

Research Article

Biopreservation of Shrimps Using Composed Edible Coatings from Chitosan Nanoparticles and Cloves Extract

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Shrimps are highly valuable and perishable foodstuff that could be rapidly spoiled. Chitosan (Cht) was extracted and transformed into nanoparticles (NPs) via ionic gelation and fortified with cloves (*Syzygium aromaticum*) buds extract (CLE) for usage as antimicrobial composites against food-borne bacterial pathogens (*Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*) and as edible coating (EC) for shrimp (*Fenneropenaeus indicus*) biopreservation throughout refrigerated storage. The synthesized CLE/Cht-NPs were electrostatically cross-linked and appeared with spherical shapes and homogenized distribution, with 159.4 nm mean size diameter and positive charge of 17.4 mV. The entire agents (Cht-NPs, CLE, and CLE/Cht-NPs) exhibited remarkable antibacterial activities toward all food-borne pathogens; CLE/Cht-NPs were significantly the most forceful. The scanning micrographs of treated *S. typhimurium* with CLE/Cht-NPs displayed NPs ability to attach and destroy bacterial cells. The ECs-treated shrimps exhibited sharp decrease in microbial groups load (aerobic microorganisms, *E. coli*, Enterobacteriaceae, and staphylococci) during refrigerated storage ($4 \pm 1^\circ\text{C}$) for 10 days. Additionally, the sensorial attributes (appearance, odor, color, and texture) of EC-treated samples preserved their elevated qualities for storage duration. The most effective EC blend contained 1.5% from Cht/NPs and 1.0% from CLE. The CLE/Cht-NPs could be impressively recommended as effectual natural composites for shrimps' biopreservation during cold storage.

1. Introduction

Chitosan (Cht) is the acquired polysaccharide from deacetylated chitin, which is the major constituent of crustacean exoskeleton [1]. Both of chitin and Cht can also exist in fungi mycelia and some insects' exoskeleton [2–4]. Cht has numerous astonishing bioactivities including antioxidation, metal chelation, and inhibition of cancerous cells and microbial pathogens (fungi, yeast, and bacteria) [2, 3, 5]. Cht was stated as effectual antibacterial materials (toward both Gram positive and negative bacteria) with approved GRAS (general recognition as safe) nature [1, 3].

Nanoparticles (NPs), synthesized from natural or synthetic polymers, are very effectual with their particles' size range from 10 to 1000 nm [6]. Polymers NPs show astonishing chemical and physical features, resulting from their effects, e.g., the macroquantum tunnel, quantum size, mini size, and surface effects. Chitosan nanoparticles (Cht-NPs) were proved as natural materials with excellent physico-chemical, biological, and structural properties, along with their eco-friendly and bioactive nature [6, 7].

Cht-NPs have the nanoparticles properties such as surface and interface effect, quantum size effects, and small size, along with the original Cht bioactive characteristics [4].

Cht-NPs can be prepared by multiple methods; the most frequent is the ionotropic gelation between Cht and sodium tripolyphosphate (TPP). Cht/TPP-NPs were frequently used as controlled-release drug carrier and for the effectual delivery of bioactive compounds [8–11].

The most important group of Crustacea (crustaceans) is shrimps; they belong to phylum Arthropoda and order Decapoda. They are distributed in the whole world and live in aquatic environments [12]. Due to shrimp's high water activity value, it is a highly perishable food and susceptible to fast spoilage [13]. The quality and shelf life of shrimp during storage are influenced by oxidation, microbiological activities, and enzymatic changes, which mostly correlated with the activity and growth of Gram negative aerobic bacteria [14].

Cloves (*Syzygium aromaticum*, Fam. Myrtaceae) are commonly used spices worldwide. The cloves oil main properties are the antifungal, antioxidant, antibacterial, and insecticidal effects; clove is also widely used for improving flavor and as antimicrobial substance in food preservation [15, 16]. Cloves are supposed to be excellent sources of minerals (manganese, calcium, and magnesium), vitamin C, dietary fiber, Ω -3 fatty acids, and vitamin K [17]. *S. aromaticum* extract and oil contain a significant amount of carbohydrates, proteins, calcium, iron, phosphorus, sodium, potassium, and also rich in vitamins C and A [18, 19]. Cloves buds extract and oil were additionally verified as potent antimicrobial agents that could hinder growth of numerous microbial pathogens including *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Escherichia coli*, many *Salmonella* spp., dermatophytic fungi, and Enterobacteriaceae [15, 16, 19–22]. Additionally, *S. aromaticum* extract was suggested for natural preservation of fisheries products (fish fingers), alone or combining with other plant extracts [23].

Edible coatings (EC) were defined as “the thin layers of materials that cover food surfaces and can be eaten and considered as a part of the whole food product” [24]; the major benefits of ECs are that they can provide additional nutrients, be consumed with the food, contain quality-enhancing antimicrobials, and enhance sensory characteristics. Edible coatings also act as barrier to external elements such as oil, moisture, and vapors, to protect food, prevent dehydration, and extend the shelf-life [24–26].

Although various investigations evaluated the incorporation of Cht and Cht-NPs with *S. aromaticum* oil, for applying as ECs to preserve foodstuffs including shrimps and pork meat [26–29], it is presumed that literature could not provide sufficient researches concerning the incorporation of cloves' whole crude extract with chitosan nanoparticles and their usage as edible coatings for shrimp biopreservation. The fortification of Cht and Cht-NPs based ECs with plant extracts was recommended to augment the antimicrobial and antioxidant attributes of the composited EC [28–30], but cloves extract was not sufficiently investigated as a fortifier in the Cht-NPs based ECs.

Accordingly, the synthesis of Cht-NPs and their fortification with clove extract were planned for this study. The evaluation of these agents as antibacterial blends, *in vitro*, and as edible coatings, and their applications for

biopreserving shrimps and upholding their qualities was also planned.

2. Materials and Methods

2.1. Chitosan Preparation. The extraction of Cht based on shells wastes of white prawn (*Fenneropenaeus indicus*), farmed in Aquaculture farm, Kafrelsheikh University. After shells cleaning and drying, they were immersed in 30 folds from 2.0 N NaOH solution (w/v, Sigma-Aldrich, MO) for 24 h; then after extensive washing with deionized water (DW), neutral materials were reimmersed in 30 folds from 2 N HCl solution (w/v, Sigma-Aldrich, MO) for 24 h, followed by extensive washing with DW. The resulting chitin was dried and immersed in 50% NaOH solution (with ratio of 1 g of chitin powder/13 ml of NaOH) and then heated in an oil bath at 125°C for 5 hours for obtaining chitosan. The molecular Cht weight was determined via CGP (chromatographic gel permeation), whereas its DD (deacetylation degree) measurement was based on Cht spectra using FTIR spectroscopic (Fourier transform infrared spectroscopy, FTS 45, Biorad, Germany).

2.2. Clove Extract Preparation. Dried cloves buds (*Syzygium aromaticum*) were obtained from ARC (Agricultural Research Centre, Giza, Egypt). The pulverized plant materials (60 mesh size) were immersed in 8 folds (w/v) from ethanol (70%, Sigma-Aldrich, MO) and agitated for 22 h at 160 x g speed in room temperature ($25 \pm 2^\circ\text{C}$). The plant residues were excluded via filtration and the result cloves extract (CLE) was rotary evaporated (IKA, RV 10, Germany) at 44°C until dryness [31]. The dried CLE was dissolved in 2% Tween 80 solution (in DW) to concentration of 1 mg/ml, and this solution was applied in further experiments.

2.3. Chitosan/Clove Extract Nanoparticles Preparation. For the synthesis of Cht-NPs and loading them with CLE, the following solutions were prepared according to Almutairi et al. [11]: Cht (1 mg/ml in 1% acetic acid solution), TPP, Sigma-Aldrich, MO (0.5 mg/ml in DW), and CLE (1 mg/ml of 2% Tween 80 solution in DW). The pH of Cht solution was adjusted to 5.2; then the TPP solution was slowly dropped onto Cht solution (while vigorously stirred at 620 x g) using a syringe needle at rate of 0.35 ml/min, until reaching equal volume from both solutions. For CLE/Cht-NPs formation, the previous steps were conducted with the addition of equal volume from CLE to Cht before TPP dropping. The formation of opalescent suspensions, after TPP dropping and persistent stirring for 110 min, indicated NPs formation. The formed NPs pellet was attained via centrifugation (SIGMA, 2–16 KL, Germany) at 11500 x g for 35 min, washing with DW and recentrifugation. The obtained NPs pellets were lyophilized and subjected to analysis.

2.4. Analysis of CLE/Cht-NPs Physiognomies

2.4.1. Structural Analysis. The morphological and organizational features of CLE/Cht-NPs (shape, size, and distribution) were appraised via spectroscopy (photon

correlation, PCS, Malvern™ Zetasizer, Malvern, UK), for determining the size, charge, and distribution of NPs, whereas the electron scanning microscopy (SEM, JEOL JEM IT-100, Tokyo, Japan) designated the CLE/Cht-NPs morphology and dispersion; the SEM imaging was conducted at 20 kV and x15000.

2.4.2. FTIR Spectral Analysis. The infrared analysis using FTIR (Fourier transform infrared spectroscopy, Perkin Elmer™ FTIR-V. 10.03.08, Germany) of synthesized Cht-NPs and CLE/Cht-NPs was perceived after samples' integration with 1% KBr, in transmission mode (at wavenumber range of 450–4000 cm^{-1}).

2.5. Antibacterial Evaluation of Natural Products

2.5.1. Bacteria Cultures. Three standard food-borne pathogens were employed for antimicrobial screening, that is, *Escherichia coli* ATCC-25922, *Salmonella typhimurium* ATCC-14028, and *Staphylococcus aureus* ATCC-25923. The entire bacterial strains were maintained and subcultured onto the nutrient agar and broth (NA and NB, Difco Laboratories, Detroit, MI), aerobically at 37°C.

2.5.2. Qualitative Antimicrobial Assay. The inhibitions zones (IZ), which appeared after disc diffusion test, were considered as indicators for antibacterial bioactivity of produced agents. Sterile Whatman No. 4 paper discs (6 mm diameter) were impregnated with 25 μL from 2% solutions of Cht-NPs, CLE, or CLE/Cht-NPs and placed onto freshly inoculated NA plate with each bacterial culture. The plates were then upturned incubated at 37°C for 18–24 h and the IZs diameters were measured using a precise caliper; then their triplicates means were calculated as \pm standard deviation [3].

2.5.3. SEM Imaging of Treated Bacteria. The morphological variations in *S. typhimurium* cell surfaces, after exposure to CLE/Cht-NPs solution (2.0%, w/v), were screened via SEM imaging, after 0, 5, and 10 h of exposure and incubation at 37°C, as formerly described [32]. The SEM micrographs were captured at 20 kV and x10000, depending on the appeared distortions in bacterial cells layouts.

2.6. Treatment of Shrimp with Cht-NPs-Based Edible Coating

2.6.1. Edible Coating (EC) Preparation. For the EC solutions preparation, 1.5% Cht-NPs (w/v) was slowly dissolved in DW (at 100°C for 45 min, with stirring at 450 x g); the solution was cooled to 45°C during stirring; then 1% (v/v) acetic acid and 0.25 mL from the plasticizer glycerol per gram of Cht-NPs were added [33]. After stirring for additional 30 minute (during which the temperature of the coating solution decreased to approximately 37°C), clove extract dispersed in 2% Tween 80 solution was added to the EC solution by gradual concentrations (w/v).

2.6.2. Application of Coatings on Peeled Shrimp. Freshly harvested shrimps (*F. indicus*), after manual deheading, deshelling, and cleaning with DW, were divided into five groups (each of them consisted of 25 shrimps with weight of $\sim 10 \pm 1$ g/shrimp). The first group was the uncoated control (C), and the other 4 groups of shrimp were immersed into Cht-NPs based EC solutions incorporated with clove extract (CLE) with the following order: group (Ch) contained Cht-NPs only and groups Ch-Cl 0.5, Ch-Cl 1.0, and Ch-Cl 1.5 were fortified with 0.5, 1.0, and 1.5% (w/v) from CLE, respectively. The shrimps coating was performed through dipping of samples in EC solutions (for 30 min at 4°C), at 1:2 ratio (w/v) from shrimp/EC solution; then coated shrimps were drained for 5 min at $25 \pm 2^\circ\text{C}$. Samples were then packaged in polystyrene trays and each tray was wrapped with plastic films and held at $4 \pm 1^\circ\text{C}$ for 10 days and at RH (relative humidity) of $65 \pm 3\%$ and sampled at 2 days intervals for further analyses [34, 35].

2.6.3. Microbiological Examination. The EC-treated and control shrimps were sampled aseptically (15 g/sample), immersed in 135 mL of buffered peptone solution (0.1%, LAB M, Lancashire, UK) in a stomacher sack, and then homogenized for 3 min using a Seward Stomacher 400 (Norfolk, UK). Serial dilutions from shrimp homogenization were made in NB and screened for the counts from different microbial groups via plating onto recommended agar media as illustrated by the standard microbiological protocols:

Total aerobic microorganisms enumeration of colony count at 30°C (**ISO 4833-1: 2013**) [36].

Enumeration of *Escherichia coli* (β -glucuronidase-positive) (**ISO 16649 -1: 2018**) [37].

Enterobacteriaceae detection and enumeration (**ISO 21528 -2: 2017**) [38].

Coagulase-positive staphylococci enumeration (**6888 -1: 2018**) [39].

2.6.4. Sensory Analysis. A well-trained panelist team (13 members; 8 females and 5 males), with experience in seafood judgments, was involved in evaluating the sensory attributes of EC-treated shrimps. The panelists were inquired to assess the samples appearance, odor, color, and texture, using a ranged hedonic scale from 1 (extremely poor) to 9 (extremely good) [31].

2.7. Statistical Analysis. The entire experiments were triplicated, with presented data as means \pm SD. The SPSS package (SPSS V-11.5, Chicago, IL, USA) was applied for statistically analyzed data using *t*-test and one-way ANOVA at $p < 0.05$.

3. Results and Discussion

Chitosan was efficaciously extracted from shrimp wastes; the produced Cht powder had a creamy-white color, a 38.3 kDa molecular weight (MW), and a DD of 89.4%. The DD of

≥70% and the MW of produced Cht confirmed the transformation of shrimp chitin to low MW chitosan [40].

3.1. Physiognomies of Synthesized Clove Extract/Chitosan Nanoparticles

3.1.1. NPs Structural Attributes. Size (including size distribution) is an important characteristic parameter for nanosuspensions [41]. Particles size distribution of synthesized clove extract/chitosan nanoparticles is shown in Figure 1(a). The CLE/Cht-NPs had a mean diameter of 159.4 and median of 165 nm and their particles' size ranged between 142.3 and 179.1 nm. The charges of synthesized NPs were 24.3 mV for Cht-NPs and 17.4 mV for the composited CLE/Cht-NPs.

Morphology and microstructure of CLE/Cht-NPs were evaluated by SEM (Figure 1(b)). Electron micrograph of the sample demonstrates presence of spherical structures, absence of cracks, and creation of a continuous layer onto NPs surfaces. The SEM image for clove extract chitosan nanoparticles illustrates spherical shape and regular distribution, which seems separated and well stable during the preparation process [42, 43].

3.1.2. Fourier Transform Infrared Spectroscopy. The FTIR spectra of Cht-NPs and composited CLE/Cht-NPs are given in Figure 2. The most significant peaks of plain Cht-NPs spectrum were as follows: 3428.59 cm^{-1} (stretching vibrations of O–H and N–H), 2927.26 cm^{-1} (aliphatic C–H stretching vibration), 3068.88 cm^{-1} (CH_2 stretching vibration), 3027.81 cm^{-1} (C–H₃ stretching vibration), 1702.25 cm^{-1} (C=O stretching of amide I), 1664.02 cm^{-1} (NH of amide II stretching vibration), 1110.29 cm^{-1} (C3–OH stretching vibration), and 1039.48 cm^{-1} (C6–OH stretching vibration) [7, 44]. Also, in Cht-NPs spectrum, the band at 3450 cm^{-1} , which has lower wideness than bulk Cht indicates reduced hydrogen bonding. The reduced intensity of hydrogen bonding in the cross-linked NPs complexes is owing to more open structure caused from cross-linking with TPP [44].

A new sharp peak at 1625 cm^{-1} appeared, the amine bending intensity at 1625 cm^{-1} goes down, which assumingly attributed to linkage between TPP and the ammonium group of Cht-NPs [9, 10]. FTIR analysis of combined CLE with Cht-NPs showed a band at 3450 cm^{-1} , which could indicate the reduced hydrogen bonding in Cht-NPs and/or the O–H groups of alcoholic CLE. Another band at 1687.5 cm^{-1} represented ester group frequency patterns C–O or the aromatic ketone group C=O that combines with more than ring. At the wavenumber of 1562.5 cm^{-1} , the detected band designates matched patterns to the aromatic carbonyl group belonging to quinine [45], which is close to the aromatic group (C=C). The bands at 780 cm^{-1} and 1330 cm^{-1} could represent the CH_2 group frequency pattern [46].

3.2. Antibacterial Activity of CLE/Cht-NPs against Food-Borne Pathogens

3.2.1. Qualitative Assay. The antibacterial activities of produced natural products, i.e., clove extract (CLE), nanochitosan (Cht-NPs), and clove extract with

nanochitosan (CLE/Cht-NPs), expressed as IZ diameters, are shown in (Table 1). The antibacterial activities, toward the entire food-borne pathogens, were significantly more forceful from CLE/Cht-NPs and then from Cht-NPs and CLE, respectively. *S. typhimurium* was the most susceptible strain to screened compounds compared to *E. coli* and *S. aureus*, as evidenced from the wider IZs. Larger IZ typically indicates higher antibacterial activity of tested solutions against challenged microbial species [20].

The Cht-NPs antimicrobial potentialities were recurrently confirmed from many investigations [4, 6, 9, 44]; they indicated that Cht-NPs had more forceful action than native Cht for inhibiting numerous pathogenic microorganisms. The Cht-NPs antibacterial action was principally attributed to their increased positive charges, which enable them to interact with microbial cells' surface and interior vital components (DNA, RNA, enzymes, etc.) and thus prohibit microbial growth and survival.

The alcoholic CLE contains plenty of bioactive compounds belonging to revealed existence of flavonoids, tannins, alkaloids, glycoside, steroids, terpenoids, ketones, aldehydes, and phenolics; more than 46 phenolic constituents were detected and quantified in CLE [22, 23], several compounds from these phytochemicals possess antimicrobial potentiality. Additionally, the high CLE content from tannins (10–19%) was also verified to augment its antimicrobial activity [21]. The major detected phytoconstituents in CLE and oil are eugenol, α - and β -caryophyllene, eugenyl acetate, limonene, α -copaene, and α -terpinolene [47]. Eugenol and caryophyllene (the highest occurring constituents in CLE and oil) were verified to possess strong antimicrobial (antibacterial, antimycotic, and antifungal) properties toward numerous pathogenic species [17]; the crude ethanolic CLE exhibited also comparable antimicrobial powers against several food-borne strains as the essential oil [16].

The synergistic action of the composited Cht-NPs and CLE was stronger than each of the individual agents; this is because the microbial pathogen cannot resist a composite from different antimicrobial agents with diverse action modes [9, 31].

3.2.2. SEM Detection of Morphological Alterations in CLE/Cht-NPs-Treated Bacteria. The SEM image shows how bacterial pathogen (*S. typhimurium*) was influenced by CLE/Cht-NPs treatment (Figure 3). At zero time of treatment, there is no apparent change in bacterial cells wall/membrane; cells appeared with regular shapes, smooth and uniformed surfaces (Figure 3(c)). After 5 h of CLE/Cht-NPs exposure, *S. typhimurium* cell appeared with swollen and partially lyses manifestation (Figure 3, 5 h), many NPs were appeared to attach/interact with exposed bacterial cells. With prolongation of CLE/Cht-NPs exposure to 10 h, most of bacterial cells were severely damaged; the lyses/explosion signs in their walls became obvious, the interior cellular components were released and composited with NPs (Figure 3, 10 h).

The CLE/Cht-NPs composite had powerful activity to deteriorate *S. typhimurium* cells; this is assumed to result

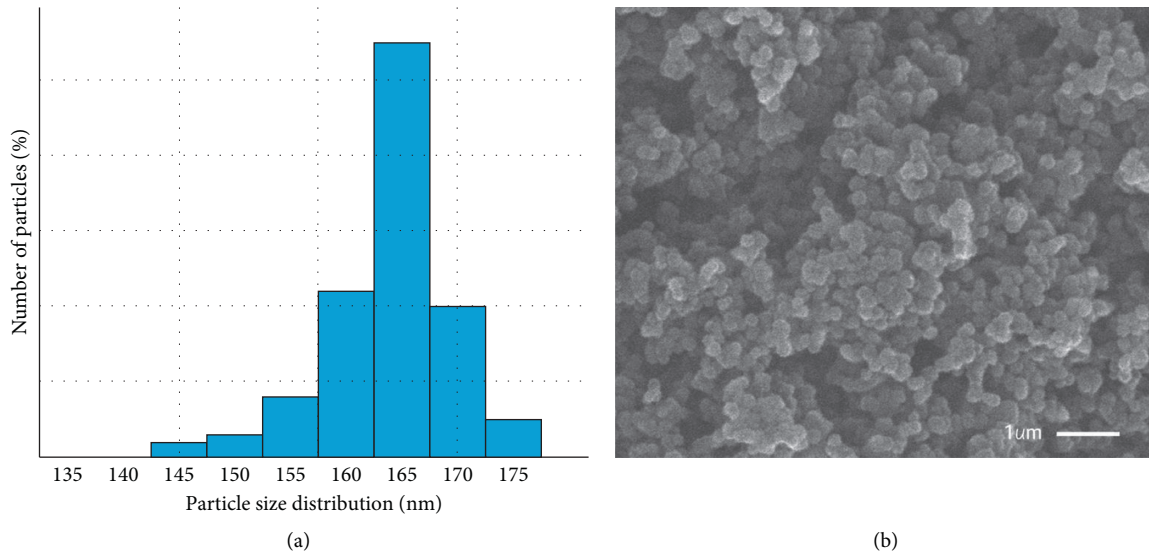


FIGURE 1: Structural features of synthesized clove extract/chitosan nanoparticles including their particles size distribution (a) and morphological attributes using scanning micrographs (b).

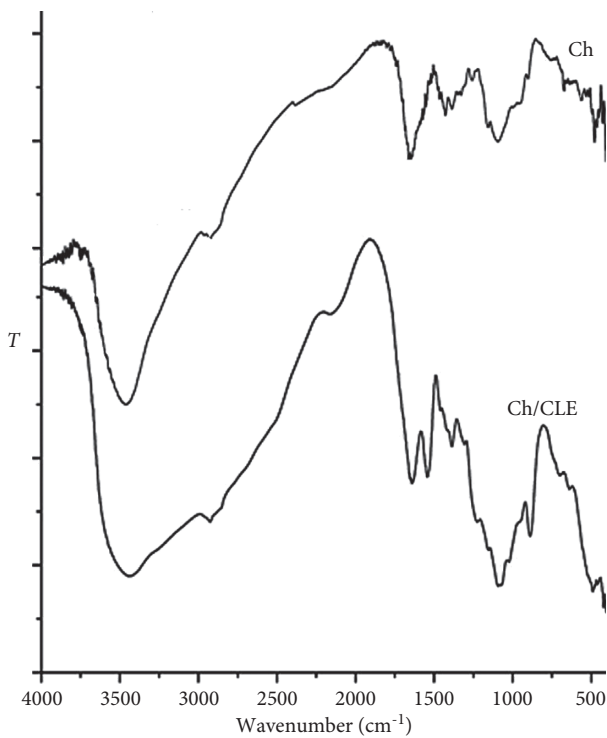


FIGURE 2: FTIR spectra of synthesized nanochitosan (Ch) and combined clove extract with nanochitosan (Ch/CLE).

from the combined bioactivities of CLE and Cht-NPs. The bioactivity of eugenol (the major component of CLE) was stated to enable it for deteriorating bacterial membrane through serial mechanisms that alter cell morphology, prompt leakage of cellular constituents, and increase permeability [48].

In addition, the electrostatic interaction between the positively charged Cht and negatively charged *S. typhimurium* walls/membranes could increase Cht-NPs attachment

to bacteria and their penetration into their interior organelles; thus they can severely alter the negative-charged bacterial cell walls/membranes (generated from their constituents of lipopolysaccharide and teichoic acid) [49], and interfere with cells' vital functions and components to stop them, hinder cells progress, and disrupt their outer membranes [1, 3, 6, 50].

The bactericidal actions of Cht, resulting from its adsorption onto microbial surfaces and induction of cell walls' lyses, were described and stated [3, 9, 31, 51]; the intensity of absorbed particles depended on Cht concentration, its MW, contact time, challenged microorganisms, and the charges on their cells. Many of preceding investigations stated the disruption/explosion of microbial cells as a consequence after their exposure to Cht [1–3].

The wide antimicrobial spectrum (antibacterial, anti-mycotic, and antifungal) from *S. aromaticum* oil, crude extract, and their constituents, was indicated [15, 52]; they suggested that *S. aromaticum* antimicrobial actions could include the interaction with microbial cells, proteins' denaturation and reactions with phospholipids in cell membranes, which suggested to affect microbial membranes' permeability.

The treated cells of pathogenic Gram-negative bacteria (*S. enteritidis* and *E. coli*) with ethanolic CLE appeared with uniform, coarse surfaces, and withered and irregular morphology, with formed adhesions and aggregations, which suggest that CLE led to severe damages in bacterial cell membranes [53].

3.3. Biopreservation of Shrimp through CLE/Cht-NPs Edible Coatings

3.3.1. Impact on Microbiological Quality of Stored Shrimps. The influences of shrimps' coating with composed EC contained 1.5% w/v from Cht-NPs and its fortified blends

TABLE 1: Antibacterial activity of produced natural products; clove extract (CLE), nanochitosan (Cht-NPs), and their composites (CLE/Cht-NPs) against examined food-borne pathogens.

Antibacterial agent	Inhibition zone (mm)*		
	<i>Salmonella typhimurium</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>
CLE	8.9 ± 0.4 ^a	8.4 ± 0.6 ^a	8.7 ± 0.7 ^a
Cht-NPs	9.3 ± 0.3 ^a	8.8 ± 0.5 ^a	9.0 ± 0.6 ^a
CLE/Cht-NPs	12.7 ± 0.9 ^b	12.1 ± 0.8 ^b	11.4 ± 1.1 ^b

*Results represent means of triplicates ± SD. **Different superscript letters in the same column indicate significant difference at $p < 0.05$.

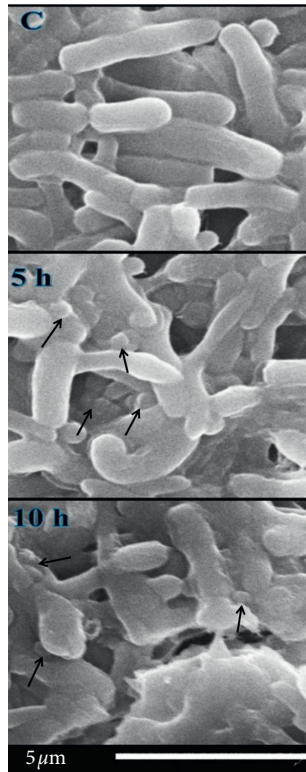


FIGURE 3: Scanning micrographs of treated *Salmonella typhimurium* cells with clove extract/chitosan nanoparticles* for 0, 5, and 10 (h) *The arrows in micrographs indicate selected examples of interacted NPs.

with CLE at percentages of 0.5, 1.0, and 1.5% (w/v), on the microbial load during refrigerated storage at $4 \pm 1^\circ\text{C}$, are illustrated (Figure 4). While all examined microbial groups (Total aerobic microorganisms, *E. coli*, Enterobacteriaceae and coagulase-positive staphylococci) tended to sharply increase, in control (uncoated) groups, with storage time prolongation, the inhibitory effects of ECs on these microbial groups were very remarkable.

The ECs formulation could significantly reduce the microbial loads in treated shrimps, compared with uncoated control samples. Generally, the EC antimicrobial efficacy increased with CLE percentage increment; the most effectual EC mixture contained 1.5% w/v from Cht-NPs with 1.0 or 1.5% from CLE. These combinations could reduce the counts of *E. coli* and coagulase-positive staphylococci to zero after 4 and 6 days of cold storage, respectively. The Cht-NPs based EC was remarkably effective in hindering microbial growth, and its efficacy notably increased with CLE fortification.

The strong antimicrobial potentialities (of both Cht and Cht-NPs) were proved and confirmed against numerous species from bacteria (Gram positives and negatives), fungi, and yeasts [1–3, 6, 9, 49, 51], either using *in vitro* evaluation or in food models [5, 24, 32, 33, 54]. These antimicrobial potentialities of Cht were attributed to its superficial bioactive positive charges, high chelation, and antioxidant capabilities, and its strong interactions with microbial cells components. The Cht-NPs were reported to have extra bioactivities and powers than bulk Cht due to their interacting surface area and their minute size that lead to more effectual interactions with microbial cells [4, 6, 8, 43]. The microbial inhibitory action of Cht-NPs coating, especially toward aerobic microorganisms, is assumingly attributed to oxygen parrying capability of Cht coating films that prevents the essential O_2 penetration for microbial breathing [23, 24, 33].

The antimicrobial consequences generated from *S. aromaticum* extract and oil were recurrently stated toward wide pathogenic microorganisms varieties, especially food-borne species [15, 16, 19, 22, 47]; they mainly attributed these microbicidal impacts to CLE and oil contents from bioactive constituents, particularly eugenol, caryophyllene, and other phenolics. The high amounts from TPC (total phenolic compounds) in CLE were suggested as the main reason for its influential antimicrobial activity [47, 53]. The combined synergistic antimicrobial effect of CLE and Cht-NPs persisted for the duration of storage time, and that was illustrated for many formulated EC from Cht and other plant extracts [24, 28, 34, 35, 55].

3.3.2. Impact on Sensorial Quality of Stored Shrimps. The influences of shrimp coating with 1.5% solution from Cht-NPs and their fortified blends with CLE (at 0.5, 1.0 and 1.5% concentrations), on the sensorial attributes after refrigerated storage ($4 \pm 1^\circ\text{C}$) for 7 days, are presented in Figure 5.

Whereas, in the beginning of the EC experiment, no significant differences were observed for the examined sensory attributes of all samples (data not included), the panelists scores for the examined attributes (appearance, odor, color, and texture) revealed that the control (uncoated) sampled became inconsumable after this time, regarding the acceptance level of 5/9 as the limiting value. The entire sensorial scores for the control group were significantly much lower than EC treated samples. The best composition of EC blends, to preserve appearance, odor, and color of stored shrimps, contained 1.5% from Cht/NPs and 1.0% from CLE, whereas the best for texture attribute contained 1.5% from both agents.

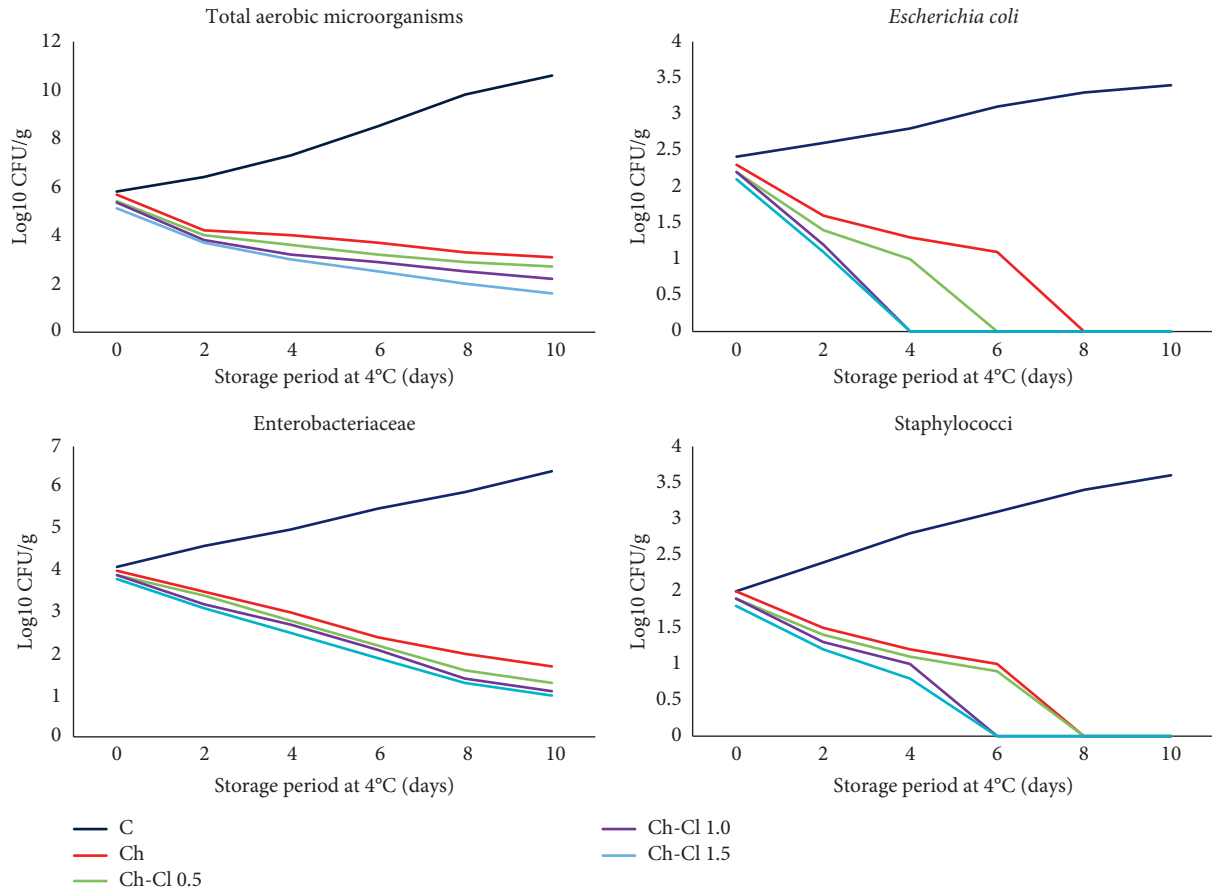


FIGURE 4: Influence of shrimp coating with 1.5% solution from nanochitosan (Ch) and fortified nanochitosan solution with 0.5, 1.0, and 1.5% (w/v) from clove extract (Ch-Cl 0.5, Ch-Cl 1.0, and Ch-Cl 1.5, respectively) on the microbial load during refrigerated storage ($4 \pm 1^\circ\text{C}$) for 10 days, compared to control (C)*. *Results represent means of triplicates.

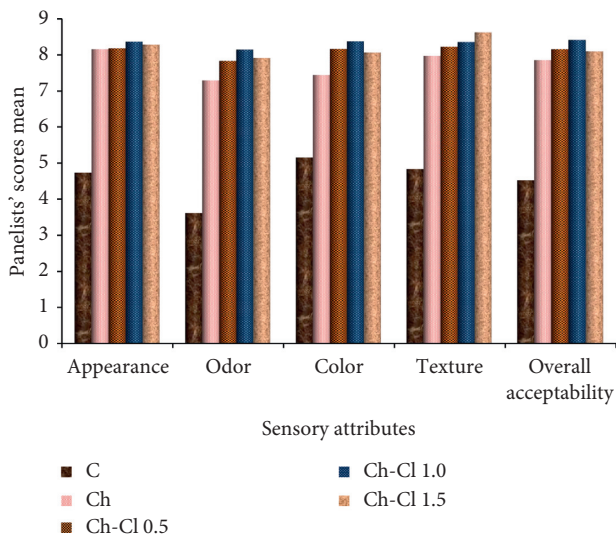


FIGURE 5: Influence of shrimp coating with 1.5% solution from nanochitosan (Ch) and fortified nanochitosan solution with 0.5, 1.0, and 1.5% (w/v) from clove extract (Ch-Cl 0.5, Ch-Cl 1.0, and Ch-Cl 1.5, respectively) on the sensory attributes after refrigerated storage ($4 \pm 1^\circ\text{C}$) for 7 days, compared to control (C)*. *Results represent means of panelists' scores ($N=13$).

The quality upholding of appearance, color, and texture attributes, in EC treated shrimps, was notably evidenced, compared with non-EC (control) shrimps (Figure 6). Many health benefits from seafood are principally attributed to their elevated contents from beneficial lipids, especially omega 3 and long-chain PUFA (poly unsaturated fatty acids) [56]. But, these valued constituents in seafood are extremely susceptible to oxidation (resulting from autoxidation, photosensitized oxidation, peroxidation, lipoxygenase, or microsomal enzymes), which lead to off-flavors emergence, at any storage conditions.

The application of Cht-NPs and CLE-Cht-NPs based ECs for shrimps biopreservation led to sensory quality maintenance through the protection from oxidation. Cht was reported as powerful antioxidant material and chelating agent; this is assumed to make it able to bind with oxidation enzymes and inhibit their effects [32]. The oxygen parrying effect of Cht-NPs-based EC also protected shrimps from oxidative reactions that regularly happened [23, 24, 32, 35, 55]. Additionally, the efficacy of Cht to control lipids oxidation in cooked cod (*Gadus morhua*) was attributed to the influential capability of it for metal chelation [57].

The sensorial quality maintenance, in CLE/Cht-NPs coated shrimps, is noticeably correlated with their lowered microbial load after EC treatments, which is assumed to

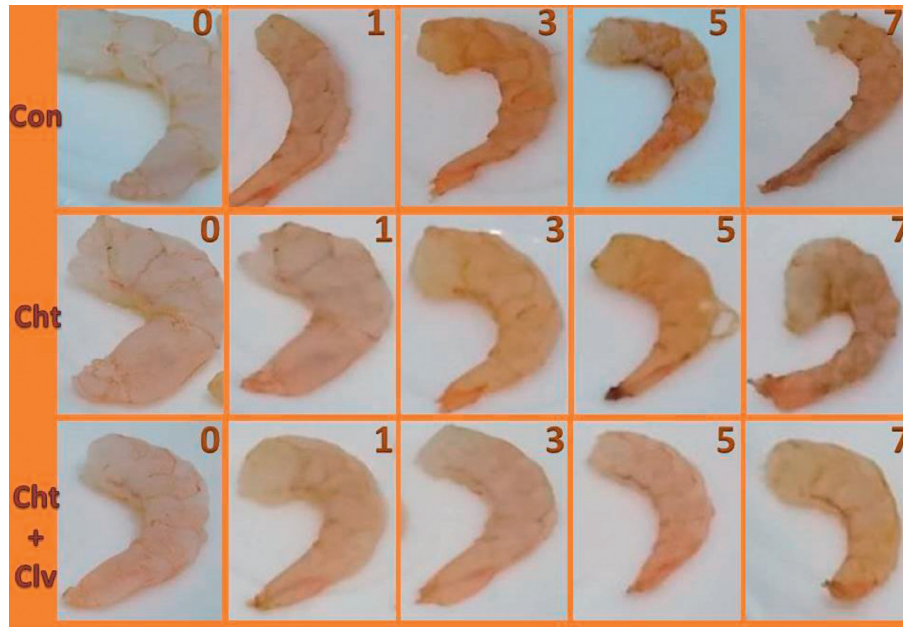


FIGURE 6: Influence of shrimp coating with 1.5% solution from nanochitosan (Cht) and fortified nanochitosan with 1% clove extract (Cht + Clv) on the appearance and texture of samples compared to control (Con) after refrigerated storage ($4 \pm 1^\circ\text{C}$) for 0, 1, 3, 5, and 7 days.

retard the microbial spoilage, melanosis formation and biochemical decompositions [35].

Many investigations recommended the application of Cht coatings and their blends with plant extracts/oils for shrimp biopreservation [24, 28, 34, 35, 55]. The achieved results here, for the microbiological and sensorial qualities, auspiciously exceeded those reported in these studies, which may be because of the usage of Cht-NPs (with higher antimicrobial and antioxidant activity than bulk Cht) in EC blends and the effectiveness of CLE for strengthen the NPs bioactivities. The CLE and oils convincingly stated to possess numerous phytoconstituents with high antimicrobial and antioxidant potentialities [16, 17, 19, 22, 47, 53]; these bioactivities are proved here to protect stored shrimps from spoilage signs and to augment the activity of Cht-NPs based coatings to uphold the shrimps' qualities during cold storage.

4. Conclusion

Toward the elimination of pathogenic food-borne bacterial pathogens and preserving the microbiological and sensorial qualities of stored shrimps, the bioactive formulations from nanochitosan (Cht-NPs) and cloves buds extract (CLE) were innovatively composited. The structural physiognomies of CLE/Cht-NPs were proved and their remarkable antimicrobial potentialities against bacterial pathogens were confirmed, along with their potential action modes. The coating treatment of unpeeled shrimps with CLE/Cht-NPs solutions resulted in sharp decrease in the microbial loads and the upholding of stored shrimps sensorial qualities during cold storage for 7 days. The CLE/Cht-NPs could be impressively recommended as effectual natural composites for the biopreservation of shrimp during cold storage.

Future investigations are suggested for further appraisal of CLE/Cht-NPs biopreservation actions in different foodstuffs and their biomedical consequences.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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