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Journal of Multidisciplinary Applied Natural Science Vol. 6 No. 3 (2026) https://doi.org/10.47352/jmans.2774-3047.466
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Research Article

Lotus Bioactive-Loaded Carboxymethyl Cellulose Films for Shrimp Preservation

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Received : January 7, 2026	Revised : April 22, 2026	Accepted : May 19, 2026	Online : July 29, 2026

Abstract
Shrimp is highly susceptible to microbial spoilage, creating a need for sustainable antimicrobial packaging systems. In the present study, a biodegradable carboxymethyl cellulose (CMC)-based composite film incorporating lotus-derived flavonoids, essential oil, and carbon nanoparticles (CNPs) was developed for shrimp preservation. A Box–Behnken design was employed to optimize film composition, resulting in a minimum film formation time of 28.2 ± 0.2 h at 0.4% essential oil, 1.65% flavonoids, and 1.2% CNPs. The films were prepared by solution casting and characterized for physicochemical, mechanical, antioxidant, antimicrobial, and biodegradability properties. The optimized film exhibited good structural integrity, a maximum tensile force of 10.5 N, moderate water vapor barrier properties, strong antioxidant activity, and rapid biodegradability under soil conditions. Shelf-life evaluation of *Litopenaeus vannamei* stored at 16–18 °C demonstrated maintenance of acceptable pH, total phenolic content, and total volatile base nitrogen levels over 15 days. A modified Kaplan–Meier approach further indicated delayed microbial spoilage in shrimp packaged with the composite film compared to conventional plastic packaging, although the differences were not statistically significant at the 95% confidence level. The results suggest that lotus bioactive-loaded CMC films have potential as sustainable active packaging materials for seafood preservation.

Keywords: Box-Behnken design, carbon nanoparticles, carboxymethyl cellulose, hydrothermal autoclaving, Kaplan-Meier curve, *Litopenaeus vannamai*

1. INTRODUCTION

Biopolymers are natural polymers produced by living organisms or synthesized from biological sources. In recent years, biopolymers have gained increasing attention as alternatives to conventional petroleum-based plastics due to environmental concerns, regulatory pressures, and the need for sustainable packaging solutions. Common biopolymers used in packaging include polylactic acid (PLA), polyhydroxyalkanoates (PHA), starch-based, chitin-based, and cellulose-based materials. Carboxymethyl cellulose (CMC), a water-soluble derivative of cellulose, a natural polymer abundantly present in plant cell walls, is widely explored in food packaging applications. Cellulose, although abundant in nature, is insoluble in water and has limited processability in its native form. To overcome these limitations, it is chemically modified through the introduction of carboxymethyl groups (–CH_2–COOH), resulting in a water-soluble derivative with improved functional properties [1]. This modification significantly enhances its applicability across industries. CMC has attracted considerable interest as a biodegradable packaging material due to its film-forming ability, biocompatibility, and eco-friendly nature. Additionally, CMC and its derivatives (E 460–466, E 468 and E 469) are approved food additives and are considered safe for direct food contact applications [2].

Despite these advantages, native CMC films exhibit limitations such as moderate mechanical strength, high moisture sensitivity, and limited inherent antimicrobial activity. To address these challenges, incorporation of bioactive compounds into CMC matrices has been widely investigated to improve functional performance, including antioxidant and antimicrobial properties [3]-[5]. Such modifications can enhance the applicability of CMC films in active packaging systems; however, the extent of improvement depends on the type and concentration of incorporated agents. Previous studies have reported the use of clove oil nanoemulsions, chitosan coatings, and metallic nanoparticle composites for seafood preservation; however, limited information is available regarding

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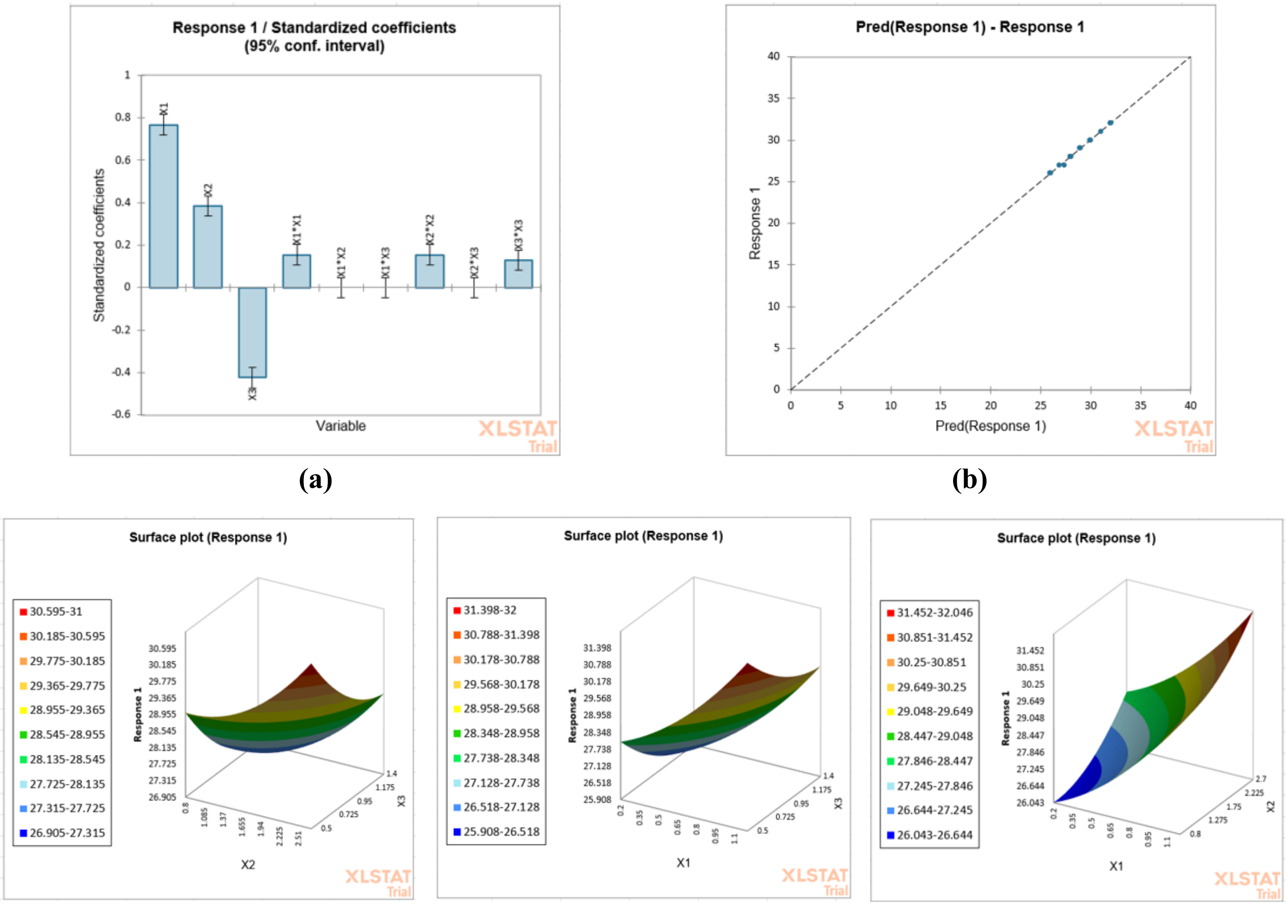


Figure 1. Response surface analysis using Box–Behnken design for optimization of film formulation variables affecting film formation time. (a) Standardized regression coefficients showing the relative influence of independent variables (X1: essential oil, X2: lotus bioactives, X3: carbon nanoparticles), where positive values indicate direct relationships and negative values indicate inverse relationships. (b) Contour plots illustrating interactions between variables, where blue regions correspond to minimum film formation time. (c) Model fit showing agreement between predicted and experimental values, indicating good suitability of the regression model ($R^2 = 0.997$). The regression equation was:

$$Response = 27.96 + 2X1 + 1X2 - 1.05X3 + 0.54X1^2 + 0.54X2^2 + 0.45X3^2$$

the combined use of lotus-derived bioactive compounds and carbon nanoparticles within CMC-based packaging systems.

Lotus (*Nelumbo nucifera*) leaf extracts are rich in bioactive compounds, including alkaloids (nuciferine), flavonoids (quercetin and kaempferol), phenolic acids (gallic acid and ellagic acid), and tannins, which have been reported to exhibit antimicrobial activity against both Gram-positive and Gram-negative bacteria [6]-[8]. These compounds are known to interact with microbial cell membranes and interfere with cellular processes. Therefore, their incorporation into CMC films may contribute to improved functional properties. However, the addition of bioactive compounds alone may not sufficiently address limitations related to mechanical strength, barrier properties, and structural stability of the films.

To overcome these limitations, the incorporation of carbon-based nanoparticles has been explored as a reinforcing strategy. Carbon nanoparticles, including graphene oxide, carbon nanotubes, and carbon dots, possess high surface area and can enhance mechanical properties and matrix interactions within polymer systems. Carbon nanoparticles (CNPs) are typically in the size range of 1–100 nm, and smaller quasi-spherical particles below 10 nm are referred to as carbon dots (CDs) [9]. Based on these considerations, it was hypothesized that the combined incorporation of lotus-derived bioactive compounds and carbon nanoparticles into the CMC matrix would improve the antimicrobial, antioxidant, and mechanical properties of the developed films while enhancing their suitability for shrimp preservation applications. In the present study, the composition of the CMC film-forming solution and the proportion of incorporated additives were systematically evaluated to obtain a stable composite film. The developed films were applied for packaging of *Litopenaeus vannamei* (white-leg shrimp), a highly perishable seafood product typically stored under refrigerated conditions. Conventionally, shrimp are packaged using plastic-based films that primarily act as passive barriers. In this study, a biodegradable CMC-based composite film was investigated as an alternative packaging material, and its potential influence on selected quality parameters and shelf-life of shrimp was evaluated under controlled storage conditions. However, comparative evaluation against conventional plastic films requires careful consideration of functional equivalence and application conditions.

2. MATERIALS AND METHODS

All chemicals used in this study were of analytical grade unless otherwise specified. Food grade CMC was procured from CK's Products (ISO 22000-2018 certified company, Kochi, India). Glycerol (analytical grade) was obtained from (HiMedia Laboratories, India). Lotus essential oil was obtained from Sparshveda (Green Vibes Biotech Private Limited, India). All microbiological media including Luria Bertani (broth and agar) and deMan Rogosa Sharpe (broth and agar), were purchased from HiMedia Laboratories (Mumbai, India).

2.1. Extraction of Lotus Flavones (LF)

Fresh lotus (*N. nucifera*) leaves were procured from a local nursery, washed, oven-dried at 50 °C, powdered, and sieved (2 mm). Flavonoids were extracted following a modified method [10]. Briefly, 100 g of leaf powder was mixed with 2 L of distilled water at 50 °C and subjected to shaking extraction (2 × 30 min). The extracts were pooled and passed through a D101 macroporous resin column. Initial elution with distilled water was continued until the effluent became colorless, followed by elution with 70% ethanol to recover flavonoid-rich fractions. The collected fractions were concentrated and spray-dried (inlet temperature: 50–60 °C) to obtain lotus flavonoid (LF) powder.

2.2. Green synthesis of CNPs

Carbon nanoparticles were synthesized using a green hydrothermal method with modifications [11]. Dried lotus leaf powder (2 g) was dispersed in 35 mL distilled water and subjected to microwave

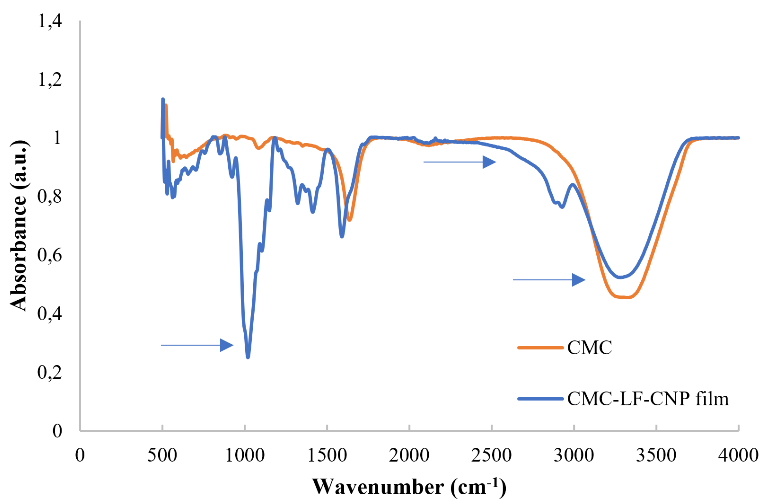


Figure 2. FTIR spectra of carbon nanoparticles (CNPs) and the CMC-LF-CNP composite film showing characteristic functional groups (C–O, C=C, C–C, C–H, and C–O–C), indicating interactions between the nanoparticles, lotus-derived bioactive compounds, and the carboxymethyl cellulose matrix.

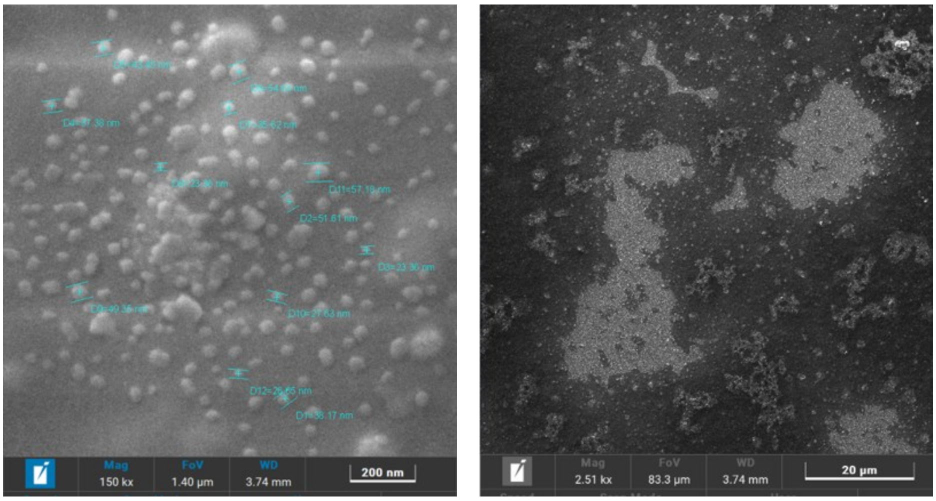


Figure 3. SEM images of carbon nanoparticles (CNPs) showing predominantly spherical morphology with particle sizes in the range of ~23–57 nm and slight aggregation, possibly due to van der Waals interactions.

heating for 15 min. The mixture was transferred into a Teflon-lined stainless-steel autoclave (Maatara Insruments, Gujarat, India) and heated at 140–200 °C for 6–12 h. After cooling, the resulting solution was centrifuged (8000 rpm, 12 min) (Beckman Coulter Allegra X-30R Centrifuge, USA) and filtered (0.2 µm syringe filter) to remove larger particles. The obtained CNP suspension was stored at 4 °C for further use. Characterization of CNPs included UV-induced photostability assessment (365 nm, 40 min), morphological analysis using Field Emission scanning electron microscopy (FESEM, MIRA, Model S6123) and functional group identification using ATR-FTIR spectroscopy (4000–500 cm^{-1}; Bruker ALPHA-II).

2.3. Preparation and Optimization of CMC Films

CMC solution was prepared by dissolving 2 g of CMC in 100 mL distilled water, followed by addition of glycerol (40% w/w of CMC) as a plasticizer. Lotus flavonoids (LF), essential oil and CNPs were incorporated into the film-forming solution. A Box–Behnken experimental design was employed to optimize three independent variables - essential oil (0.2–1.2%), lotus flavones (0.8–2.7%) and carbon nanoparticles (0.5–1.4%) with film formation time as the response variable. The optimized formulation (0.4% essential oil, 1.65% LF, and 1.2% CNPs) was selected for further analysis. The film-forming solution was cast onto silicone molds and dried under controlled conditions (35% RH, 40 °C) until complete film formation.

2.4. Physicochemical Characterization of Films

Water vapor transmission rate (WVTR) was measured using a WVTR-C1 analyzer (Systester Instruments, India) following the cup method over 24 h [12]. These measurements were conducted under standard laboratory conditions as per instrument protocol for comparability. Tensile strength was determined using an auto tensile tester (TSL-1002, Systester Instruments) at 23 °C and 30% RH, with a crosshead speed of 100 mm/min, according to GB1040 standards. Film samples (20 mm × 100 mm; thickness: 143 µm) were used. Thermal properties were analyzed using differential scanning calorimetry (DSC-60, Shimadzu) at a heating rate of 10 °C/min. Color parameters (L*, a*, b*) were measured using a ColorFlex EZ colorimeter, and yellowness index (YI) was calculated according to ASTM D1925. A white standard plate was used as the background (L = 72.84, a = −2.00, b = 2.61). Three locations were randomly selected for three repeated measurements of the film. The YI value was calculated according to ASTM D1925 protocol.

2.5. Antioxidant Properties of Films

The DPPH radical scavenging assay was performed to evaluate antioxidant activity. Film extracts were diluted and reacted with DPPH solution (200 µmol/L in ethanol) for 30 min in the dark. Absorbance was measured at 517 nm, and inhibition (%) was calculated as Equation (1);

$$\text{DPPH Inhibition } (\%) = 1 - \left(\frac{A_i - A_j}{A_0}\right) \times 100\ \% \quad (1)$$

where A_i = absorbance of sample, A_j = absorbance value of blank reagent, and A_0 = absorbance of blank control.

2.6. Antimicrobial Properties of Films

Escherichia coli was selected as a model Gram-negative organism due to its relevance in seafood contamination and food safety studies. For broth assay, 0.1 mL overnight culture was inoculated into 10 mL peptone medium, followed by addition of 50 mg film sample and incubation at 37 °C for 24 h. Bacterial growth inhibition was calculated from optical density (620 nm), using Equation (2);

$$\% \, Inhibition = \frac{OD_b - OD_s}{OD_b} \times 100\% \quad (2)$$

where, OD_b and OD_s represent bacterial density with and without the film, respectively [13]. For agar diffusion assay, 100 µL culture was spread on agar plates, and film discs (5 mm) were placed on the surface. After incubation (37 °C, 24 h), inhibition zones were measured. Plate count agar (PCA) was used for bacterial enumeration. Sterile discs without film served as negative controls. All experiments were performed in triplicate.

2.7. Biodegradability Studies

Soil biodegradation was assessed following ASTM D5988 guidelines. Film samples (4 × 4 cm) were buried in garden soil under controlled moisture (60% WHC), temperature (27–30 °C), and pH (6–7). After 30 days, samples were recovered, dried, and weight loss was determined. Water solubility was evaluated by immersing pre-weighed film discs (2 cm diameter) in 50 mL water at 28 °C for 24 h. Residues were dried and reweighed to calculate percentage solubility [14].

2.8. Shelf-life Evaluation of Shrimp

All seafood samples used in the study were procured from commercial sources intended for human consumption and handled according to

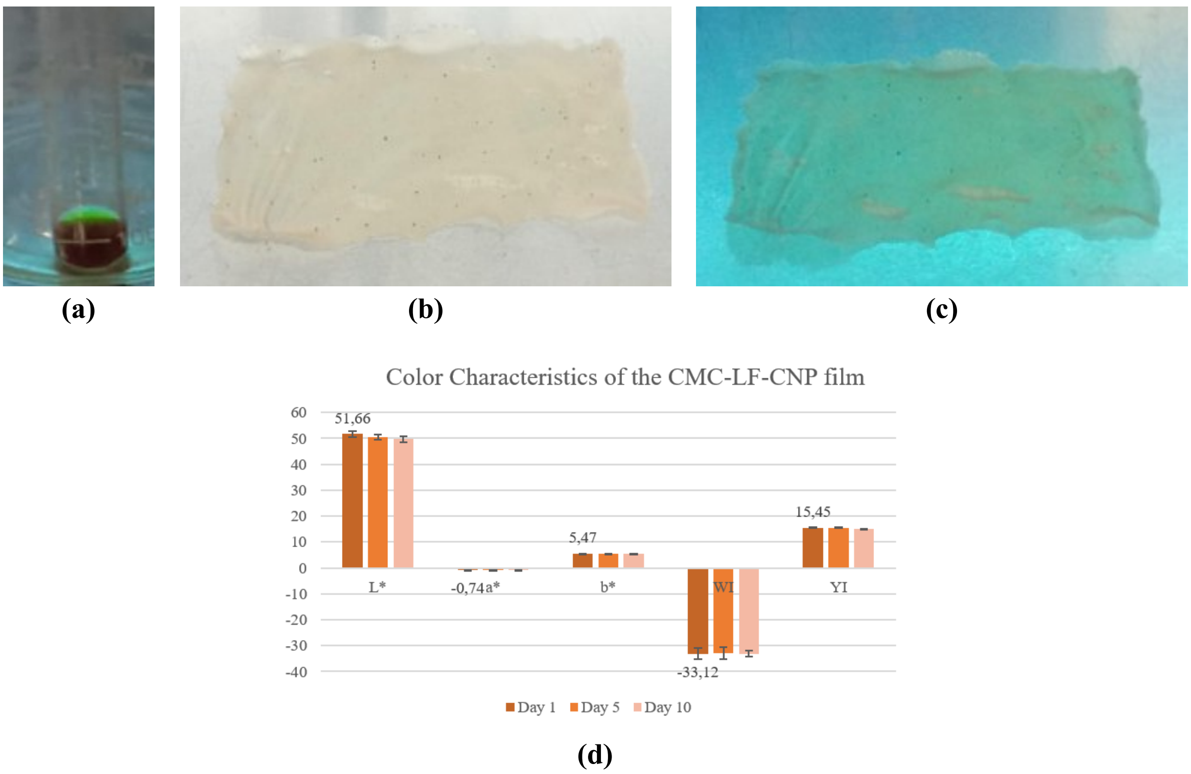
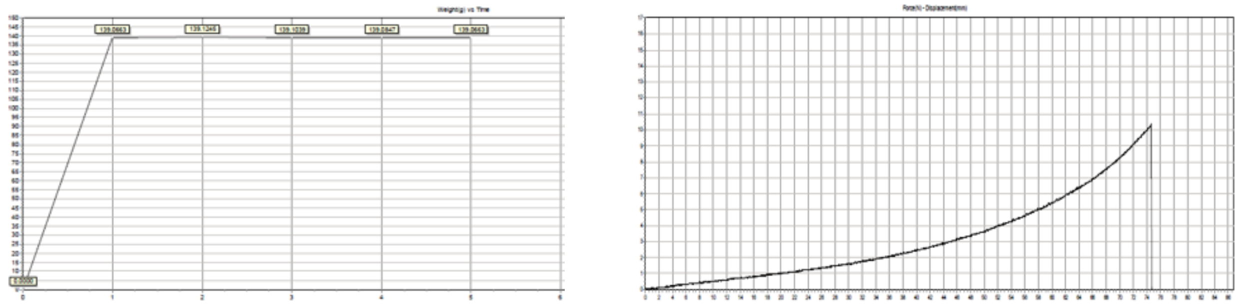


Figure 4. Optical and fluorescence properties of the carbon nanoparticle (CNP)-infused CMC-LF-CNP composite film. (a) Hydrothermally synthesized carbon nanoparticle solution obtained from lotus leaf extract. (b) Visual appearance of the composite film under white light. (c) Fluorescence emission of the film under UV irradiation showing characteristic greenish-blue luminescence. (d) Color parameters of the film represented in the CIELAB color space (L*, a*, b*), indicating changes in luminosity and chromaticity.



(a) (b)

Figure 5. Physical properties of the CMC-LF-CNP composite film. (a) Water vapor transmission rate (WVTR) profile expressed as g/m²/24 h under controlled temperature and relative humidity conditions. (b) Tensile behavior of the film showing its mechanical stability and flexibility.

standard laboratory food safety and hygiene practices. Fresh *L. vannamei* were transported to the laboratory under ice within 1 h and divided into two groups. Shrimp samples were randomly divided into control and treatment groups, with three shrimp samples included per group at each storage interval. All analyses were performed in triplicate, and results are reported as mean ± standard deviation. Control groups were wrapped in a plastic film and the treatment group were wrapped in a CMC-LF-CNP film. Samples were wrapped with optimized CMC-LF-CNP films and stored at 16–18 °C. Analyses were performed at 3-day intervals over 15 days. Statistical analysis was performed using Microsoft Excel. Differences between groups were evaluated using paired t-tests at a significance level of $p < 0.05$. All experiments were conducted in triplicate and expressed as mean ± standard deviation. pH was measured using a digital pH meter (ISO 2917:1999/Amd 1:2013). Total phenolic content was determined using the Folin–Ciocalteu method and expressed as gallic acid equivalents (GAE/g) [15]. Total viable count (TVC) was determined using plate count agar (ISO 4833-2:2013). Total volatile base nitrogen (TVB-N) was measured via distillation and titration [16]. TVB-N was calculated as Equation (3).

$$TVB\_N\ mg/100\ g = \frac{\text{Vol of sulphuric acid} \times N \times 100 \times 14}{W} \quad (3)$$

Additionally, a modified Kaplan–Meier approach was used as an exploratory tool to model the time required for samples to reach a defined microbial threshold (10^6 CFU/mL), enabling comparative assessment of spoilage progression between treatment and control groups.

3. RESULTS AND DISCUSSIONS

CMC is a water-soluble, semi-synthetic, anionic polymer derived from cellulose, widely used in applications such as biodegradable films, stabilizers and controlled release systems due to its film-forming ability and biocompatibility.

3.1. Preparation of Film

The development of CMC-based films from alternative biomass sources has gained increasing attention due to environmental and economic considerations. Previous studies have demonstrated that CMC derived from agro-waste sources such as sugarcane bagasse and oil palm residues can produce biodegradable films with favorable mechanical and barrier properties [17][18]. Furthermore, incorporation of plant-derived extracts and nanoparticles has been shown to enhance the functional properties of CMC films, including antimicrobial activity and structural stability [3] [19]. In this context, lotus (*N. nucifera*) leaves represent a promising bioresource due to their richness in flavonoids, phenolic compounds, and other bioactive compounds with known antimicrobial and antioxidant properties [20][21]. Similar classes of bioactive compounds have also been identified in other plant systems, supporting their broader relevance in biomaterial development [22][23]. These compounds may contribute to functional enhancement when incorporated into polymer matrices. In addition, essential oils, primarily composed of volatile terpenoid compounds, have been reported to improve antimicrobial properties and dispersion behavior in biopolymer films [24]-[27]. CNPs may further influence film performance by improving mechanical and barrier properties and matrix stability due to their high surface area and nanoscale interactions [28]-[30]. Similar nanocomposite approaches in CMC systems have demonstrated improvements in physicochemical and antimicrobial properties [31]-[36]. These findings provide a rationale for combining lotus-derived bioactives and CNPs within a CMC matrix to develop multifunctional films.

In the present study, a Box–Behnken design (BBD) was employed to optimize the concentrations of essential oil (X_1), lotus bioactives (X_2), and carbon nanoparticles (X_3), with film formation time as the response variable. Response surface analysis indicated that the interaction between bioactives and CNPs had a relatively stronger influence on film formation compared to other factor combinations (Figure 1). The contour plot suggested that reduced film formation time was achieved at approximately 1.65% bioactives and 0.9–1.2% CNPs, whereas the interaction between essential oil and bioactives showed a comparatively weaker effect. The optimized formulation—0.4% essential oil, 1.65% bioactives, and 1.2% CNPs—resulted in a film formation time of 28.2 ± 0.2 h. This observation suggests that while essential oils contribute to functional characteristics, the combined presence of bioactives and nanoparticles may play a more prominent role in influencing matrix formation. The interaction terms were statistically non-significant ($p > 0.05$), indicating that the response was primarily governed by the individual effects of the variables rather than their interactions ($R^2 = 0.997$). The reduction in film formation time may be attributed to enhanced intermolecular interactions within the composite system. Hydroxyl-rich bioactive compounds can facilitate hydrogen bonding with CMC chains, while CNPs may act as nucleation sites, promoting network formation. Similar trends have been reported in nanocomposite CMC systems incorporating plant-derived additives and nanoparticles [3][19].

Structural characterization further confirmed the successful synthesis and incorporation of CNPs. FTIR analysis indicated the presence of functional groups such as C–O, C=C, and C–C bonds, consistent with reported carbon nanostructures [32]-[35] (Figure 2). SEM imaging revealed predominantly spherical nanoparticles with sizes ranging from approximately 23.36 nm to 57.18 nm (Figure 3), with slight aggregation likely due to Van der Waals interactions. Variability in particle size was observed, which is common in hydrothermal synthesis methods. Such nanoscale dispersion is known to enhance mechanical and barrier properties in biopolymer films [36]. Additionally, previous studies have shown that incorporation of

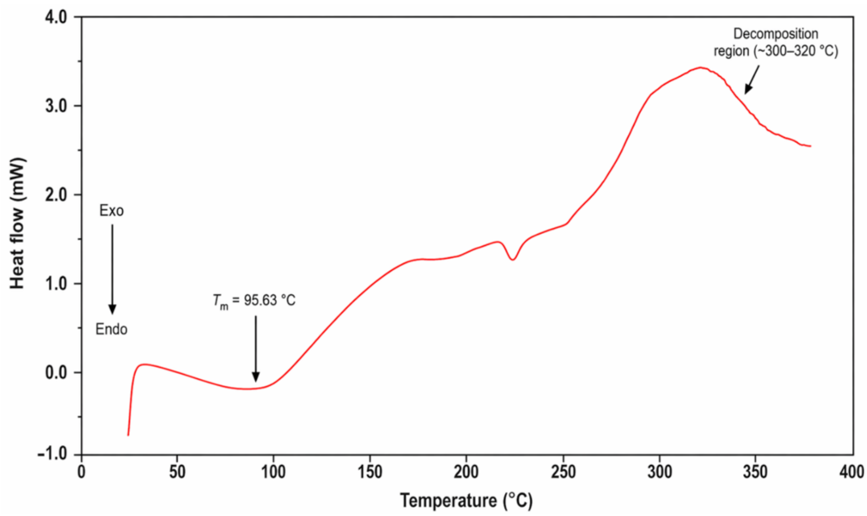


Figure 6. Differential Scanning Calorimetry (DSC) thermogram of the CMC-LF-CNP film showing heat flow as a function of temperature.

Table 1. Antioxidant and antimicrobial activities of the CMC-LF-CNP composite film, including DPPH radical scavenging activity (%) and antibacterial efficacy against *Escherichia coli*, expressed as percentage inhibition and zone of inhibition.

Sample	% Inhibition	Diameter of zone of Inhibition (mm)
CMC	0	0
CMC+CNP	59 ± 1.7	13 ± 2
CMC+Extract	48 ± 0.9	12 ± 2
CMC+Lotus EO	79 ± 1.8	15 ± 1
CMC-LF-CNP	92 ± 1.2	18 ± 2

nanoparticles into cellulose-based films can enhance antimicrobial functionality without necessarily compromising biocompatibility [37]. This supports the potential applicability of the developed CMC-LF-CNP film in food packaging systems. The results demonstrate that optimized integration of lotus-derived bioactive compounds and carbon nanoparticles into a CMC matrix enables the development of structurally stable and functionally enhanced films with potential applications in antimicrobial food packaging.

3.2. Characterization of the Film

Characterization of CNPs and their incorporation into the CMC matrix is essential to ensure the functionality and performance of the developed films. At the nanoscale, variations in particle size, morphology and surface chemistry can influence the optical, mechanical, thermal, and barrier properties of the composite material. The hydrothermally synthesized carbon nanoparticles appeared as a dark brown suspension under visible light (Figure 4(a)). Upon exposure to UV light, a greenish-blue fluorescence was observed (Figures 4 (b) – 4(c)), which is characteristic of carbon nanoparticles, particularly carbon dots. This fluorescence arises from π–π^* electronic transitions and surface functional groups, with excitation at 350 and 385 nm producing emission in the ranges of 440–490 and 500–540 nm, respectively [38]. These observations are consistent with reported optical behavior of carbon-based nanostructures and indicate successful synthesis of fluorescent nanoparticles. Such optical properties may also reflect surface functionalization and interactions within the polymer matrix.

The incorporation of CNPs and lotus-derived bioactive compounds influenced the optical appearance of the film. The film appeared pale beige under visible light, consistent with the presence of dispersed additives. Color analysis based on the CIELAB system (Figure 4(d)) showed an L* value of 50.76, indicating a medium-gray tone, while the a* value (−0.74) suggested a slight shift toward green and the b* value (5.47) indicated a yellowish tint. The yellowness index (15.1) and whiteness index (−33.127) further confirmed deviation from a pure white reference. These changes support successful incorporation of additives within the matrix, although some degree of discoloration is expected in films containing natural extracts and nanoparticles.

The barrier and mechanical properties of the films were evaluated through water vapor transmission rate (WVTR) and tensile measurements. The WVTR value of 4.92 $g/m^2/24$ h (Figure 5(a)) indicates moderate resistance to moisture transfer, which is relevant for packaging applications involving perishable seafood systems such as shrimp. The weight gain profile showed an initial rapid increase followed by a plateau, corresponding to moisture permeation through the film and stabilization of the system. Tensile analysis (Figure 5(b)) showed that the film withstood a maximum force of 10.5 N and exhibited an elongation at break of 66.44%, indicating a balance between mechanical strength and flexibility. The initial linear region of the curve represents elastic deformation, followed by plastic deformation leading to fracture. This behavior may be attributed to enhanced intermolecular interactions within the polymer matrix. Similar trends have been reported in CMC-based films containing plant-derived additives, where modifications in tensile strength and elongation were observed [39].

Thermal properties of the films were analyzed using differential scanning calorimetry (DSC) (Figure 6). DSC measures heat flow (expressed in mW) associated with thermal transitions such as melting, crystallization, and decomposition. The thermogram showed characteristic endothermic and exothermic transitions corresponding to melting and crystallization processes. The melting temperature (Tm) was observed at 95.63 °C with an enthalpy of fusion of −79.25 J/g, while broader peaks at higher temperatures indicated thermal decomposition. The incorporation of CNPs may contribute to improved structural organization and thermal behavior, consistent with previous reports on CMC-based composite films [27][40]. Although high thermal resistance is not critical for refrigerated applications, these observations suggest adequate thermal stability of the developed film.

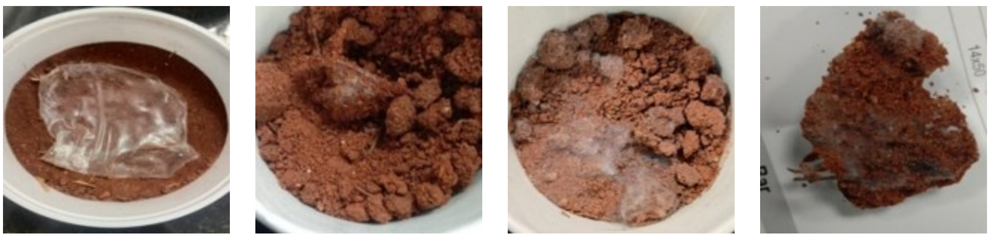
The antioxidant activity of the composite films was evaluated using the DPPH assay (Table 1), which showed demonstrable radical scavenging activity. This effect can be attributed to the presence of essential oils and lotus-derived bioactives. Similar mechanisms have been reported in CMC-based films, where phenolic compounds stabilize free radicals through hydrogen donation [41][42].

The antimicrobial properties of the films were evaluated against *Escherichia coli*. The composite film exhibited a percentage inhibition of 87.23 ± 2.17% and an average zone of inhibition of 18 ± 0.04 mm (Table 1). These values indicate notable antimicrobial activity. Compared to previously reported systems, such as gelatin–CMC films containing essential oils with inhibition zones of 3–10 mm [43], the observed activity in the present study is comparatively higher, which may be attributed to the combined effect of bioactive compounds and nanoparticles. *Escherichia coli* was selected as a representative Gram-negative bacterium commonly associated with seafood contamination and food safety evaluation. However, seafood spoilage is influenced by a broader range of refrigeration-associated spoilage bacteria and Gram-positive pathogens. Therefore, future studies on this composite film should include expanded microbial analysis involving multiple spoilage and pathogenic organisms to comprehensively evaluate the antimicrobial spectrum of the developed films.

Biodegradability studies revealed progressive degradation of the films under soil conditions (Figure 7). Structural integrity was lost within the first week, with approximately 79% weight loss observed by the second week, and near-complete degradation by the end of the study period. This degradation behavior is consistent with hydrolysis of glycosidic bonds and microbial activity reported in biodegradable polysaccharide systems. The films were also completely soluble in water within 15 min, leaving no visible residue. While these observations indicate high degradability, further quantitative analysis of degradation kinetics would be required for detailed evaluation.

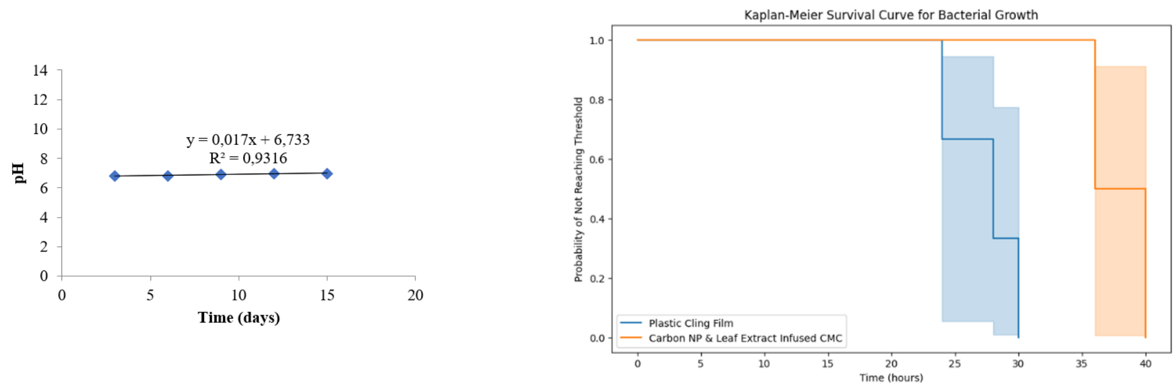
3.3. Shelf-life Study of the Shrimp Packaged with the Composite Film

Shelf-life evaluation is a critical step in assessing the practical applicability of active packaging systems. In India, the *L. vannamei* export industry faces substantial economic losses due to microbial spoilage and disease incidence [44]. Conventionally, shrimp are stored and transported under refrigerated conditions using plastic-based



(a) (b) (c) (d)

Figure 7. Soil biodegradation of the CMC-LF-CNP composite film (4 cm × 4 cm) over 30 days under controlled conditions: (a) Day 0, (b) day 7, (c) day 15, and (d) day 30, showing progressive degradation of the film matrix over time.



(a) (b)

Figure 8. (a) Changes in pH of *Litopenaeus vannamei* during refrigerated storage when packaged with the CMC-LF-CNP composite film, indicating maintenance of acceptable freshness throughout the storage period. (b) Modified Kaplan–Meier survival curves representing bacterial growth dynamics in shrimp samples, where the event was defined as reaching a threshold bacterial load of 10^6 CFU/mL, comparing the CMC-LF-CNP composite film with conventional plastic packaging.

packaging materials. In the present study, the developed CMC-LF-CNP composite film was evaluated as an alternative packaging material by monitoring physicochemical and microbiological quality parameters over a storage period of 15 days.

The pH of shrimp samples remained below 7.0 throughout the storage period (Figure 8(a)), indicating maintenance of acceptable freshness levels. Total phenolic content (TPC) was initially measured at 17.26 ± 0.18 GAE/g on day 3 and showed a slight decrease to 15.98 ± 0.35 GAE/g by day 15. Statistical analysis using a paired t-test ($n = 3$, $\alpha = 0.05$) yielded a calculated t-value of 1.46, which was lower than the critical value (±2.776), indicating no statistically significant difference over time. This suggests that the CMC-LF-CNP film performs comparably to conventional plastic films in preserving phenolic content. The relatively stable TPC values may be attributed to the presence of lotus flavones, which can chelate metal ions and reduce oxidative degradation. Similar effects have been reported in shrimp treated with natural antioxidants such as grape seed extracts [45].

Total volatile basic nitrogen (TVB-N), an indicator of protein degradation and spoilage, showed a gradual increase from 13 ± 2.1 mg/100 g on day 3 to 15 ± 1.8 mg/100 g on day 15. These values remained within acceptable limits (<20 mg/100 g) for fresh shrimp, indicating effective preservation during storage. Comparable reductions in TVB-N levels have been observed in CMC-based coatings incorporated with clove oil nanoemulsions and plant-derived extracts, where antimicrobial activity contributed to delayed spoilage [46][47]. Additionally, no visible melanosis was observed throughout the storage period, further supporting the effectiveness of the composite film in maintaining product quality. The absence of a control group limits the ability to attribute these effects solely to the film, and future studies should include direct comparisons with conventional plastic packaging. While previous studies have demonstrated the safety of certain nanoparticles such as silver nanoparticles in food-contact applications, the specific toxicological profile of the CNPs and lotus-derived bioactive compounds used in this study was not evaluated. Therefore, comprehensive toxicity and migration studies are required before large-scale food packaging applications.

Most studies evaluating microbiological safety in seafood rely on mesophilic and psychrophilic bacterial counts measured over the storage period [48]. In this study, microbial growth dynamics were further analyzed using a modified Kaplan–Meier approach (Figure 8(b)), where the "event" was defined as reaching a threshold bacterial load of 10^6 CFU/mL. This method enabled visualization of the probability of bacterial populations remaining below spoilage levels over time. Samples packaged with conventional plastic films reached the threshold earlier (approximately 25–30 h) compared to those packaged with CMC-LF-CNP films (approximately 40 h), indicating delayed bacterial proliferation in the latter. Furthermore, validation under pilot-scale refrigeration and transportation conditions would be necessary to assess industrial feasibility of the developed packaging system.

Although the Kaplan–Meier curves suggested improved microbial stability, statistical analysis using the log-rank test yielded a p-value of 0.06, indicating that the difference between the survival curves was not statistically significant at the 95% confidence level. This indicates that no statistically significant difference was observed between treatments; however, this does not establish equivalence, and the observed trend toward delayed bacterial growth suggests potential functional benefits. Therefore, the results suggest comparable performance under the tested conditions, but further validation with larger sample sizes and additional parameters is required.

The antimicrobial efficacy observed in this study is consistent with previous reports on bioactive films incorporating nanoparticles and plant-derived compounds. For instance, films containing ZnO, SiO_2, and CuO nanoparticles demonstrated strong antibacterial activity [49], while chitosan-based films enriched with essential oils and plant extracts were effective in retarding spoilage microorganisms [50]. Compared to several such previously reported biodegradable shrimp packaging systems incorporating clove oil, chitosan, ZnO nanoparticles, and plant-derived extracts, the present CMC-LF-CNP composite film demonstrated comparable preservation performance while additionally exhibiting rapid biodegradability and strong antioxidant activity. Previous studies have primarily focused on single bioactive systems or metallic nanoparticles, whereas the present study integrates lotus-derived flavonoids, essential oils, and carbon nanoparticles within a CMC matrix. This combination may provide multifunctional benefits through simultaneous antioxidant, antimicrobial, and structural reinforcement effects. Furthermore, the use of lotus-derived bioactive compounds represents a comparatively underexplored approach in seafood packaging applications, thereby contributing additional novelty to the developed system. The results suggest that the CMC-LF-CNP composite film is capable of maintaining physicochemical quality and delaying microbial growth in shrimp during refrigerated storage. While the findings demonstrate comparable or improved performance relative to conventional plastic packaging, further studies with larger sample sizes and extended storage conditions are required to establish statistical significance and validate industrial applicability.

4. CONCLUSIONS

The present study contributes to the development of biodegradable and functional food packaging materials aligned with the United Nations Sustainable Development Goals (SDG 11: Sustainable Cities and Communities and SDG 12: Responsible Consumption and Production). A composite film based on CMC, incorporated with lotus-derived flavones, essential oils, and carbon nanoparticles (CMC-LF-CNP), was successfully developed and evaluated. The film exhibited measurable physicochemical and functional properties, including moderate barrier characteristics, acceptable tensile behavior, antioxidant activity, and antimicrobial effects under the tested conditions. When applied to the packaging of *Litopenaeus vannamei*, the composite film maintained selected quality parameters such as pH, total phenolic content, and TVB-N within reported acceptable limits during refrigerated storage. Microbial assessment, including an exploratory application of a modified Kaplan–Meier approach, indicated a trend toward delayed bacterial growth in samples packaged with the composite film compared to conventional plastic packaging. However, these differences were not statistically significant at the 95% confidence level and should therefore be interpreted cautiously. The findings suggest that the developed film performs comparably to conventional packaging under the evaluated conditions while offering the additional advantage of biodegradability. The integration of lotus-derived bioactive compounds and carbon nanoparticles within a biodegradable CMC matrix also demonstrates the potential of multifunctional composite films for sustainable seafood packaging applications. Nevertheless, the authors acknowledge that the study has several limitations, including limited microbial scope, small sample size, restricted storage conditions, and laboratory-scale evaluation. Further investigations under larger-scale industrial conditions, with expanded microbial analysis, migration studies, extended storage durations, and cost-effectiveness assessment, are required to validate commercial feasibility, safety, and long-term performance of the developed packaging system.

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Conflicts of Interest

The authors have no competing interests to declare that are relevant to the content of this article. The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by Gandhi Institute of Technology and Management Seed Grant (GITAM RSG (F.2022/0184)) conferred to the first author.

DECLARATION OF GENERATIVE AI

During the preparation of this work, Chat GPT (Open AI Version 4.0) was used to paraphrase draft content and perform general English language corrections to improve readability.

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