DEVELOPMENT OF CHITOSAN-BASED ANTIBACTERIAL BIOPLASTIC FROM SHRIMP SHELL WASTE WITH THE ADDITION OF PATCHOULI OIL (*Pogostemon cablin Benth*)

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Abstract: Plastic waste pollution remains a critical global issue, with fossil-based plastics contributing significantly to environmental damage. Bioplastics derived from organic residues are a promising alternative for sustainable packaging. In this study, shrimp shell waste was converted into chitosan and combined with patchouli essential oil (PEO) and cellulose nanocrystals (CNC) from corn cob waste to develop antibacterial bioplastic films. PEO was incorporated using both Tween-80 and CNC-stabilized Pickering emulsions at different oil concentrations (20%, 30%, and 40%). The fabricated films were characterized for thickness, tensile strength, elongation, water solubility, moisture content, biodegradability, antibacterial activity, and morphology. Results showed that Tween-80 stabilized films exhibited the highest tensile strength (6.32 MPa), whereas CNC-based Pickering emulsions improved flexibility and antibacterial properties. Increasing PEO content in Pickering emulsions enhanced antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with the C_2E_{40} film achieving the largest inhibition zones (22.00 mm and 17.92 mm, respectively). Films containing CNC also displayed reduced moisture content and improved biodegradability, with up to 74.03% degradation in soil burial tests. SEM analysis confirmed more porous structures with increasing PEO concentration, particularly in Pickering emulsion films. Overall, CNC-stabilized emulsions effectively enhanced the incorporation and release of PEO, producing bioplastic films with improved antibacterial properties and biodegradability for potential food packaging applications.
Keywords: chitosan; patchouli oil; antibacterial bioplastic

Abstrak: Pencemaran limbah plastik masih menjadi isu global yang krusial, di mana plastik berbasis fosil memberikan kontribusi besar terhadap kerusakan lingkungan. Bioplastik yang berasal dari residu organik menjadi salah satu alternatif berkelanjutan untuk kemasan ramah lingkungan. Pada penelitian ini, limbah cangkang udang diolah menjadi kitosan dan dikombinasikan dengan minyak nilam (patchouli essential oil/PEO) serta nanokristal selulosa (CNC) dari limbah tongkol jagung untuk menghasilkan film bioplastik antibakteri. PEO diinkorporasikan menggunakan dua metode, yaitu emulsi dengan Tween-80 dan emulsi Pickering yang distabilkan oleh CNC pada berbagai konsentrasi minyak (20%, 30%, dan 40%). Film yang dihasilkan dikarakterisasi

berdasarkan ketebalan, kekuatan tarik, elongasi, kelarutan air, kadar air, biodegradabilitas, aktivitas antibakteri, dan morfologi. Hasil menunjukkan bahwa film dengan emulsi Tween-80 memiliki kekuatan tarik tertinggi (6,32 MPa), sedangkan emulsi Pickering berbasis CNC meningkatkan fleksibilitas dan aktivitas antibakteri. Peningkatan konsentrasi PEO pada emulsi Pickering memperkuat aktivitas antibakteri terhadap *Staphylococcus aureus* dan *Escherichia coli*, dengan film C2E40 menghasilkan zona hambat terbesar (22,00 mm dan 17,92 mm). Penambahan CNC juga menurunkan kadar air dan meningkatkan biodegradabilitas hingga 74,03% pada uji tanam tanah. Analisis SEM menunjukkan struktur lebih berpori seiring meningkatnya konsentrasi PEO. Secara keseluruhan, emulsi Pickering yang distabilkan CNC efektif meningkatkan distribusi dan pelepasan PEO, menghasilkan film bioplastik dengan sifat antibakteri dan biodegradabilitas yang lebih baik untuk aplikasi kemasan pangan.
Kata kunci: kitosan; minyak nilam; bioplastik antibakteri

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Introduction

Global plastic waste pollution is a critical environmental issue, with fossil-based plastic production reaching 400.3 million tonnes in 2022 (Dhali et al., 2024). Proposed solutions include microbial degradation (Perez et al., 2023), recycling (Alaghemandi, 2024), and biodegradable alternatives such as bioplastics (Flury & Narayan, 2021); (Ahsan et al., 2023). Shrimp shell waste is a promising bioplastic feedstock due to its high chitin content, which can be deacetylated to yield chitosan (Teixeira-costa, 2021). However, chitosan films have inherent limitations in water resistance and antimicrobial activity, driving research into incorporating additives to enhance their food packaging performance.

Patchouli essential oil (PEO; *Pogostemon cablin Benth*) is a promising antibacterial additive for chitosan-based packaging (Bakar et al., 2022). Its key bioactive compounds patchouli alcohol, patchoulene, and pogostone inhibit S. aureus at MIC as low as 0.25 µL/mL (Pandey et al., 2022); (Szewczuk et al., 2023). However, PEO's hydrophobic nature hinders uniform incorporation into hydrophilic chitosan matrices, necessitating an appropriate emulsification strategy. Pickering emulsions stabilized by cellulose nanocrystals (CNC) from corn cobs offer a sustainable solution, as CNC possesses a high aspect ratio, excellent barrier properties, and mechanical strength that reinforces biopolymer films (Amara et al., 2018); (Bahsaine et al., 2023). CNC can irreversibly adsorb at oil-water interfaces to form highly stable emulsions, enhancing dispersion of hydrophobic compounds (Ji & Wang, 2023a).

Recent developments have also emphasized the importance of valorizing underutilized natural fibers and agricultural by-products in composite material production. For instance, (Pardi et al., 2024) demonstrated that purun fiber

(*Eleocharis dulcis*) can be effectively incorporated into polyester composites, significantly improving their mechanical, morphological, and thermal properties. This underscores the broader potential of utilizing abundant local biomaterials not only to enhance material performance but also to contribute to sustainable waste management strategies. Previous studies have reported the incorporation of various essential oils into chitosan-based films to enhance antibacterial activity and improve food preservation performance (Choo et al., 2021; Zhang et al., 2021). In addition, CNC have been widely explored both as reinforcing agents in biopolymer films and as Pickering emulsion stabilizers for hydrophobic bioactive compounds (Ji & Wang, 2023b). However, most reported systems mainly utilize commonly studied essential oils such as oregano, thyme, and cinnamon oil, while studies involving patchouli essential oil remain very limited (Pavlátková et al., 2023). Furthermore, conventional emulsification approaches often rely on surfactantsm that may reduce emulsion stability and compromise the environmental sustainability of the resulting materials.

In this study, Tween 80-stabilized emulsion was employed as a reference system to compare with the CNC-stabilized Pickering emulsion. Tween 80 is one of the most commonly used surfactants in essential oil emulsification due to its ability to reduce interfacial tension and facilitate oil dispersion in hydrophilic polymer matrices (Guzmán-Pincheira et al., 2025). However, surfactant-based emulsions may exhibit limited long-term stability and lower environmental sustainability compared to particle-stabilized systems. Therefore, the comparison between Tween 80 and CNC-stabilized Pickering emulsions was intended to evaluate the effectiveness of CNC as a sustainable stabilizing agent for improving essential oil dispersion and enhancing the functional performance of chitosan-based bioplastic films.

In the present formulation, each component was designed to perform a specific role within the bioplastic system. Chitosan served as the primary biodegradable polymer matrix, while patchouli essential oil (PEO) functioned as the active antibacterial agent. CNC were incorporated not only as reinforcing nanofillers to improve film stability, but also as solid particles for stabilizing Pickering emulsions. Meanwhile, Tween 80 was used as a conventional surfactant reference to compare its emulsification behavior with the CNC-stabilized system. This formulation design allowed the evaluation of how different stabilization approaches influence the mechanical, structural, and antibacterial properties of the resulting bioplastic films.

Although several studies have separately investigated chitosan-essential oil systems and CNC-stabilized Pickering emulsions (Zhang et al., 2021), limited attention has been given to the simultaneous integration of shrimp shell-derived chitosan, corn cob-derived CNC, and patchouli essential oil within a single sustainable bioplastic matrix. To date, the incorporation of patchouli essential oil into chitosan matrices using a CNC-stabilized Pickering emulsion from corn cob

biomass has not been reported. Therefore, this study proposes a novel strategy for developing chitosan-based bioplastic films incorporating patchouli essential oil through a CNC-stabilized Pickering emulsion approach. This novel approach not only ensures better oil distribution and enhanced antibacterial activity but also contributes to agricultural waste valorization, offering a sustainable advancement in bioplastic development for food packaging applications.

Methodology

Materials

Shrimp shells and corn cobs were obtained from local markets and agricultural waste in Banda Aceh. PEO was purchased from a local distillation facility. S. aureus (ATCC 25923) and E. coli (ATCC 25922) were used for antibacterial tests. Analytical-grade chemicals included HCl, NaOH, H_2SO_4, $NaClO_2$, acetic acid, and Tween 80. Mueller-Hinton Agar, physiological saline, and EM4 solution were used for antibacterial and biodegradability tests.

Preparation of chitosan

Shrimp shells were cleaned, dried, and ground into powder. The demineralization process was conducted using 1 M HCl, followed by deproteinization with 1 M NaOH. The chitin obtained was then deacetylated using 50% NaOH at 90 °C for 4 hours to produce chitosan. The resulting chitosan was washed and dried for further use.

Isolation of cellulose and production of CNC

Corn cobs were washed, dried, and ground. The powder was subjected to alkali treatment with 4% NaOH, followed by bleaching using a mixture of $NaClO_2$ and acetic acid. The cellulose was hydrolyzed with 65% H_2SO_4 at 45 °C for 1 hour under stirring to obtain CNC. The suspension was washed repeatedly via centrifugation and dialyzed until neutral.

Preparation of pickering emulsion

Two emulsification systems were prepared: (1) Tween 80-stabilized emulsion (C_0E_{30}) as a conventional reference, and (2) CNC-stabilized Pickering emulsions (C_2E_{20}, C_2E_{30}, and C_2E_{40}) at varying PEO concentrations (20%, 30%, and 40%), to compare sustainable CNC stabilization against traditional surfactant-based approaches.

Film fabrication

Films were prepared by mixing 28.5 mL chitosan solution with 1.5 mL of emulsion or additive. Formulations were coded: C_0E_0 (chitosan only), C_0E_{30} (chitosan + Tween-80-PEO emulsion), C_2E_0 (chitosan + 2% CNC), and C_2E_{20},

C_2E_{30}, C_2E_{40} (chitosan + CNC Pickering emulsions at 20%, 30%, and 40% PEO). Mixtures were cast in petri dishes and dried under controlled conditions.

Characterization

C_0E_{30} was completely water-soluble, as Tween-80 is highly hydrophilic and causes rapid film disintegration. C_0E_0 showed moderate solubility (~47%) from glycerol leaching. CNC alone (C_2E_0) increased solubility to ~71% via hydroxyl group-facilitated water penetration. Pickering emulsion films (C_2E_{20}, C_2E_{30}) reached ~75% and ~66% solubility, while C_2E_{40} decreased to ~58.9%, as the higher oil volume fraction formed a more continuous hydrophobic barrier slowing water ingress (Kong et al., 2022a).

Results and Discussion

Mechanical properties of films

Film thickness ranged from 60 to 150 µm (Table 1). C_0E_0 was thinnest (60 µm), as expected for a neat chitosan-glycerol matrix. Thickness increased with CNC and PEO addition, as nanoparticles and dispersed oil droplets elevate solid content (J. Liu et al., 2022). Combined CNC-PEO films (C_2E_{20}-C_2E_{40}) were thickest, suggesting a synergistic effect on film dimensions.

Table 1. Thickness and mechanical properties of bioplastic films

Sample	Thickness (µm)	Elongation at break (%)	Tensile strength (MPa)
C_0E_0	60	20	3,88
C_0E_{30}	90	8,57	6,32
C_2E_0	75	16,83	4,75
C_2E_{20}	140	20,74	4,28
C_2E_{30}	150	11,62	3,55
C_2E_{40}	120	9,25	2,58

The films with CNC-stabilized PEO Pickering emulsions exhibited diverse mechanical characteristics. The C_2E_0 film (2% CNC without PEO) exhibited a moderate enhancement in tensile strength (4.75 MPa) relative to the control, suggesting that CNC particles contributed to the reinforcement of the chitosan matrix via hydrogen bonding and mechanical interlocking (Talebi et al., 2022). The amalgamation of CNC with 20% PEO (C_2E_{20}) yielded mechanical qualities that harmonized strength and flexibility, sustaining a tensile strength of 4.28 MPa akin to the CNC-only film while retaining significant flexibility with 20.74% elongation. Nonetheless, as the concentration of PEO escalated to 30% and 40% (C_2E_{30} and C_2E_{40} films), both tensile strength and elongation exhibited a steady decline. The C_2E_{40} film demonstrated the lowest tensile strength (2.58 MPa) and elongation (9.25%) among the formulations incorporating CNC. The reduction in mechanical performance indicates that increased oil content breaks the continuity of the polymer matrix and hinders stress transfer mechanisms, possibly due to phase

separation and the creation of weak surfaces within the composite structure (Yang et al., 2024). The findings demonstrate that CNC stabilization can partially preserve mechanical properties at reduced PEO concentrations (20%). However, the Tween-80 stabilization approach was markedly more efficient in augmenting tensile strength. The exceptional performance of the C_0E_{30} film indicates that traditional surfactant stabilization may be more effective for producing PEO-loaded chitosan films with improved mechanical properties than Pickering emulsion methods utilizing CNC, given the processing circumstances applied in this investigation.

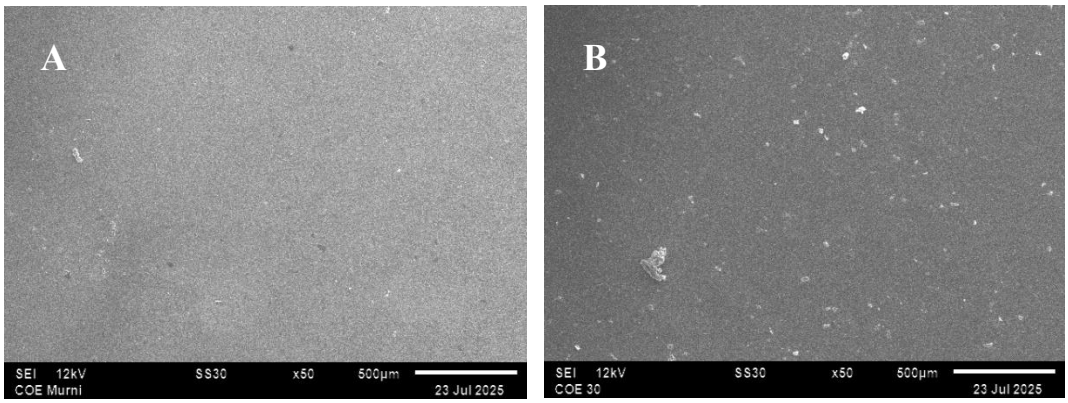


(a) (b) (c)

Figure 1. Photographs of the fabricated chitosan-based bioplastic films: (a) C_0E_0 pure chitosan film without emulsion, (b) C_0E_{30} film containing Tween 80-stabilized PEO emulsion, and (c) C_2E_{30} film containing CNC-stabilized Pickering emulsion with 30% v/v PEO.

Thickness and morphology of films

Film thickness ranged from 60 to 150 µm. C_0E_0 was the thinnest (60 µm) as expected for a pure chitosan-glycerol matrix. Thickness increased substantially with CNC and PEO addition, as solid nanoparticles and dispersed oil droplets raise overall solid content and occupy volume in the matrix (Y. Liu et al., 2024). Combined CNC-PEO films (C_2E_{20}-C_2E_{40}) were the thickest, indicating a synergistic effect of CNC nanoparticles and oil droplets on film dimensions.



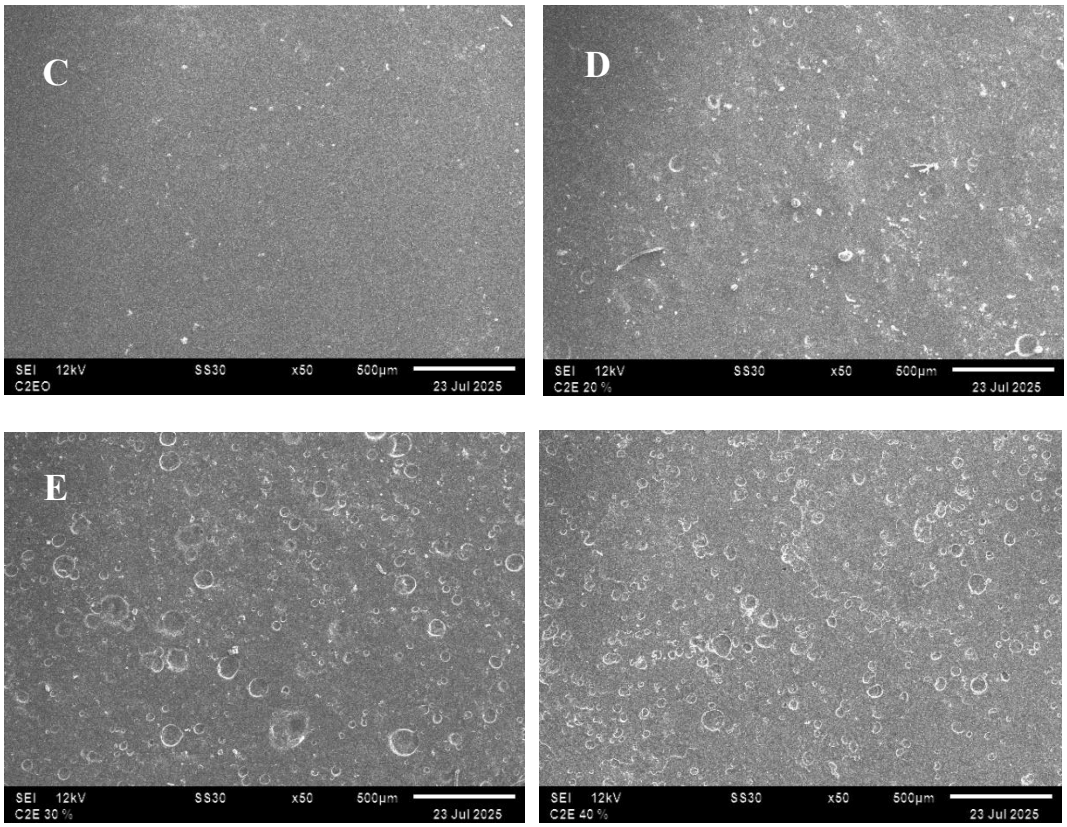


Figure 2. SEM micrographs of chitosan-based films: (a) C_0E_0 (pure chitosan), (b) C_0E_{30} (chitosan containing Tween-80 stabilized patchouli oil emulsion), (c) C_2E_0 (chitosan containing CNC), (d) C_2E_{20} (CNC-stabilized Pickering emulsion, 20% v/v patchouli oil), (e) C_2E_{30} (30% v/v), and (f) C_2E_{40} (40% v/v).

SEM analysis revealed distinct morphological differences among formulations (Fig. 2). C_0E_0 showed a smooth, dense, homogeneous surface typical of neat chitosan matrices (Yadav et al., 2020). C_0E_{30} (Tween-80) produced a slightly rougher but continuous microstructure with well-dispersed oil droplets (Elshamy et al., 2021), while C_2E_0 showed surface irregularities from CNC aggregation (Díaz-Cruz et al., 2022).

CNC-stabilized Pickering films (C_2E_{20}-C_2E_{40}) showed spherical pores and cavities from oil droplet evaporation during drying (Chaabane et al., 2023). Pore abundance increased with PEO concentration, reflecting larger droplets at higher oil loadings (Bu et al., 2022). These porous structures facilitated antibacterial compound diffusion and release (Ran et al., 2023), explaining higher inhibition activity compared to the denser Tween-80 film, which produced more continuous structures with smaller droplets (Cahyana et al., 2022).

Moisture content and solubility in water

C_0E_0 showed 17.00% moisture, reflecting hydrophilic chitosan and glycerol (Silva et al., 2024). On the other hand, C_0E_{30} was slightly higher (20.45%) due to

the hygroscopic surfactant (Chen et al., 2024) C_2E_0 had the highest value (22.38%) due to CNC hydroxyl groups. Conversely, Pickering emulsion films (C_2E_{20}–C_2E_{40}) showed significantly reduced moisture (12.73–14.81%), as hydrophobic PEO created barriers within the matrix. CNC adsorption at the oil–water interface also reduced hydroxyl group exposure to water (Haouache et al., 2022); (Pires et al., 2025).

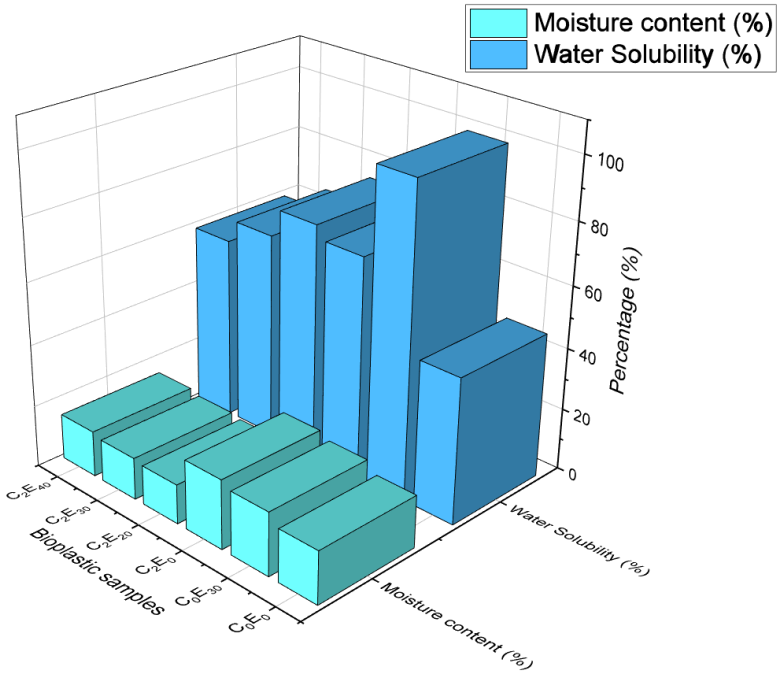


Figure 3. Water solubility and moisture content of bioplastic films

The interaction between the chitosan matrix, plasticizer, additives, and water dictates the solubility behavior of the films, as illustrated in Figure 1. Sample C_0E_{30} showed complete solubility in water, likely because Tween-80 is a highly hydrophilic, water-soluble surfactant that disperses readily in water, causing rapid disintegration of the film's structure. The control film (C_0E_0) exhibited moderate solubility (~47%), attributed mainly to leaching of glycerol and minor fragmentation, although the chitosan backbone remains largely intact in neutral pH. With the addition of hydrophilic CNC alone (C_2E_0), solubility increased to ~71%, likely because the hydroxyl groups on CNC facilitate water penetration and disrupt polymer network integrity (Wigati et al., 2025). Pickering emulsion films (C_2E_{20} and C_2E_{30}) exhibited the highest solubility values (~75% and ~66%, respectively), suggesting that the hydrophilic CNC shell surrounding numerous small oil droplets accelerated film breakdown in water (Chen et al., 2024). However, when the PEO concentration increased further (C_2E_{40}), the solubility decreased to ~58.9%, implying that a higher volume fraction of hydrophobic oil formed a more continuous barrier that slowed water ingress and dissolution (Kong et al., 2022b).

Biodegradability test

Biodegradability results after 7 days in EM4 solution are shown in Figure 4. C_0E_{30} degraded completely (100%), as chitosan and glycerol are readily digestible and Tween-80 facilitates microbial access (Zehra et al., 2022). C_0E_0 showed 63.74% degradation, consistent with the biodegradable nature of chitosan and glycerol (Wrońska et al., 2023).

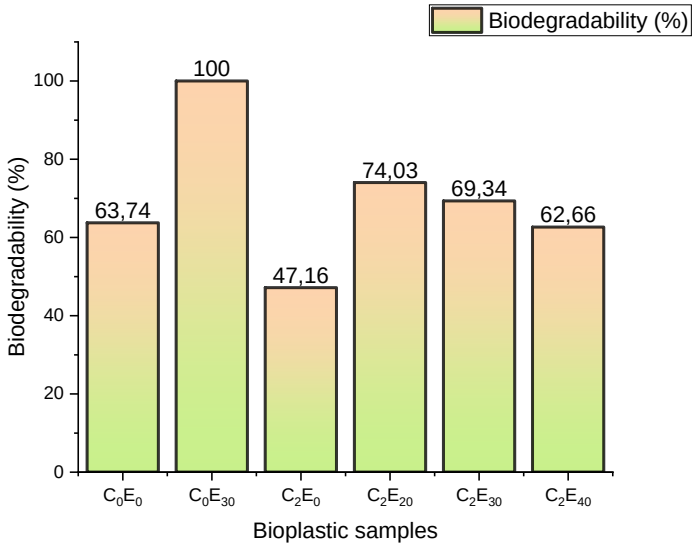


Figure 4. Biodegradability of bioplastic films

C_2E_0 showed lower biodegradation (47.16%), as CNC reinforcement densified the matrix and reduced enzymatic access (Silva et al., 2024). Pickering emulsion films (C_2E_{20}: 74.03%; C_2E_{30}: 69.34%) had higher degradation, as the emulsion microstructure provides microbial colonization sites and controlled oil release (Daeialiakbar et al., 2025). C_2E_{40} showed slightly lower but still substantial degradation (62.68%), suggesting that high antimicrobial PEO concentrations may somewhat limit microbial activity.

The EM4-based biodegradation method was used as a rapid preliminary evaluation under controlled laboratory conditions. EM4 contains mixed beneficial microorganisms, including lactic acid bacteria, yeast, and photosynthetic bacteria, which can accelerate the degradation of biodegradable polymers and organic compounds (Ezeagu et al., 2023). Although it is not as standardized as conventional soil burial testing, EM4 provides a simplified environment with high microbial activity that enables faster comparison of biodegradation behavior among film formulations. The observed degradation trends were consistent with soil burial principles, where films with more hydrophilic and less compact structures degraded more rapidly due to easier water and microbial penetration into the polymer matrix.

For food packaging applications, biodegradability should be balanced with adequate structural stability during use. In this study, C_2E_{20} exhibited the most balanced performance by combining good mechanical properties with high biodegradability. In contrast, although C_0E_{30} achieved complete degradation, its high water solubility may limit its practical application. These results indicate that optimizing PEO and CNC contents is essential to achieve an appropriate balance between functional durability and environmental degradability.

Antibacterial test

The disk diffusion experiment was carried out to assess the antibacterial effectiveness of the synthesized bioplastic films against the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli*. The results, determined by the diameter of the inhibition zone (IZ), are illustrated in Figure 5 and demonstrate a distinct correlation with the film's composition.

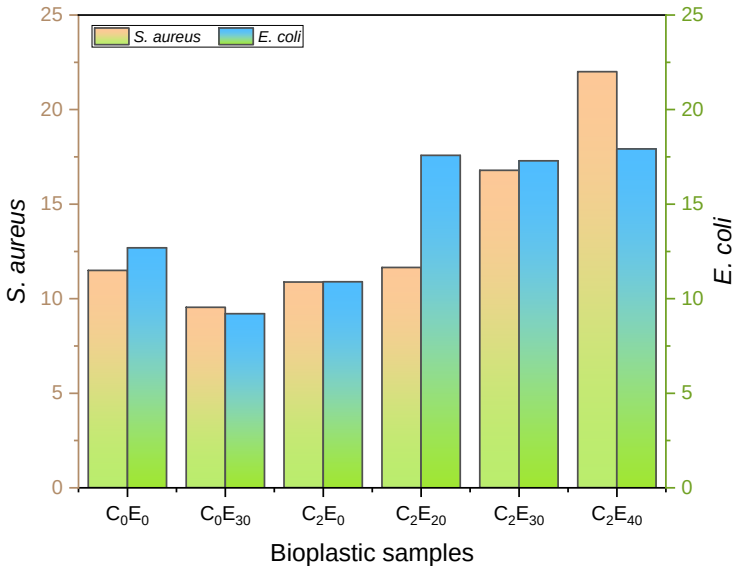


Figure 5. Inhibition zones of bioplastic films against *S.aureus* and *E.coli*

Pure chitosan (C_0E_0) showed intrinsic antibacterial properties, producing inhibition zones of 11.49 mm (S. aureus) and 12.69 mm (E. coli), due to interaction of positively charged chitosan with negatively charged bacterial cell membranes (Silva et al., 2024). C_0E_{30} (Tween-80-PEO) exhibited the lowest antibacterial activity, suggesting that the surfactant-based emulsion limits effective PEO release or causes its inactivation. C_2E_0 (CNC only) did not significantly improve antibacterial activity relative to the control, as CNC reinforces but does not enhance active compound release ((J. Liu et al., 2022)).

CNC-Pickering emulsion films showed dose-dependent antibacterial activity: C_2E_{40} achieved inhibition zones of 22.00 mm (S. aureus) and 17.92 mm (E. coli) (Elshamy et al., 2021). Beyond concentration, CNC stabilization improved PEO dispersion and controlled release, reducing droplet coalescence and enabling uniform diffusion of active compounds, contributing further to larger inhibition zones (Wang et al., 2024). The CNC-Pickering structure acts as a controlled-release reservoir for PEO (J. Liu et al., 2022). All Pickering films showed particularly pronounced activity against Gram-positive S. aureus in C_2E_{30} and C_2E_{40}, likely reflecting cell wall structural differences between Gram-positive and Gram-negative bacteria (Mulla et al., 2024).

Conclusions

This study developed antibacterial chitosan-based bioplastic films incorporating patchouli oil via CNC-stabilized Pickering emulsions. SEM confirmed progressively porous structures with increasing PEO, facilitating antibacterial release. C_2E_{40} achieved the highest inhibition zones (22.00 mm vs. S. aureus; 17.92 mm vs. E. coli). CNC reduced moisture content; higher PEO concentrations reduced tensile strength due to matrix discontinuity. C_2E_{20} offered the best balance between mechanical stability and antibacterial performance. CNC-stabilized Pickering emulsions show strong potential for biodegradable active food packaging.

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