where methanolic extracts of neem leaves can yield up to 85.05 mg QE/100 g, and root bark and seed extracts reached 98.00 mg QE/100 g (Kiranmai *et al.*, 2012). The relatively lower values here likely result from chemical interactions among coating components that may have altered flavonoid structures, specifically the binding of flavonoids within the chitosan–gelatin matrix, which may change their structure

Table 2. Total flavonoid content of edible coatings formulated at different liquid smoke pyrolysis temperatures and concentrations

Sample	Total flavonoid (µg QE/g extract)
A1C1	48.87
A1C2	70.52
A1C3	16.05
A2C1	146.25
A2C2	19.40
A2C3	50.58
A3C1	33.85
A3C2	36.28
A3C3	26.11

or make them less detectable. Chitosan carries a positive charge under acidic–neutral conditions and contains reactive amino and hydroxyl groups capable of interacting with phenolic structures (Ding et al., 2019; Mamand et al., 2025; Fierri et al., 2024), while gelatin is known to bind polyphenols through hydrogen bonding and protein–polyphenol complexation (Feng et al., 2023; Poungchawanwong et al., 2020). These interactions promote hydrogen bonding and ion–dipole complex formation (Fierri et al., 2024; Wang et al., 2025), which can lead to the binding or entrapment of flavonoids within the chitosan-gelatin network. Such matrix effects may alter flavonoid structures or reduce their extractability and, consequently, their detectability in colorimetric assays.

At previous study has widely shown interactions between chitosan-gelatin and flavonoid (via hydrogen bonding and electrostatic interactions) and in certain instances, covalent cross-linking through Schiff base reactions between amino groups and carbonyl donors (Zhang et al, 2023; Dellali et al., 2021). Although these interactions can stabilize the polymer matrix but may also reduce the availability or release of the bioactive compounds embedded in it (Qiao et al., 2017). Similarly, Zaidi *et al.*, (2023) reported a much higher TFC (583.72 mg QE/100 g fresh weight) in a ginger-arabic gum coatings applied to "Surahi" guava fruit, highlighting methodological differences: TFC in their study was measured in coated fruit tissue, while in this work, it was determined in the coating solution prior to application.

MIC and MBC

The antibacterial activity of the coatings was further evaluated using MIC and MBC assays against *S. typhimurium*. The MIC represents the lowest concentration of an antimicrobial agent that inhibits bacterial growth, while MBC is the minimum concentration that completely eliminates viable bacterial cells (Krochmal and Wicher, 2021; Tao *et al.*, 2022). The results are presented in Tables 3 and 4.

The MIC results (Table 3) showed that the edible coating containing 1% liquid smoke prevented visible growth at all three pyrolysis temperatures. However, media containing 2% and 3% liquid smoke became turbid, indicating a loss of inhibitory activity. This observation was consistent with the MBC results (Table 4), where the number of microbial colonies was markedly higher at the 2% and 3% concentrations. Therefore, both the MIC and MBC were determined to be at the 1% liquid smoke concentration. These findings indicate that increasing the concentration of liquid smoke does not linearly enhance antimicrobial activity and may even reduce it. This phenomenon may be attributed to the Eagle effect, or paradoxical effect, first described by Eagle, (1948) in studies on penicillin, where higher concentrations of an antimicrobial agent led to reduced efficacy. A similar observation was reported by Wu *et al*. (2015), who found that high concentrations of fluoroquinolone decreased bactericidal effectiveness against *Mycrobacterium smegmatis*.

Table 3. Minimum inhibitory concentration of edible coatings against *S. Typhimurium*

	A1	A2	A3
C1	Clear	Clear	Clear
C2	Turbid +	Clear	Turbid+
C3	Turbid ++	Turbid +	Turbid +++
K+	Clear	Clear	Clear
K-	Turbid +++	Turbid +++	Turbid +++

Table 4. Minimum bactericidal concentration of edible coatings against *S. typhimurium*

Replicate	A1			A2			A3			K+	K-
	C1	C2	C3	C1	C2	C3	C1	C2	C3		
1	2	6	22	1	5	10	0	14	22	0	>500
2	1	10	46	1	5	50	0	12	50		

Note: Values are means ± standard deviation (n = 3). Means within the same column followed by the same letter are not significantly different according to Tukey's test at $P \leq 0.05$.

The present findings contrast with those reported byMunira and Nasir (2023), who investigated the antibacterial activity of *Chromolaena odorata* (kirinyuh) leaf extract. Their study demonstrated an MIC of 5% and an MBC of 7% against *S. aureus*, with bacterial colony numbers decreasing proportionally with increasing extract concentration. The discrepancy observed in this study is likely due to the complex composition of liquid smoke and neem extract, which contain diverse active compounds such as phenols, carbonyls, and organic acids. At lower concentrations, these constituents may have synergistic antimicrobial effect, but at higher concentrations, antagonistic interactions may occur, diminishing overall efficacy. This interpretation aligns with Vaou *et al.* (2022), who emphasized that mixtures of plant-derived bioactive compounds do not always act synergistically as certain combinations may instead exert antagonistic effects that reduce overall antimicrobial performance.

Antibacterial activity of edible coating solutions

Antibacterial activity was assessed against two Gram-negative (*E. coli, S. typhimurium*) and two Gram-positive (*S. aureus, B. subtilis*) bacteria commonly associated with food spoilage. Activity was indicated by clear inhibition zones around treated paper discs impregnated with the coating solutions. The inhibition zone diameters are shown in Figure 2, and the detailed patterns for each bacterium are illustrated in Figures 3–6.

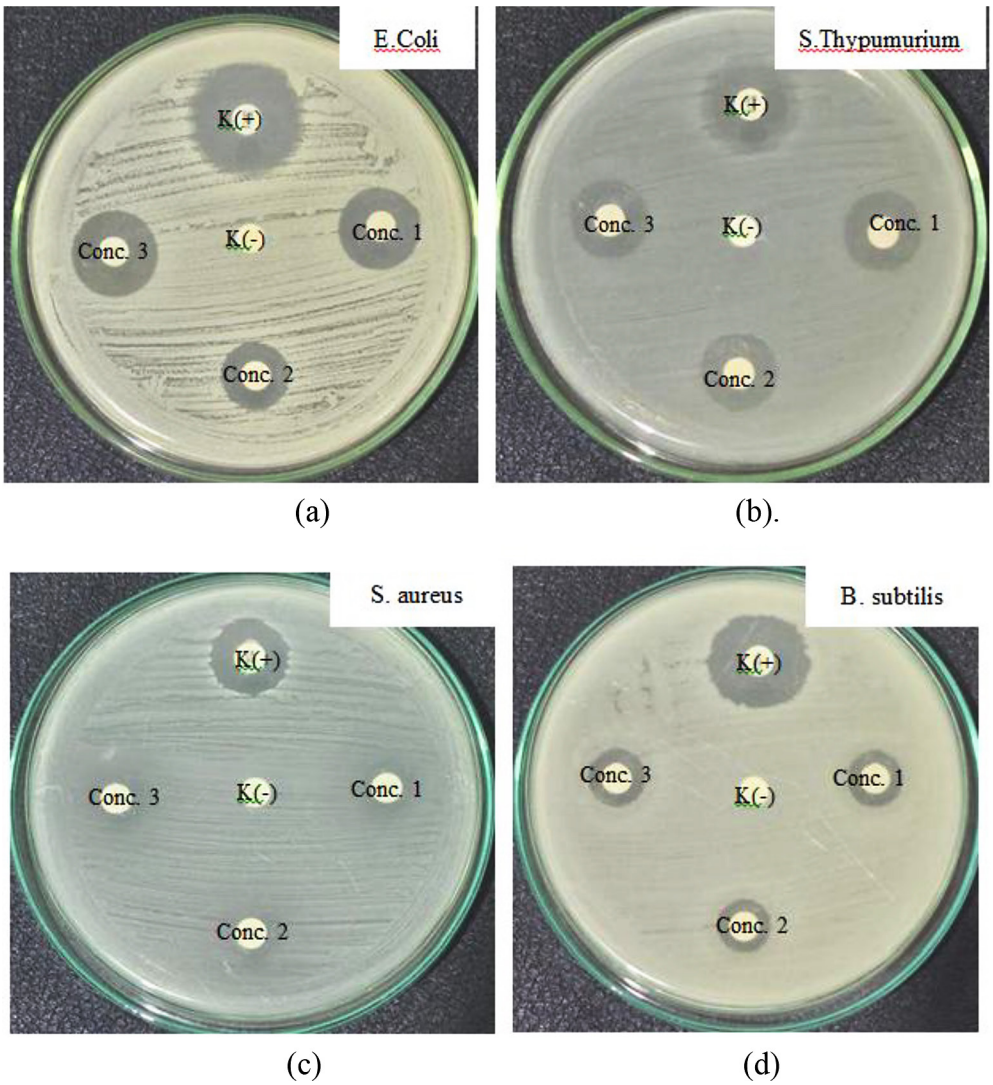

Figure 2. Comparison for antibacterial test results of edible coatings against (a) *S. typhimurium;* (b) *E. coli*; (c) *S. aereus*; and (d) *B. subtilis*

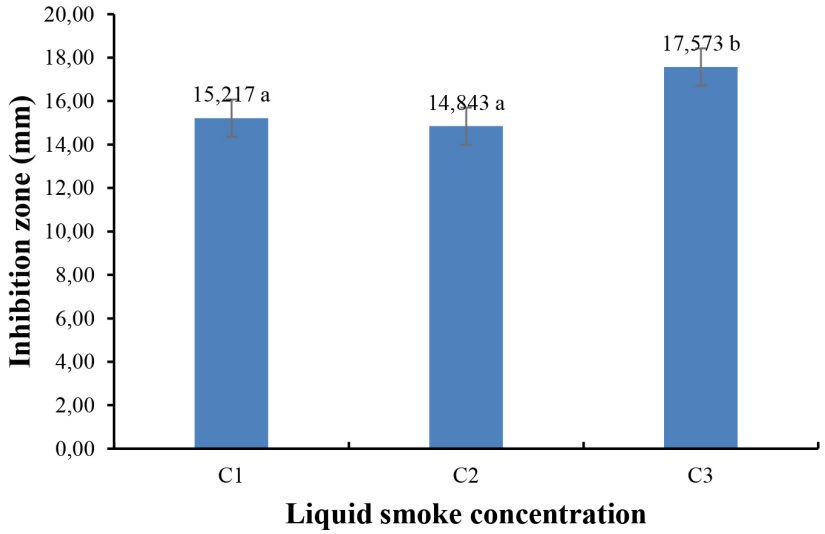

Figure 3. Antibacterial inhibition against *E. coli*

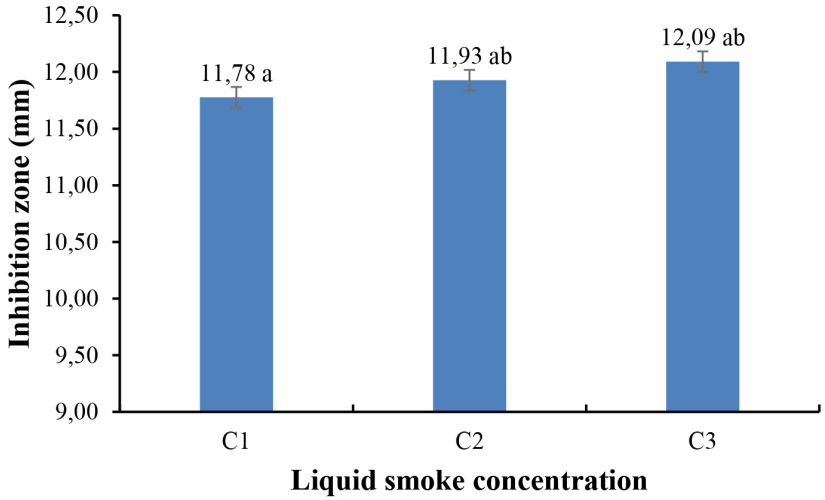

Figure 4. Antibacterial inhibition against *S. typhimurium*

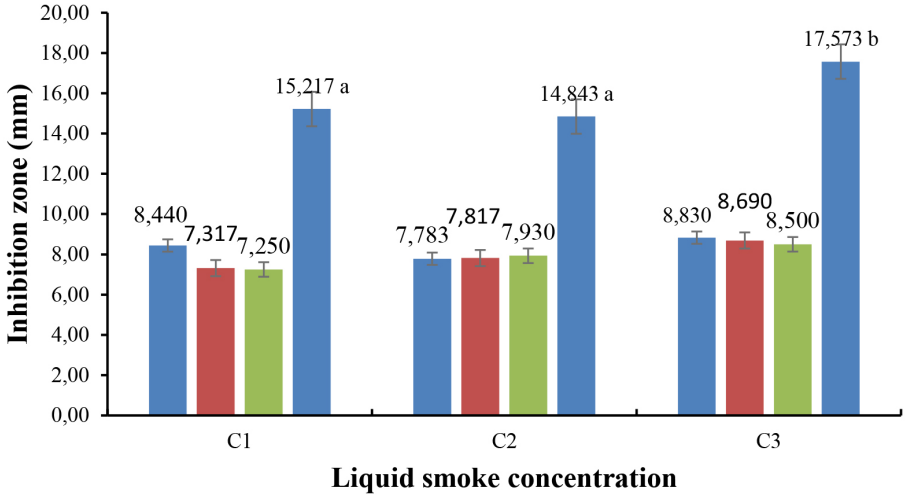

Figure 5. Antibacterial inhibition against *S. aureus*

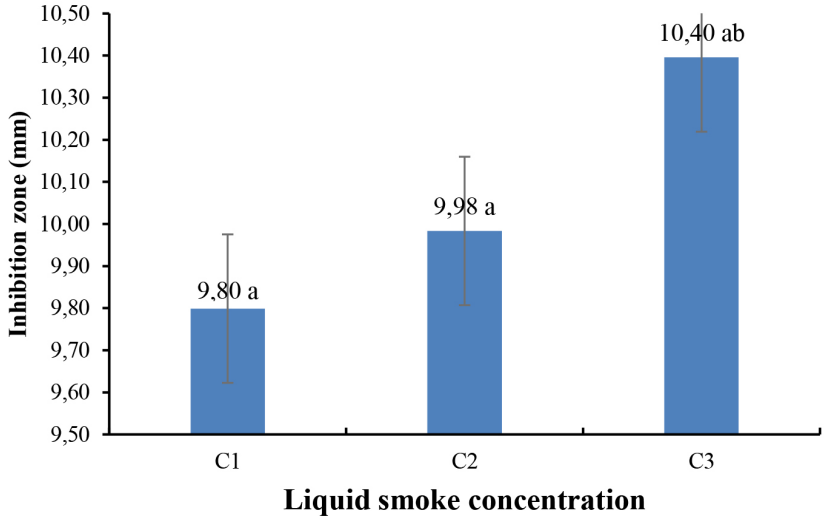

Figure 6. Antibacterial inhibition against *B. subtilis*

As shown in Figures 3–6, the four tested bacteria exhibited different sensitivities to the edible coating formulations. The diameter of the inhibition zones was generally proportional to the pyrolysis temperature and the concentration of liquid smoke, chitosan concentration, and neem extract. This relationship likely reflects the increasing levels of phenolic, carbonyl, and organic acid compounds from the liquid smoke, combined with bioactive compounds from the neem extract, which collectively enhance antibacterial efficacy (El-Beltagi *et al.*, 2024).

Based on the classification by Liah *et al.* (2023), where >20 mm indicates very strong inhibition, 11–20 mm strong, 6–10 mm moderate, and 5 mm weak, the coatings exhibited strong activity against *E. coli* (14.87–17.57 mm, Figure 3) and *S. typhimurium* (11.77–12.09 mm, Figure 4). In contrast, moderate inhibition was observed against *S. aureus* (7.25–8.44 mm, Figure 5) and *B. subtilis* (9.79–10.39 mm, Figure 6).

These results are consistent with Desvita *et al.* (2023), who found that edible coatings formulated with rice husk liquid smoke and chitosan were more effective against Gram-negative bacteria. The greater susceptibility of Gram-negative bacteria may be attributed to phenolic compounds disrupting the outer membrane, increasing permeability, and causing leakage of intracellular components. Li and Zhuang (2020) also noted that, beyond differences in Gram-type, coating viscosity influences efficacy, as low-viscosity coatings may not form a uniform barrier, potentially explaining the smaller zones for *S. aureus*. Furthermore, neem extract enhances antibacterial potential through its phenolic, flavonoid, and limonoid constituents, which disrupt bacterial cell membrane integrity, leading to protein and nucleic acid leakage and ultimately cell lysis (Putsakum et al., 2018).

CONCLUSIONS

The chitosan-based edible coating enriched with oil palm empty fruit bunches liquid smoke and neem leaf extract demonstrates strong potential as an eco-friendly natural preservative with effective antimicrobial activity. The combination exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. FTIR characterization revealed the presence of –OH, –NH, and aromatic functional groups, indicating hydrogen bonding and the successful incorporation of bioactive compounds from neem and gelatin, which enhanced structural integrity and antimicrobial and antioxidant capacity. The coating formulated with 1% liquid smoke produced at 340 °C exhibited the highest flavonoid content and optimal antimicrobial performance, whereas higher liquid smoke concentrations showed an antagonistic effect, likely due to the Eagle effect. Utilizing these agricultural waste materials supports circular economy principles, and the coating developed in this study shows promise for industrial-scale application. Further research is recommended

to evaluate its performance on various food matrices, its compatibility with different products, and its stability during storage under diverse environmental conditions.

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REFERENCES

1. Acharya, S.K. (2025). Neem leaf extract in wound healing: mechanisms and efficacy. *International Journal of Sciences and Innovation Engineering*, *2*(5), 822–830.
2. Ali, G., Sharma, M., Salama, E.S., Ling, Z., & Li, X. (2024). Applications of chitin and chitosan as natural biopolymer: potential sources, pretreatments, and degradation pathways. *Biomass Conversion and Biorefinery*, *14*(4), 4567–4581.
3. Arundina, I., Frimayanti, N., Surboyo, M.D.C., Budhy, T.I., & Iskandar, B. (2024). 6-Octadecenoic and oleic acid in liquid smoke rice husk showed COVID-19 inhibitor properties. *Advances in pharmacological and pharmaceutical sciences*, *2024*(1), 8105595.
4. Bajer, D., Janczak, K., & Bajer, K. (2020). Novel starch/chitosan/aloe vera composites as promising biopackaging materials. *Journal of Polymers and the Environment*, *28*(3), 1021–1039.
5. Brustolin, A.P., Soares, J.M., Muraro, K., Schwert, R., Steffens, C., Cansian, R.L., & Valduga, E. (2024). Investigating antimicrobial and antioxidant activity of liquid smoke and physical-chemical stability of bacon subjected to liquid smoke and conventional smoking. *Journal of Food Science*, *89*(11), 7217–7227.
6. Desvita, H., Faisal, M., Mahidin., & Suhendrayatna. (2022). Antimicrobial potential of wood vinegar from cocoa pod shells (Theobroma cacao L.) against Candida albicans and Aspergillus niger. *Materials Today: Proceedings*, *63*, S210–S213.
7. Desvita, H., Faisal, M., & Mahidin, M. (2023). Natural antimicrobial properties of liquid smoke derived from cocoa pod shells in meatball preservation. *South African Journal of Chemical Engineering*, *46*(1), 106–111.
8. Desvita, H., Faisal, M., Mahidin., & Suhendrayatna. (2025). Optimization of wood vinegar from cacao pod shells by response surface methodology. *Baghdad Science Journal*, *22*(3), 822–829.
9. Delmondes, P.H., & Stefani, R. (2016). Computational study of natural phenolic acid solubility and their interactions with chitosan. *MOL2NET.*
10. Dellali, M., Iurciuc, C.E., Savin, C.L., Spahis, N., Djennad, M.H., & Popa, M. (2021). Hydrogel films based on chitosan and oxidized carboxymethylcellulose optimized for the controlled release of curcumin with applications in treating dermatological conditions. *Molecules*, *26*(8), 2185.
11. Ding, L., Huang, Y., Cai, X., & Wang, S. (2019). Impact of pH, ionic strength and chitosan charge density on chitosan/casein complexation and phase behavior. *Carbohydrate Polymers*, *208*, 133–141.
12. Eagle, H. (1948). The Classic: aminopterin in the treatment of acute leukemia in children. *Ca: A Cancer Journal For Clinicians*, 55(5), 99–131. https://doi.org/10.3322/Canjclin.55.5.343.
13. El-Beltagi, H.S., Ragab, M., Osman, A., El-Masry, R.A., Alwutayd, K.M., Althagafi, H.,... & El-Saber, M.M. (2024). Biosynthesis of zinc oxide nanoparticles via neem extract and their anticancer and antibacterial activities. *PeerJ*, *12*, e17588.
14. Faisal, M., Kamaruzzaman, S., & Desvita, H. (2025a). Natural meatball preservative using edible coating from modified shrimp shells chitosan and durian skin liquid smoke. *South African Journal of Chemical Engineering*.
15. Faisal, M., Abubakar, Y., Heriansyah, M.B., & Desvita, H. (2025b). Application of edible coating based on durian seed starch and peel liquid smoke as natural preservatives for tomatoes (Solanum Lycopersicum L.). *South African Journal of Chemical Engineering*.
16. Feng, Y., Jin, C., Lv, S., Zhang, H., Ren, F., & Wang, J. (2023). Molecular mechanisms and applications of polyphenol-protein complexes with antioxidant properties: A review. *Antioxidants*, *12*(8), 1577.
17. Fierri, I., Chignola, R., Stranieri, C., Di Leo, E. G., Bellumori, M., Roncoletta, S.,... & Zoccatelli, G. (2024). Formulation, characterization, and antioxidant properties of chitosan nanoparticles containing phenolic compounds from olive pomace. *Antioxidants*, *13*(12), 1522.
18. Kiranmai, M., Ushasri, B., Mohammed Ibrahim, M.I., & Kumar, C.B.M. (2012). Antioxidant activity and total flavonoids content of different parts of Azadirachta indica A. Juss.
19. Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. *Pathogens*, *10*(2), 165.

20. Liah, J. L., & Rauf, R. F. (2023). The Effect of additional lemongrass extract (Cymbopogon Nardus L.) on the characteristics of edible film sodium alginate and arabic gum. *Formosa Journal of Science and Technology*, *2*(8), 2055–2068.
21. Lin, S., & Xiao, J. (2024). Impact of thermal processing on dietary flavonoids. *Advances in Food and Nutrition Research*, *108*, 1–34.
22. Li, J., & Zhuang, S. (2020). Antibacterial activity of chitosan and its derivatives and their interaction mechanism with bacteria: Current state and perspectives. *European Polymer Journal*, *138*, 109984.
23. Mamand, D.M., Hussen, S.A., & Aziz, S.B. (2025). Green approach to synthesis polymer composites based on chitosan with desired linear and non-linear optical characteristics. *Scientific Reports*, *15*(1), 3130.
24. Munira, M., & Nasir, M. (2023). Uji Kadar Hambat Minimum (KHM) dan Kadar Bunuh Minimum (KBM) ekstrak daun kirinyuh (Chromolaena odorata) dari geothermal Ie Seum Aceh Besar terhadap Staphylococcus aureus. *Jurnal SAGO Gizi Dan Kesehatan*, *4*(2), 179–185.
25. Milinovic, J., Figueiredo, F.M., Noronha, J.P., & Sardinha, J. (2022). Application of ED-XRF spectra for determination of macroelements in edible seaweeds. *Journal of Food Composition and Analysis*, *110*, 104559.
26. Mohanasundaram, P., & Antoneyraj, M. (2025). A systematic review of neem flower (Azadirachta indica): a promising source of bioactive compounds with pharmacological and immunomodulating properties. *Tradit Med Res*, *10*(7), 41.
27. Nunes, C., Silva, M., Farinha, D., Sales, H., Pontes, R., & Nunes, J. (2023). Edible coatings and future trends in active food packaging–fruits' and traditional sausages' shelf life increasing. Foods, 12(17), 1–23. http://doi.org/10.3390/foods12173308
28. Pan, Q., Ma, H., Liu, Z., Pan, C., Zuo, X., Cheng, S.,... & Guo, A. (2024). Carboxymethyl chitosan: Synthesis, functional properties, and applications in sustainable food packaging material. *Comprehensive Reviews in Food Science and Food Safety*, *23*(6), e70061.
29. Pandey, V.K., Shafi, Z., Choudhary, P., Pathak, A., & Rustagi, S. (2025). Navigating the functional and bioactive landscape of sodium alginate-based edible coatings for modernized fruit preservation. *Journal of Food Safety*, *45*(3), e70020.
30. Paul, S.K., Sarkar, S., Sethi, L.N., & Ghosh, S.K. (2018). Development of chitosan based optimized edible coating for tomato (Solanum lycopersicum) and its characterization. *Journal of food science and technology*, *55*(7), 2446–2456.
31. Patil, D.K., Bornane, M.D., Borse, K.P., Pardhesi, P.K., *et al.* (2024). phytochemical composition and antibacterial/antimicrobial properties of Azadirachta Indica (Neem). *International Journal of Pharmaceutical Research and Applications*. 9(3), 283–298.
32. Pellegrino, M., Elechi, J.O.G., Plastina, P., & Loizzo, M.R. (2024). Application of natural edible coating to enhance the shelf life of red fruits and their bioactive content. *Applied Sciences*, *14*(11), 4552.
33. Poungchawanwong, S., Klaypradit, W., Li, Q., Wang, J., & Hou, H. (2020). Interaction effect of phenolic compounds on Alaska Pollock skin gelatin and associated changes. *Lwt*, *133*, 110018.
34. Putsakum, G., Lee, D. S., Suthiluk, P., & Rawdkuen, S. (2018). The properties of gelatin film-neem extract and its effectiveness for preserving minced beef. *Packaging Technology and Science*, *31*(9), 611–620.
35. Qiao, C., Ma, X., Zhang, J., & Yao, J. (2017). Molecular interactions in gelatin/chitosan composite films. *Food chemistry*, *235*, 45–50.
36. Rostamabadi, H., Demirkesen, I., Colussi, R., Roy, S., Tabassum, N., de Oliveira Filho, J.G.,... & Falsafi, S.R. (2024). Recent trends in the application of films and coatings based on starch, cellulose, chitin, chitosan, xanthan, gellan, pullulan, Arabic gum, alginate, pectin, and carrageenan in food packaging. *Food Frontiers*, *5*(2), 350–391.
37. Siddique, I. (2024). Exploring functional groups and molecular structures: a comprehensive analysis using FTIR spectroscopy. *Chemistry Research Journal*, *9*(2), 70–76.
38. Shanbhag, C., Shenoy, R., Shetty, P., Srinivasulu, M., & Nayak, R. (2023). Formulation and characterization of starch-based novel biodegradable edible films for food packaging. *Journal of Food Science and Technology*, *60*(11), 2858–2867.
39. Singh, A., Jaiswal, A., Yadav, A., Mandal, S., & Sahoo, J. P. (2024). Nature's pharmacy: exploring potential therapeutic roles of neem (Azadirachta indica L.). *Vigyan Varta*, *5*, 149–54.
40. Tao, J.J., Xiang, J.J., Jiang, M., Kuang, S.F., Peng, X.X., & Li, H. (2022). A microtitre plate dilution method for minimum killing concentration is developed to evaluate metabolites-enabled killing of bacteria by β-lactam antibiotics. *Frontiers in Molecular Biosciences*, *9*, 878651.
41. Vanhoof, C., Bacon, J.R., Fittschen, U.E., & Vincze, L. (2021). Atomic spectrometry update–a review of advances in X-ray fluorescence spectrometry and its special applications. *Journal of Analytical Atomic Spectrometry*, *36*(9), 1797–1812.
42. Vaou, N., Stavropoulou, E., Voidarou, C., Tsakris, Z., Rozos, G., Tsigalou, C., & Bezirtzoglou, E. (2022). Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. *Antibiotics*, *11*(8), 1014.
43. Wang, W., Xue, C., & Mao, X. (2020). Chitosan:

Structural modification, biological activity and application. *International Journal of Biological Macromolecules*, *164*, 4532–4546.

44. Wang, J., Chen, J., Jiang, Y., Yang, B., & Wen, L. (2025). Effect of phenolic hydrogen on the formation of chitosan-prenylated flavonoids nanocomplexes. *Food Hydrocolloids*, 111523.
45. Wu, M. L., Tan, J., & Dick, T. (2015). Eagle effect in nonreplicating persister mycobacteria. *Antimicrobial agents and chemotherapy*, *59*(12), 7786–7789.
46. Yan, J., He, S., Chen, L., Chen, H., Ouyang, K., & Wang, W. (2024). Effect of gelatin-chitosan-Cyclocarya paliurus flavonoids edible coating film on the preservation of chilled beef. *LWT-Food Science and Technology*, *199*.
47. Zaidi, M., Akbar, A., Ali, S., Akram, H., Ercisli, S., Ilhan, G.,... & Anjum, M.A. (2023). Application of plant-based edible coatings and extracts influences the postharvest quality and shelf life potential of "Surahi" guava fruits. *ACS omega*, *8*(22), 19523–19531.
48. Zhang, C., Wang, J., Du, H., Han, X., Li, C., Huang, D., & Yu, X. (2023). Antioxidant, and physical properties of chitosan/gelatin composite film incorporated with Caulerpa lentillifera extract for pork preservation. *International Journal of Food Science & Technology*, *58*(3), 1474–1486.