

Green extraction of chitosan from *pinctada maxima* shells: Comprehensive physicochemical properties and antibacterial performance for biomedical applications

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ABSTRACT

The growing demand for eco-friendly biomaterials with sustained bacterial activity highlights chitosan as a promising medical material, yet conventional extraction often yields inconsistent quality. This research established a sustainable extraction route by varying isolation sequence, NaOH concentration (40–60%), and microwave-assisted deacetylation time (0–20 min). Chitosan was characterized by FTIR, ¹H NMR, XRD, viscometry, EDX, and FESEM, and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was evaluated by broth microdilution and plate-count assays. The optimal process demineralization followed by deacetylation with 60% NaOH for 15 minutes under microwave irradiation (Ch-MW60-15) produced a high degree of deacetylation (95.37%), reduced molecular weight (206–148 kDa), lower viscosity (3.17–2.45 cP L/g), and higher crystallinity (60.89%). XRD and EDX indicated residual CaCO₃, so the product is best described as a high-DD chitosan-CaCO₃ composite. ¹H NMR showed characteristic chitosan resonances without a prominent acetyl-methyl signal (~2 ppm), confirming a high-DD backbone. FESEM revealed an interconnected porous-layered morphology with uniform particles (273.98 ± 10.49 nm), supporting improved antibacterial performance. Ch-MW60-15 reduced *Escherichia coli* (3.12 to ~2.0 log CFU/mL) and *Staphylococcus aureus* (3.0 to ~1.92 log CFU/mL) at 20 ppm, with complete inhibition at 80 ppm, indicating suitability for biomedical applications.

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1. Introduction

The rising worldwide alarm about antimicrobial resistance needs immediate solutions which require scientists to create sustainable biomaterials which possess natural antibacterial properties. Chitosan is a natural polysaccharide that scientists obtain from chitin through deacetylation. Chitosan has become a popular research material because its biocompatibility and biodegradability enable multiple biomedical uses such as wound dressings and scaffolds and drug delivery systems and implant coatings (Nurmaulida et al., 2023). Chitosan has medical applications but also serves as a functional biopolymer that manufacturers use in food packaging and agricultural products (Sánchez et al., 2021).

The high purity chitosan standard requires physicochemical property control which makes it hard to maintain consistent structural strength and operational capability. The functional and structural attributes of chitosan are determined by extraction parameters which include alkali type and processing sequence and deacetylation method (Dong et al., 2023). The traditional extraction method needs to apply strong alkaline treatment which damages polymer structure and creates more environmental pollution problems (Chandha et al., 2022). The development of extraction methods which use less energy and produce less waste should be our objective. The process of microwave-assisted deacetylation improves both reaction speed and product yield because it decreases the time and material requirements for chemical operations (Rahayu et al., 2025).

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The research field lacks sufficient systematic studies which assess multiple extraction parameters for chitosan derived from marine materials like *Pinctada maxima* shells (Nurlaili et al., 2022). The extraction method inconsistencies lead to chitosan physicochemical property changes which restrict its use in medical applications. The primary challenge lies in devising environmentally friendly isolation strategies that yield chitosan with controlled attributes (Yarnpakdee et al., 2022). The three processing parameters which include sequential isolation steps and alkali concentration and microwave-assisted deacetylation parameters need optimization to create high-quality chitosan that meets biomedical standards and supports sustainable development (Nurmaulida et al., 2023).

Researchers who want to improve chitosan extraction efficiency need to research chitosan extraction methods which apply green chemistry principles. The use of microwave-assisted deacetylation method leads to higher acetyl group cleavage efficiency which results in chitosan production with greater degrees of deacetylation (DD) and better solubility than traditional methods produce (Sánchez et al., 2021). The method achieves consistent temperature distribution which speeds up chemical reactions while protecting material integrity and maintaining operational capacity (Ways et al., 2018). The optimization of both alkali concentration and reaction time will produce direct changes to chitosan molecular weight and crystallinity and antibacterial properties. The use of higher NaOH concentrations leads to increased acetyl group cleavage which results in the creation of active amino functionalities that enable antimicrobial activity (Dong et al., 2023).

Marine materials, especially *Pinctada maxima*, are becoming popular as sustainable alternatives to traditional chitosan sources which come from crustaceans. The use of pearl oyster shell waste for chitosan production not only solves environmental issues from aquaculture by-products but also demonstrates its capability to create premium chitosan (Yarnpakdee et al., 2022). The initial research results indicate that the special mineral-to-protein ratio found in *Pinctada maxima* shells affects the deacetylation efficiency and the resulting chitosan characteristics, but detailed research on this topic is still limited (Nurlaili et al., 2022).

Researchers predominantly examine individual extraction parameters without considering their synergistic effects on chitosan's structural integrity and antibacterial efficacy, particularly against clinically significant pathogens, such as *Staphylococcus aureus* and *Escherichia coli* (Yarnpakdee et al., 2022). This gap necessitates a systematic approach that correlates processing conditions with both structural characteristics and bioactivity, contributing to the establishment of standardized protocols for producing high purity chitosan (Ani et al., 2023).

The research investigates new green isolation methods for chitosan that scientists develop from *Pinctada maxima* shell waste through their research on different processing techniques which affect the material's chemical properties and its ability to fight bacteria. The research establishes optimal processing conditions which enhance chitosan through its implementation of an integrated approach that tests multiple sequential isolation methods with different alkali levels and microwave deacetylation times. The study measures essential factors through the assessment of degree of deacetylation, molecular weight, crystallinity, elemental composition, surface morphology, and antibacterial effectiveness against *Staphylococcus aureus* and *Escherichia coli* pathogens. The research combines green processing methods with functional performance testing to create a framework which enables the production of sustainable chitosan materials that meet high standards for biomedical uses.

2. Materials and methods

2.1. Materials

Shell waste of *Pinctada maxima* (Jameson, 1901) was collected from aquaculture sites in Lombok, Indonesia (8°23'23.7"S 116°06'05.1"E) (MolluscaBase, 2026). The shells were thoroughly washed, dried at 60°C, and ground into fine powder using a conventional milling unit (FGD-Z100, Fomac, Indonesia). Analytical-grade reagents were employed, including sodium hydroxide (NaOH, 40–60%, Merck, Germany), hydrochloric acid (HCl, 1 M, Mallinckrodt, USA), ethanol (95%, Merck, Germany), and sodium hypochlorite (NaOCl, Merck, Germany). Deionized water was used for all solution

preparations. Commercial chitosan (CDH, India) was used as a reference control. The main instruments utilized for preparation and isolation included a pulp grinder (SY-150, Yamamoto, Indonesia), milling unit (FGD-Z100, Fomac, Indonesia), magnetic stirrer and hot plate (IKA-CMAG HS7, Germany), drying oven (Mito, Indonesia), and microwave unit (Sharp, Indonesia).

2.2. Green isolation of chitosan

The isolation of chitosan from *Pinctada maxima* shells was conducted through a sequence of environmentally friendly steps, namely deproteinization, demineralization, decolorization, and deacetylation. Initially, the shells were thoroughly cleaned, oven-dried at 60°C, and ground into fine powder (<200 µm). For deproteinization, 80 g of shell powder was treated with 3.5% NaOH solution (1:10 m/v) at 80°C for 1 h, followed by repeated washing with deionized water until neutral pH and drying. Demineralization was carried out using 1 M HCl (1:15 m/v) at room temperature with continuous stirring for 3 h, followed by neutralization and drying to yield purified chitin. Decolorization was performed with 0.5% NaOCl (1:10 m/v) at 75°C for 1 h, and the obtained residue was washed and dried (Figure 1).

The crucial deacetylation step was performed using either conventional heating or microwave-assisted treatment. In the conventional method, the chitin powder was dispersed in 40% NaOH (1:20 m/v) and heated at 120°C for 3 h, generating samples Ch-Cv40-P (isolation sequence initiated with deproteinization) and Ch-Cv40-M (isolation sequence initiated with demineralization). In the microwave-assisted method, chitin was treated with NaOH solutions of 40%, 50%, or 60% (w/v), homogenized for 1 h, and subjected to microwave irradiation at 600 W for different durations (0, 5, 10, 15, and 20 min). This produced the Ch-MW40-15, Ch-MW50-15, and Ch-MW60-15 variants, along with the Ch-MW60-0, Ch-MW60-5, Ch-MW60-10, Ch-MW60-15, and Ch-MW60-20 series. Following deacetylation, all samples were thoroughly washed with deionized water until neutral pH, oven-dried at 60°C, and stored in desiccators. The systematic variation of isolation sequences, alkali

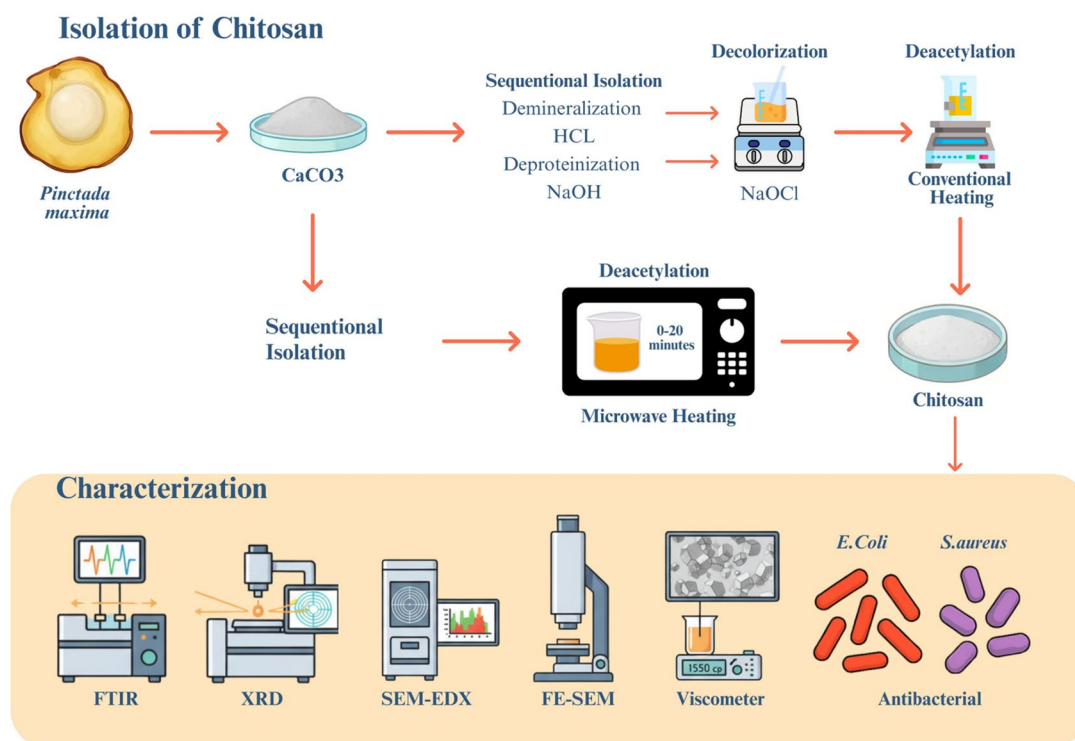


Figure 1. Illustration of the process of chitosan isolation from pearl oyster (*Pinctada maxima*) shells, showing sequential steps of demineralization (HCl), deproteinization (NaOH), and microwave-assisted deacetylation leading to the formation of medical-grade chitosan.

concentration, and microwave irradiation times provided a comparative framework to evaluate the efficiency of green extraction and the resultant quality of medical-grade chitosan.

2.3. Physicochemical characterization

The isolated chitosan samples underwent complete physicochemical analysis through multiple analytical methods which are displayed in Figure 1. The researchers conducted functional group identification and degree of deacetylation (DD) measurement using Fourier-transform infrared spectroscopy (FTIR) which operated between 4000 and 500 cm^{-1} through KBr pellets. The scientists utilized X-ray diffraction (XRD, Bruker D8 Advance) analysis with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) between 5 and 60° to study crystallographic properties while using standard equations to determine crystallite size, crystallinity index, dislocation density, and microstrain. The Ubbelohde viscometer determined intrinsic viscosity of chitosan samples which were tested at 25 °C in 0.3 M acetic acid/0.2 M sodium acetate and the Mark–Houwink equation provided molecular weight estimation. Energy-dispersive X-ray spectroscopy (EDX) combined with field emission scanning electron microscopy (FESEM, Quanta FEG650, FEI USA) was used to analyze elemental composition and surface morphology which included uniformity, porosity, particle distribution, connectivity, carbon, oxygen, nitrogen, calcium, and magnesium quantification. Nuclear magnetic resonance (NMR) spectroscopy served as a verification method to confirm the chemical structure of the organic fraction in the chosen sample by comparing it with standard chitosan NMR spectra which were obtained at 500 MHz in D₂O under proton-decoupled conditions. The researchers established a complete understanding of chitosan structural and compositional characteristics through their research on different methods of isolating chitosan.

2.4. Antibacterial activity

The antibacterial activity of the synthesized chitosan powders was assessed against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) using the broth microdilution method, which applied standard microbiological protocols that underwent minor changes during execution. For the determination of the Minimum Inhibitory Concentration (MIC), chitosan samples were initially dissolved in 1% (v/v) acetic acid and then researchers diluted the solution to create different concentrations between 20 and 80 ppm. Researchers prepared bacterial suspensions in Mueller-Hinton broth and then adjusted the turbidity to match the 0.5 McFarland standard, which represents a bacterial density of approximately 1×10^6 CFU/mL. The researchers added 200 μL of each bacterial inoculum to sterile 96-well microplates, which contained serial dilutions of the chitosan solutions. The experimental setup included: negative control (bacteria without chitosan), positive control (bacteria with commercial chitosan), and blank control (no chitosan).

The plates were incubated at 37 °C for 24 h under aerobic conditions. Bacterial growth was monitored by using a UV–vis spectrophotometer to measure the optical density at 410 nanometers through a microplate reader. A McFarland calibration curve was created through the process of plotting absorbance values against known bacterial concentrations which scientists used to determine cell density in liquid cultures. The Minimum Inhibitory Concentration (MIC) was established as the lowest chitosan concentration that produced no visible turbidity and showed no significant increase in absorbance when compared to the negative control.

The experimenters used 10 μL samples from growth-free wells to make 10^{-1} dilutions by aseptically mixing the samples with 9 mL Buffered Peptone Water (BPW) solution. Serial dilutions (10^{-2} to 10^{-5} , as required) were prepared by transferring 1 mL of the previous dilution into 9 mL BPW. From each dilution, 1 mL aliquots were plated in duplicate onto sterile Petri dishes. Subsequently, 15–20 mL of Plate Count Agar (PCA), cooled to $45 \pm 1^\circ\text{C}$, was poured into each dish, gently mixed, and allowed to solidify. The plates were incubated at $35 \pm 1^\circ\text{C}$ for 24 to 48 hours with their inverted position. The Minimum Bactericidal Concentration (MBC) was defined as the lowest chitosan concentration resulting in complete bacterial inactivation, indicated by the absence of colony growth (0 CFU per plate).

Plates containing 25–250 colonies were selected for enumeration. The viable bacterial count was calculated using the following equation:

$$\text{CFU / mL} = (\Sigma C) / (n \times V) \times d$$

The formula to calculate total colonies shows that ΣC equals the total number of colonies counted and n represents the number of plates and V denotes the plated volume in milliliters and d stands for the dilution factor. The report indicated colony counts which exceeded the range of 25 to 250 according to the established notation system. Spreader colonies were interpreted according to standard total plate count guidelines and included in the calculation when applicable. All experiments were conducted in duplo ($n=2$), and results are presented as mean $\pm$ standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant. The antibacterial assessment utilized representative photographs of agar plates together with colony count statistics as supporting evidence.

3. Results

The ongoing problem of bacterial resistance requires development of sustainable biomaterials that can provide consistent antibacterial protection. Chitosan serves as a potential material because its natural properties enable it to work as a biocompatible and bioactive substance. This research study developed optimal methods to extract high purity chitosan from *Pinctada maxima* shells through testing various processing steps and alkali concentrations and microwave-assisted deacetylation methods on material properties and antibacterial effectiveness.

3.1. Chemical structure and macromolecular properties

The FTIR spectra results demonstrated that different extraction methods produced unique structural characteristics in the chitosan samples which were tested (Figure 2). The samples displayed typical absorption patterns which included O–H stretching at approximately 3400 cm^{-1} and C–H stretching

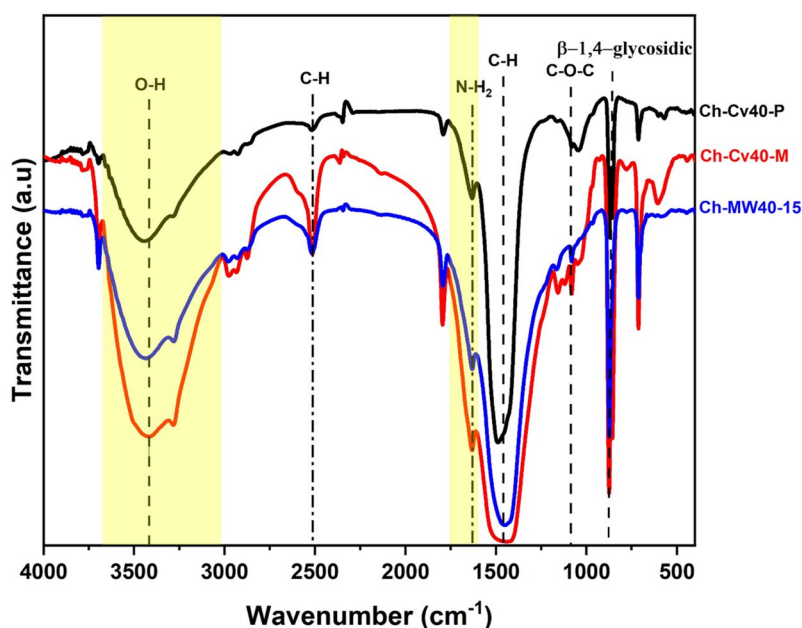


Figure 2. Infrared spectral profiles of chitosan obtained through different sequential processing steps, illustrating variations in characteristic absorption bands corresponding to O–H, N–H, and C–O–C vibrations that confirm successful chitin-to-chitosan conversion.

at about 2900 cm^{-1} and N-H bending of primary amines at 1630 cm^{-1} and C-O-C vibrations of glycosidic linkages which occurred at 1080 cm^{-1} . Ch-Cv40-M showed greater amine absorption with an 81.20% DD value because it used demineralization as its first step while Ch-Cv40-P which used deproteinization as its first step reached a 75.56% DD value. Microwave-assisted deacetylation. Ch-MW40-15 produced sharper bands in the amine region which achieved the highest DD of 95.37% because the processing sequence and microwave treatment determined chitosan's structural quality. The FTIR analysis results showed that NaOH concentration changes during deacetylation induced significant chemical structure alterations in chitosan (Figure 3). The samples all showed the essential chitosan functional groups which included hydroxyl (O-H) stretching at around 3400 cm^{-1} and C-H

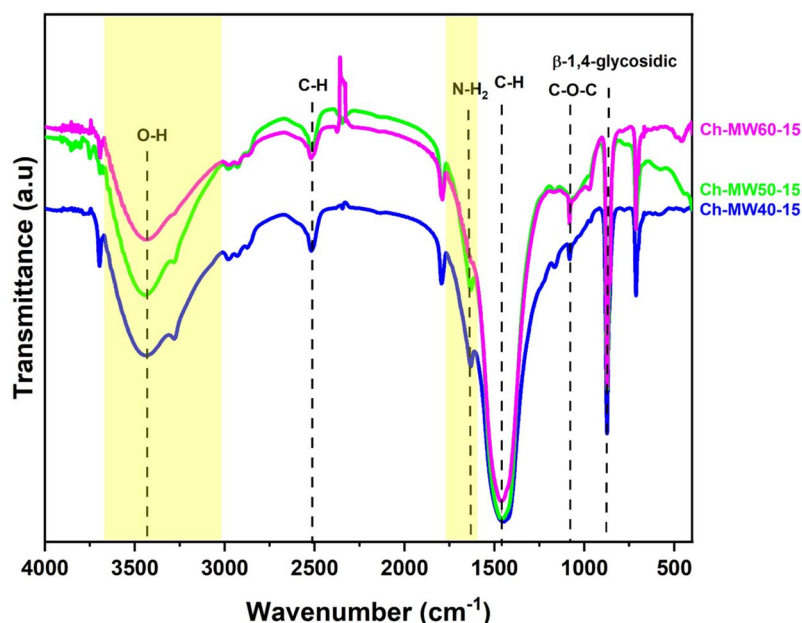


Figure 3. Infrared spectra of chitosan samples obtained using different NaOH concentrations during the deacetylation process, showing variations in the intensity of characteristic absorption bands related to hydroxyl, amine, and glycosidic linkages that reflect changes in the degree of deacetylation.

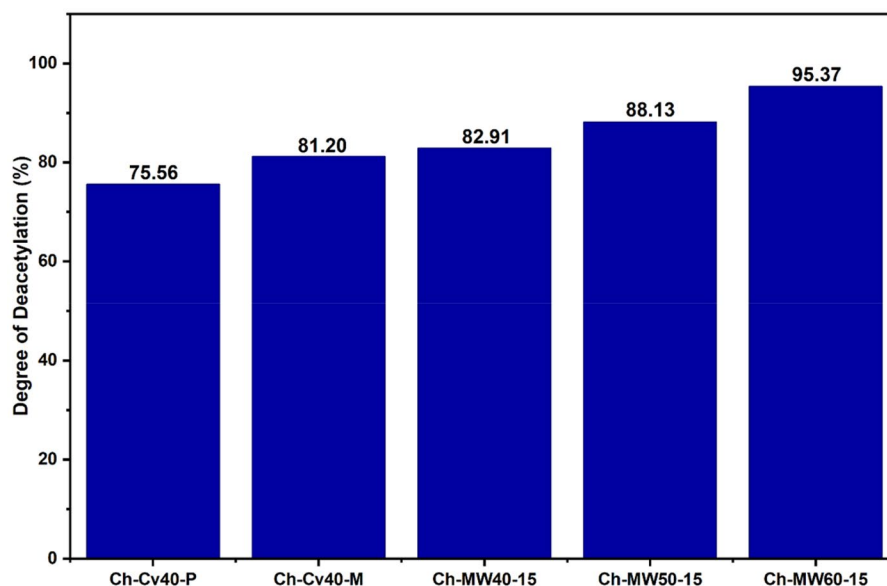


Figure 4. Effect of different isolation methods on the degree of deacetylation (DD) of chitosan, demonstrating how variations in sequential processing influence the efficiency of acetyl group removal and the structural conversion of chitin into chitosan.

stretching at near 2900 cm^{-1} and N-H bending of primary amines at $\sim 1630\text{ cm}^{-1}$ and glycosidic C-O-C vibrations at near 1080 cm^{-1} . The treatments produced different band intensity results according to the different methods which were applied.

Chitosan obtained with 40% NaOH (Ch-MW40-15) exhibited moderate amine absorption, corresponding to a DD of 82.91%. Increasing the NaOH concentration to 50% (Ch-MW50-15) enhanced the sharpness of the amine band, yielding a higher DD of 88.13% (Figure 4). The 60% NaOH solution produced the highest absorption value which resulted in a 95.37% degree of deacetylation for Ch-MW60-15. The process of acetyl group cleavage becomes more efficient when NaOH concentrations increase which results in better chemical conversion from chitin to chitosan.

The FTIR spectra of chitosan obtained using varying microwave durations showed characteristic bands for hydroxyl ($3434\text{--}3449\text{ cm}^{-1}$), C-H stretching ($2519\text{--}2521\text{ cm}^{-1}$), primary amine bending ($1630\text{--}1655\text{ cm}^{-1}$), C-H bending ($1447\text{--}1461\text{ cm}^{-1}$), glycosidic C-O-C ($\sim 1082\text{ cm}^{-1}$), and β -1,4 linkages ($872\text{--}874\text{ cm}^{-1}$) (Figure 5). The study found that the O-H and amine bands experienced a shift which showed elevated levels of free amine production and deacetylation in the Ch-MW60-15 sample through its 1655 cm^{-1} band measurement. The polysaccharide backbone remained untouched during all treatment processes. The DD values started from 83.29% at Ch-MW60-0 and reached 95.37% at 15 minutes before dropping to 91.15% at 20 minutes according to Figure 6. The study showed that microwave irradiation increased deacetylation efficiency until it reached its peak point. The study presents Table 1 as further evidence of how different microwave exposure times affect the molecular weight and viscosity aspects of chitosan macromolecular characteristics.

3.2. Crystal structure and elements

The X-ray diffraction (XRD) patterns of chitosan obtained using different microwave exposure times are presented in Figure 7. The samples showed their semicrystalline chitosan structure by displaying characteristic peaks that occurred between 2θ values of 10 degrees and 36 degrees. The study showed that microwave treatment generated major changes to the crystallographic characteristics. The crystallite size ranged from 592.17 nm to 847.67 nm while the crystallinity index showed values between 40.50% and 60.89%. The exposure time of Ch-MW60-0 who received no microwave treatment resulted in 44.44% crystallinity but Ch-MW60-5 who received five minutes of microwave exposure achieved

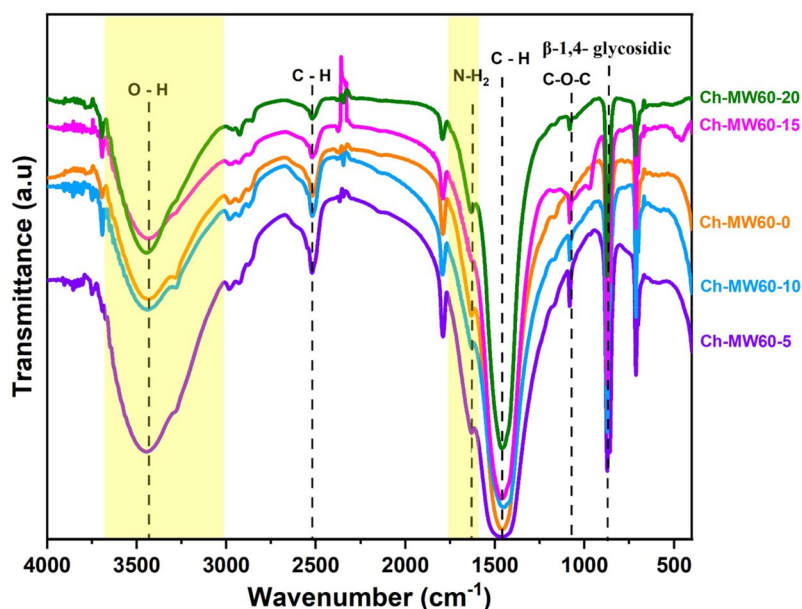


Figure 5. Infrared spectral analysis of chitosan as influenced by microwave-assisted deacetylation duration, illustrating variations in characteristic absorption bands corresponding to hydroxyl, amine, and glycosidic linkages that indicate progressive structural modification with increasing irradiation time.

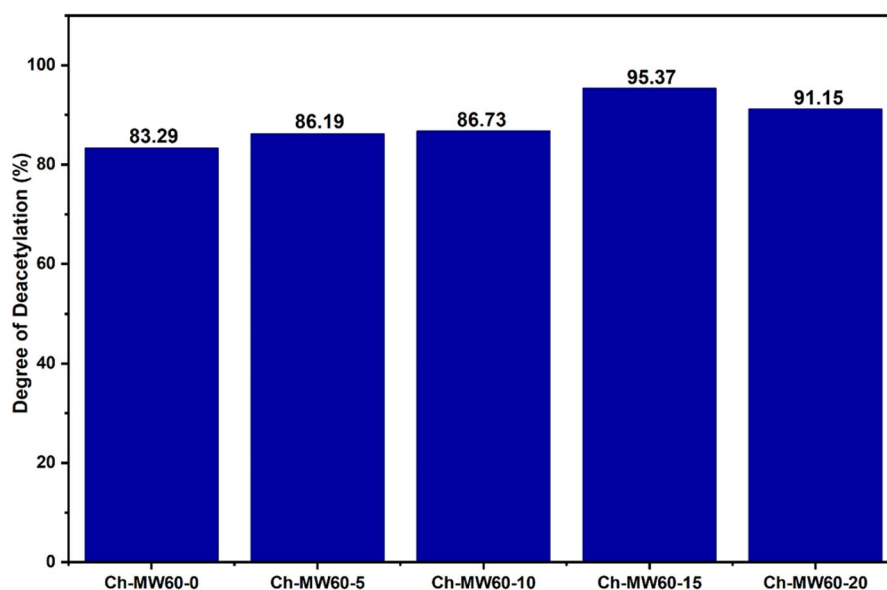


Figure 6. Effect of microwave-assisted deacetylation duration on the degree of deacetylation (DD) of chitosan, showing how increased microwave exposure enhances acetyl group cleavage efficiency up to the optimal 15-minute treatment before declining at longer durations due to partial degradation.

Table 1. Macromolecular characteristics of chitosan as influenced by variations in microwave exposure.

Sample	Viscosity (Cp)	Molecular Weight (kDa)	Moisture Content (%)	Protein Content (%)
Ch-MW60-0	3.17	206.08	0.85	0.91
Ch-MW60-5	2.99	195.20	0.56	1.21
Ch-MW60-10	2.92	188.91	0.64	1.39
Ch-MW60-15	2.45	148.20	1.70	0.70
Ch-MW60-20	2.83	180.90	3.23	1.43

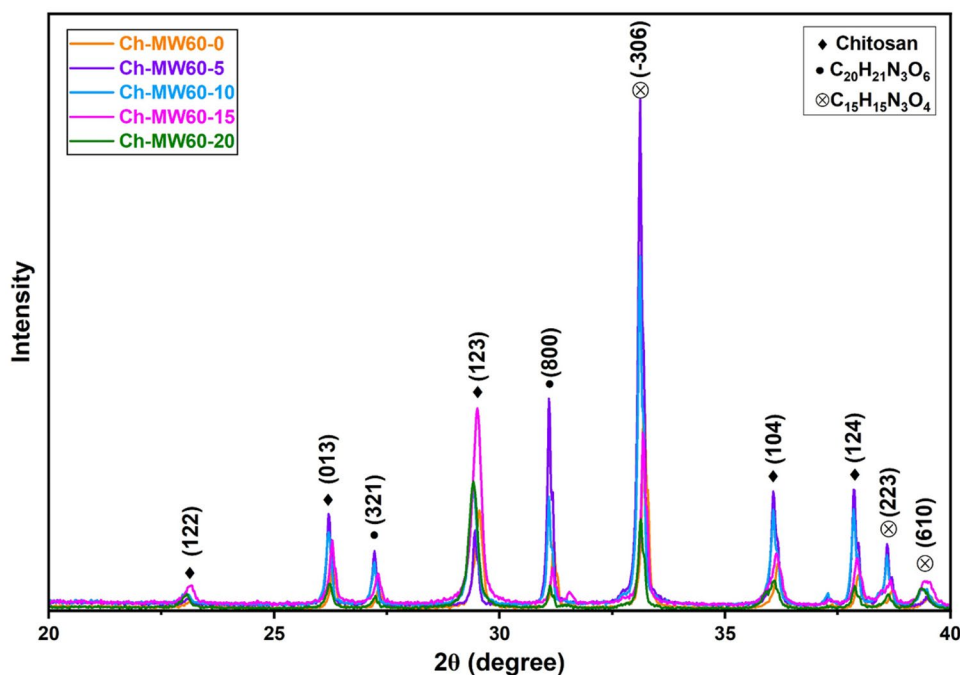


Figure 7. Effect of microwave-assisted deacetylation time on the crystalline structure of chitosan, showing variations in peak intensity and crystallinity index that indicate molecular rearrangement and improved crystal order up to the optimal 15-minute irradiation period.

56.67% crystallinity. Prolonged heating for 10 min (Ch-MW60-10) reduced the crystallite size to 592.17 nm, with 54.58% crystallinity. The highest crystallinity (60.89%) was observed at 15 min (Ch-MW60-15), with the corresponding crystallite size being 747.14 nm. After extended exposure (Ch-MW60-20), crystallinity decreased to 40.50%, with a crystallite size of 602.54 nm. Furthermore, EDX analysis showed a reduction in residual mineral elements, such as Ca and Mg, across samples, indicating an improvement in the elemental purity of chitosan with optimized microwave treatment.

The elemental compositions of chitosan obtained using different microwave-assisted deacetylation durations are presented in [Figure 8](#). The residual mineral elements, particularly Ca and Mg, progressively decreased as microwave exposure increased. The Ca content of the untreated sample (Ch-MW60-0) was high (22.12%), but longer microwave treatments substantially lowered this value, reaching 2.91% at 20 min (Ch-MW60-20). Similarly, Mg contents decreased from 0.09% in Ch-MW60-0 to 0.19%–0.24% in the microwave-treated samples, indicating the partial removal of mineral impurities. The C fraction increased from 16.38% (Ch-MW60-0) to 64.43% (Ch-MW60-20), whereas the O content decreased, consistent with progressive deacetylation. The study showed that N levels decreased during extended treatment from 6.44% to 2.01%. The study demonstrated that optimized microwave exposure process led to improved elemental purity for chitosan because it removed mineral contaminants and increased organic material content.

3.3. Morphology and particle characteristics

The study found that chitosan samples which underwent different microwave treatments showed unique surface structure changes according to their microwave treatment duration. The untreated sample (Ch-MW60-0) exhibited a rough surface with compact, irregularly arranged structures. The microwave treatment for 5 minutes (Ch-MW60-5) caused the surface to become denser while the particles aggregated together because of their partial structural changes. The morphology of the sample which underwent 10 minutes of treatment (Ch-MW60-10) developed a more organized structure with layered patterns that formed smoother surfaces, which helped the molecules to achieve better alignment. The sample treated for 15 min (Ch-MW60-15) displayed a porous yet compact structure with well-distributed surface features; this morphology was the most homogeneous across all samples. However, prolonged exposure (20 min; Ch-MW60-20) increased the surface roughness and particle agglomeration, reflecting a loss of uniformity and partial structural collapse. These morphological variations indicate that the microwave exposure duration strongly influences surface uniformity and porosity, providing a structural basis for differences in the particle characteristics of chitosan ([Figure 9](#)).

FESEM and particle size analyses ([Figure 10](#)) revealed that microwave-assisted deacetylation markedly influenced the morphology and size distribution of chitosan powders. The untreated sample (Ch-MW60-0) showed irregular, agglomerated granular structures and a broad particle size range (100–800 nm, mean 273.59 ± 12.09 nm). The powders reached their maximum density after 5 minutes of treatment (Ch-MW60-5) because the material became denser and maintained its structure between 100 and 250 nanometers while averaging 164.20 ± 2.19 nanometers. The treatment for 10 minutes (Ch-MW60-10) resulted in secondary particle aggregation which created denser particle clusters that reached an average diameter of 349.83 ± 8.03 nanometers. The 15-minute treatment (Ch-MW60-15) produced the best particle shape which showed evenly spread small particles and a continuous surface with moderate-sized particles that measured between 200 and 400 nanometers and had an average size of 273.98 ± 10.49 nanometers, which showed improved stability. The extended treatment duration (Ch-MW60-20) resulted in the formation of compact uniform spherical particles which displayed a narrow size range that extended from 200 to 300 nanometers and had an average diameter of 235.18 ± 4.69 nanometers. The duration of microwave treatment needed to produce stable chitosan powders reached its best point at 15 minutes because this time produced particles of specific size and maintained their physical structure throughout the process. The biomedical applications gain advantages from nanoscale uniformity and morphological compactness because these features increase solubility and bioactivity and compatibility with scaffold and composite systems.

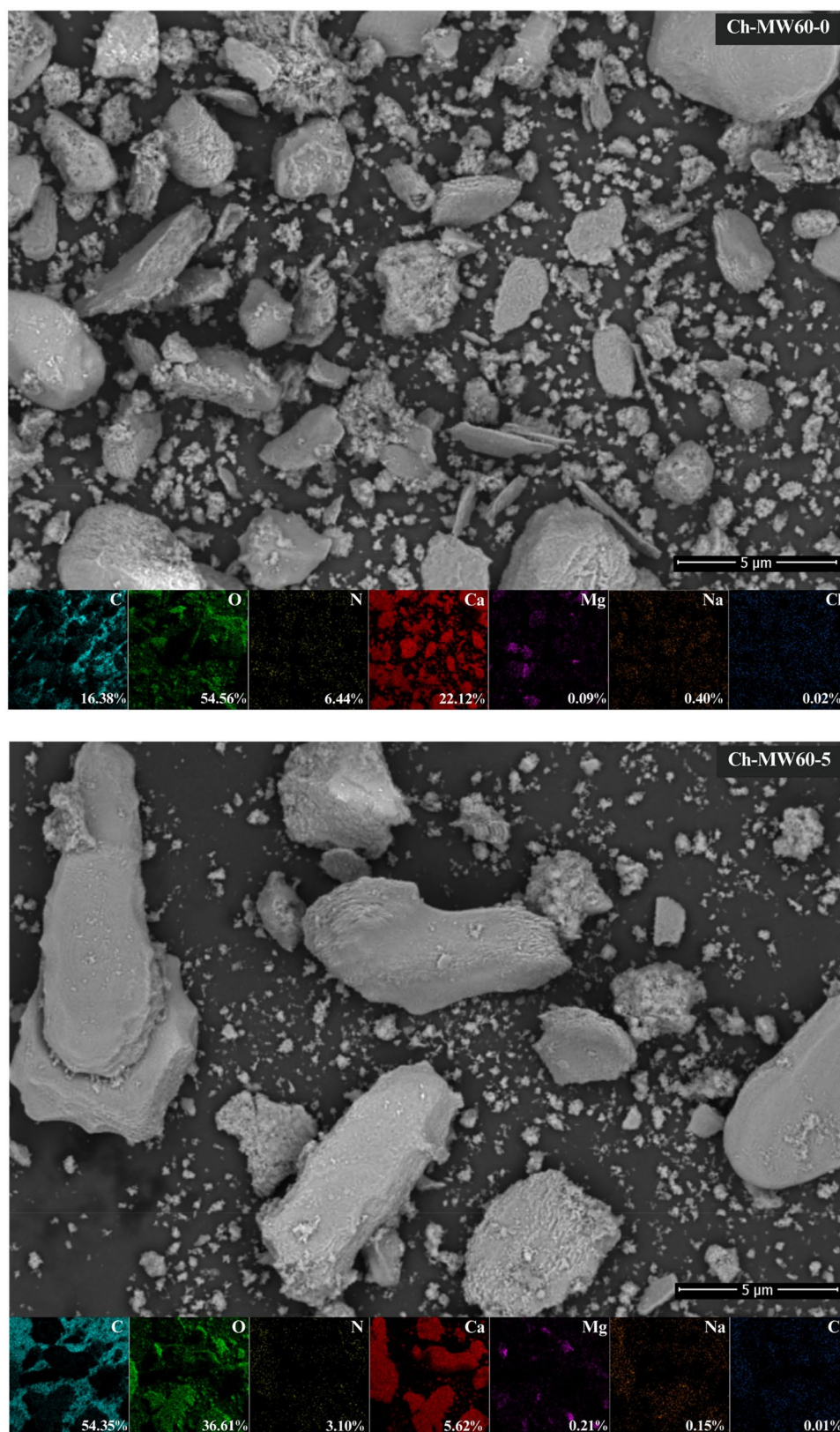


Figure 8. Effect of microwave-assisted deacetylation duration on the elemental composition of chitosan as determined by energy-dispersive X-ray spectroscopy (EDX). The analysis reveals a systematic decrease in residual inorganic elements (Ca and Mg) and a proportional enrichment of carbon and nitrogen content, reflecting improved purity and enhanced organic backbone integrity at optimal exposure durations. An EDX compositional profile comparing chitosan samples treated with varying microwave-assisted deacetylation times. The figure demonstrates that increasing irradiation time significantly reduces mineral residues and enhances the carbon-to-nitrogen ratio, indicating superior deacetylation efficiency and compositional refinement consistent with medical-grade chitosan characteristics.

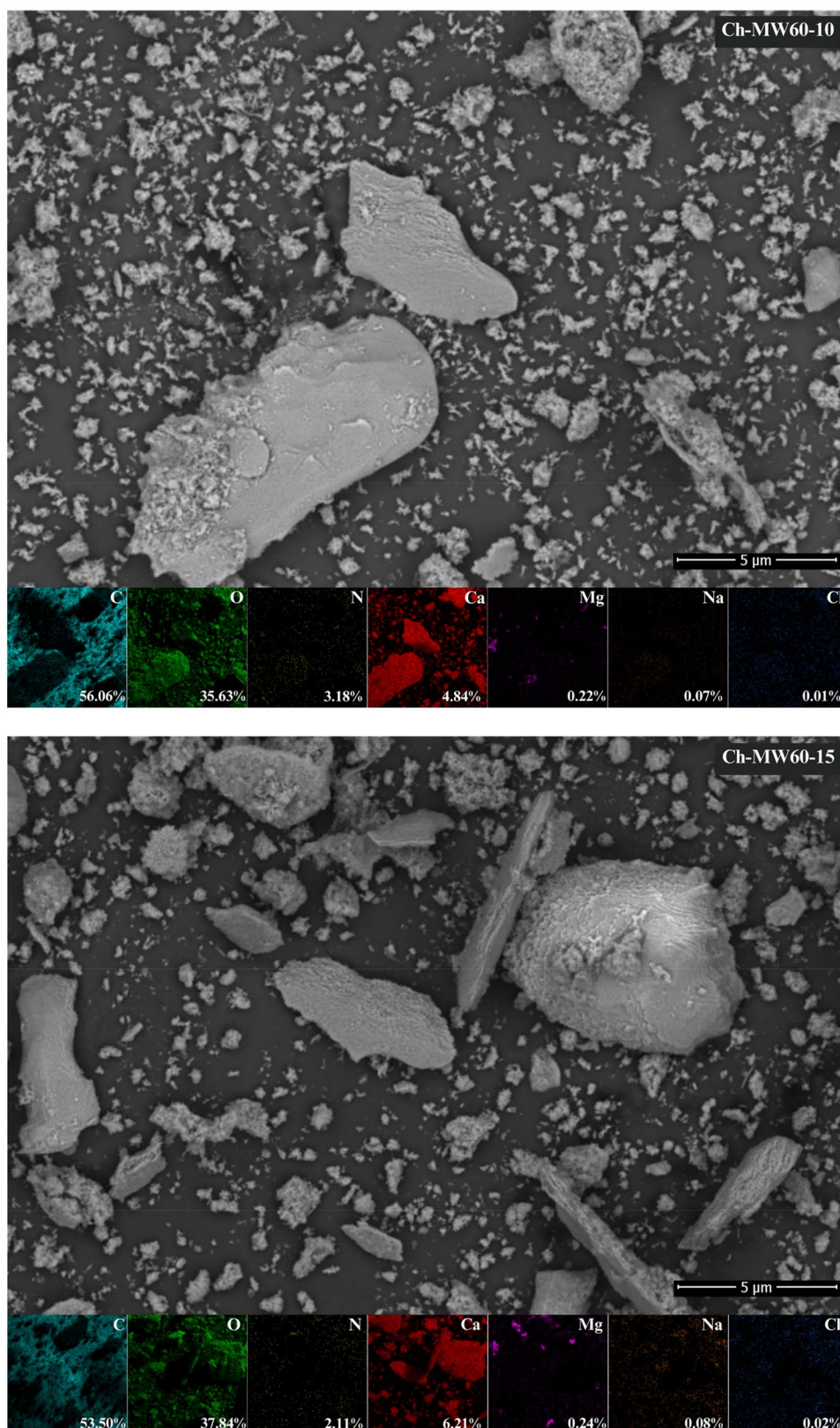


Figure 8. Continued.

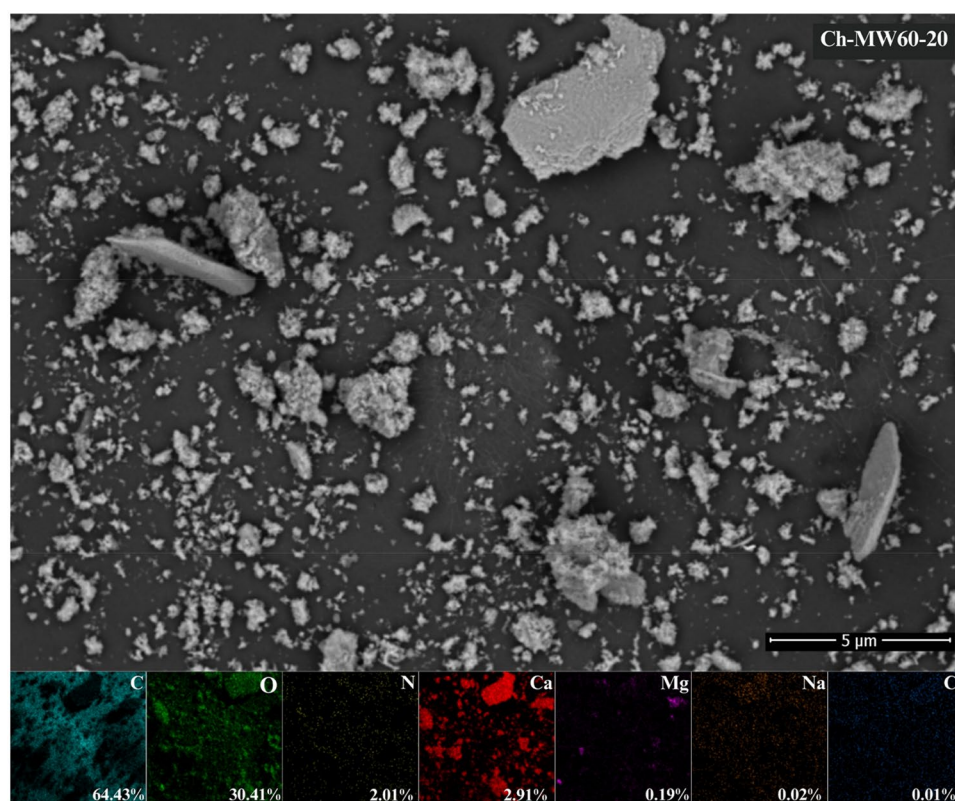


Figure 8. Continued.

3.4. Chemical structure and purity

Nuclear magnetic resonance (NMR) spectroscopy served as a confirmatory method which confirmed both the chemical structure and purity testing of the organic fraction from the selected sample (Ch-MW60-15) through comparison with standard chitosan. The ^1H NMR spectra were recorded at 500 MHz in D_2O (with water presaturation for the isolated sample). The ^1H NMR spectrum of the chitosan standard shows the typical chitosan polysaccharide-region resonances (carbohydrate protons in the $\sim 3.0\text{--}4.0$ ppm region) together with a signal at $\delta \approx 2.03$ ppm assigned to the acetyl methyl protons ($-\text{NHCOCH}_3$), indicating the presence of residual N-acetylated units (GlcNAc) in the reference material. The D_2O solution exhibits a strong HDO/water resonance that appears near $\delta \approx 4.79$ ppm and this resonance creates an obstruction which affects the visibility of the anomeric region. The ^1H NMR spectrum of the isolated sample (Ch-MW60-15) shows an abdominal region which contains its main signals that extend from peak-picks at δ 4.651–4.864 ppm while the acetyl methyl resonance near $\delta \approx 1.9\text{--}2.1$ ppm is not prominent, qualitatively supporting a higher degree of deacetylation (DD) relative to standard chitosan. The analysis shows a small resonance which appears at $\delta \approx 2.671$ ppm and this resonance does not match the characteristics of native chitosan but it most likely originates from trace impurities or leftover manufacturing materials.

3.5. Antibacterial performance

Chitosan showed distinct antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* (Tables 2 and 3; Figures 11 and Li & Wu, 2019). The data in Tables 2 and 3 show antibacterial results which Figure 11 confirms through its demonstration of how Ch-MW60-15 inhibits *Escherichia coli* (Figure 11(a)) and *Staphylococcus aureus* (Figure 11(b)) during 24 hours of testing. The negative control plates in both panels exhibit dense bacterial colonies, confirming normal microbial growth in the absence of chitosan. The experiment shows that increasing chitosan concentrations from 20 to 80 ppm leads

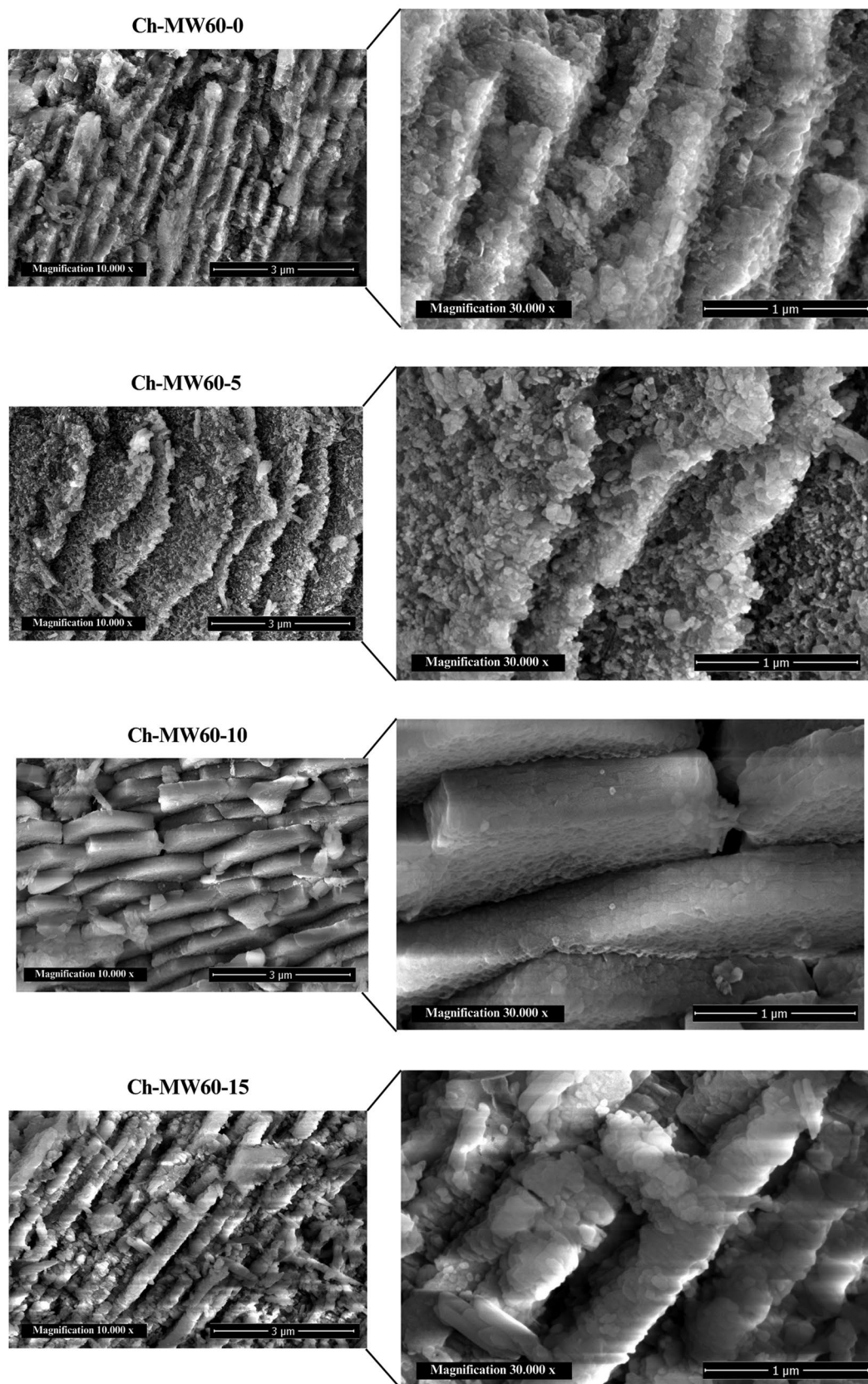


Figure 9. Surface morphology of chitosan as influenced by variations in microwave-assisted treatment, showing distinct structural transformations from compact, irregular surfaces in untreated samples to a uniform, interconnected porous structure with homogeneous particle distribution at optimal exposure duration. A series of FESEM micrographs illustrating the morphological evolution of chitosan under different microwave-assisted deacetylation times. The untreated sample exhibits dense, irregular surfaces, while 15-minute exposure yields a well-defined, interconnected porous morphology with layered arrangement and uniform particle size ($\sim 273.98 \pm 10.49$ nm), indicative of enhanced structural organization.

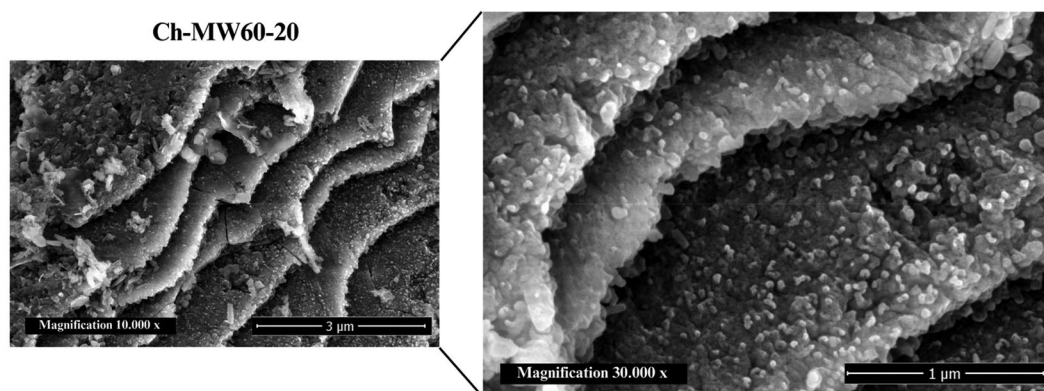


Figure 9. Continued.

to decreasing colony density which establishes a strong dose-response link between the treatment and the log CFU/mL reductions shown in the quantitative results.

Colony count data obtained from the plate counting method were statistically analyzed and expressed as mean $\pm$ standard deviation (SD) based on duplicate measurements ($n=2$) for each treatment group. Colony-forming units (CFU) were converted to log CFU/mL prior to statistical comparison to normalize variance and ensure consistency with graphical representations. The mean and SD values were calculated using standard statistical equations, where the mean was determined by dividing the total colony counts by the number of replicates and SD was calculated from the variance of replicate values. For *Escherichia coli*, Ch-MW60-15 exhibited a clear concentration-dependent reduction in colony counts. At 20 ppm, the bacterial count decreased to 117 ± 22.63 CFU, derived from replicate values of 101 and 133 CFU. Increasing the concentration to 40 ppm further reduced bacterial growth to 69 ± 8.49 CFU. A marked decline was observed at 60 ppm, where the mean colony count dropped to 5 ± 2.83 CFU, indicating strong bactericidal activity. Complete suppression was achieved at 80 ppm, with a mean value of 0.5 ± 0.71 CFU. In comparison, commercial chitosan demonstrated near-complete inhibition beginning at 20 ppm (4.5 ± 2.12 CFU) and total inhibition at concentrations ≥ 40 ppm. These findings confirm a statistically significant concentration-dependent reduction in *Escherichia coli* viability ($p < 0.05$). A similar trend was observed for *Staphylococcus aureus*, although the inhibitory response was more gradual. At 20 ppm, the mean colony count was reduced to 87 ± 5.66 CFU (replicates: 83 and 91 CFU). At 40 ppm, bacterial growth decreased to 76.5 ± 6.36 CFU. A substantial decline occurred at 60 ppm, where counts reached 8.5 ± 3.54 CFU, followed by nearly complete inhibition at 80 ppm (0.5 ± 0.71 CFU). Commercial chitosan demonstrated a more effective suppression of bacteria which resulted in 4 ± 0 CFU at 20 ppm and complete bacteria suppression at 40 ppm and higher concentrations. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to evaluate differences among concentration groups, with statistical significance established at $p < 0.05$. The analysis confirmed that increasing chitosan concentration significantly reduced bacterial colony counts for both *Escherichia coli* and *Staphylococcus aureus*. The experiments were repeated two times ($n=2$), and the representative plate images shown in Figure 11 match the calculated mean values. The strong agreement between numerical CFU data, log-transformed graphical trends, and plate photographs demonstrates the reproducibility and reliability of the antibacterial assessment.

For *Escherichia coli*, optimized microwave treatment (Ch-MW60-15) resulted in dose-dependent inhibition, reducing bacterial counts from 3.12 log CFU/mL to ~ 2.0 log CFU/mL at 20 ppm, comparable to those of commercial chitosan. Further reductions to 1.80 and 0.48–0.85 log CFU/mL were observed at 40 and 60 ppm, respectively, with complete inhibition at 80 ppm. By contrast, the commercial chitosan sample showed faster kinetics, achieving near-complete inhibition at 20 ppm and total pathogen suppression from 40 ppm. *Staphylococcus aureus* showed a different inhibition profile. Ch-MW60-15 reduced bacterial counts from 3.0 log CFU/mL at 0 ppm to approximately 1.9 log CFU/

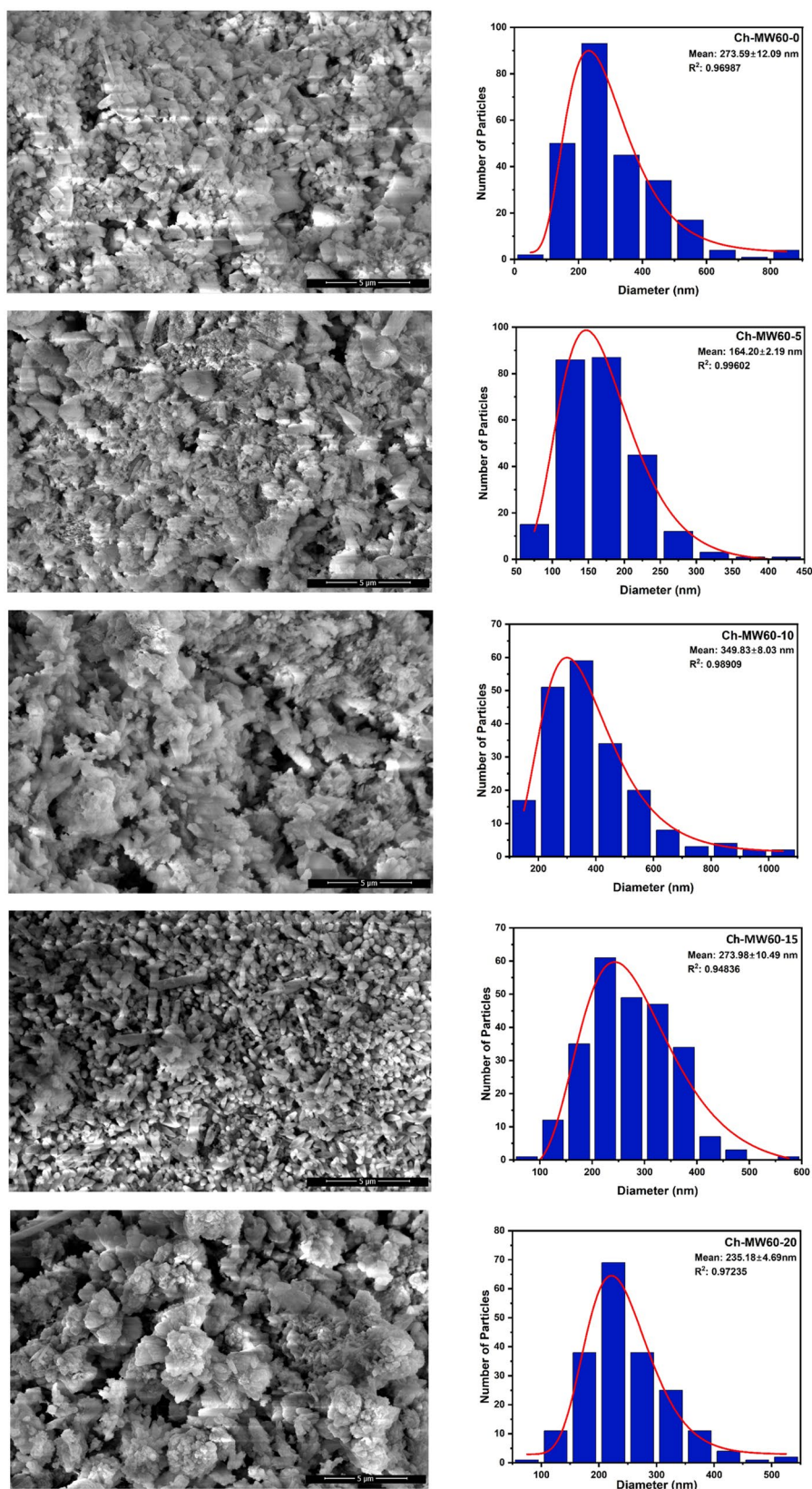


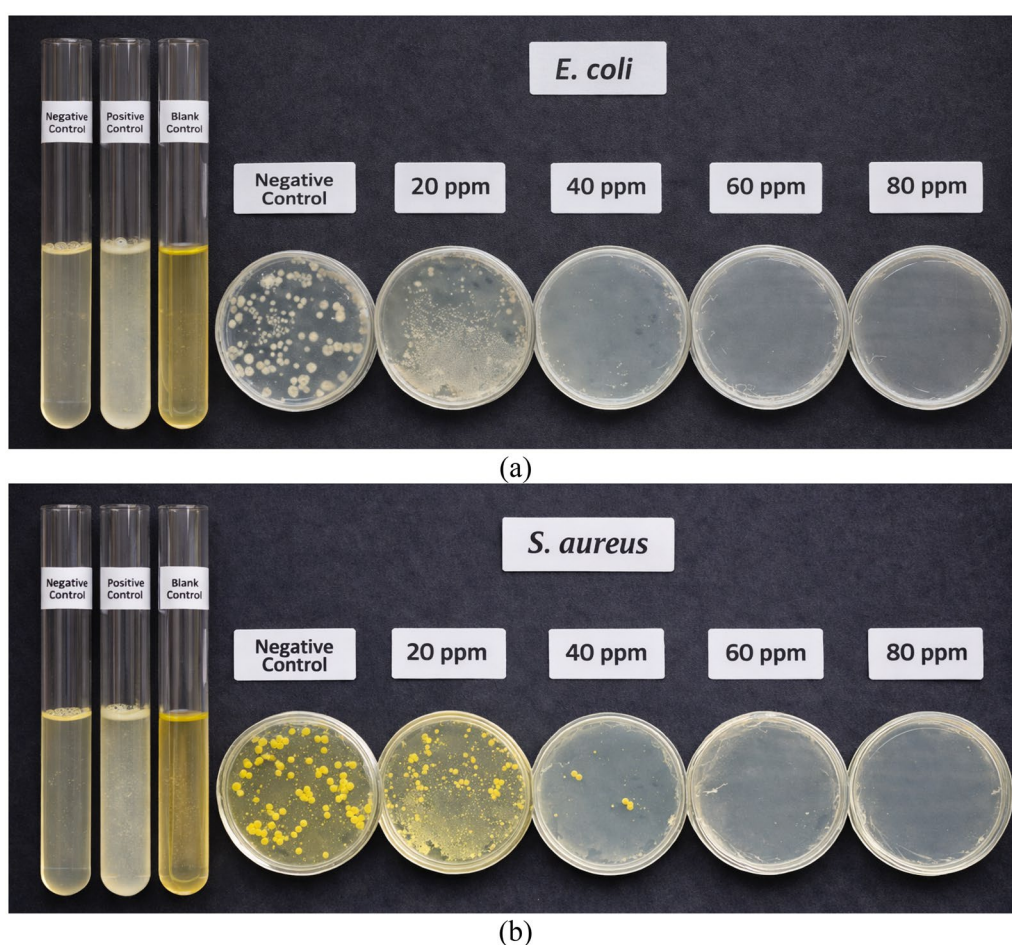
Figure 10. Particle size distribution of chitosan as affected by microwave-assisted deacetylation, demonstrating the influence of exposure duration on particle homogeneity and aggregation behavior. The optimized 15-minute treatment produced uniform, moderately sized particles (200–400 nm) with enhanced stability and reduced polydispersity.

Table 2. Antibacterial activity of chitosan samples against *Escherichia coli*.

Sample	Number of colonies (CFU)				
	0 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Ch-MW60-15-Rep 1	1310	101	63	7	0
Ch-MW60-15-Rep 2		133	75	3	1
Ch-Rep 1		3	0	0	0
Ch-Rep 2		6	0	0	0

Table 3. Antibacterial activity of chitosan samples against *Staphylococcus aureus*.

Sample	Number of colonies (CFU)				
	0 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Ch-MW60-15-Rep 1	1001	83	72	11	0
Ch-MW60-15-Rep 2		91	81	6	1
Ch-Rep 1		4	0	0	0
Ch-Rep 2		4	1	0	0

**Figure 11.** Antibacterial performance of microwave-assisted green-isolated chitosan evaluated against *Escherichia coli* (a) and *Staphylococcus aureus* (b) after 24 h incubation. The figure presents broth turbidity controls (negative, positive, and blank) alongside plate count results at different chitosan concentrations (20–80 ppm). A concentration-dependent reduction in bacterial colonies is observed for both species. Complete growth inhibition was achieved at 80 ppm, confirming the enhanced antibacterial efficacy of the optimized chitosan formulation.

mL at 20 ppm and about 1.0 log CFU/mL at 60 ppm, with complete inhibition occurring at 80 ppm. Although the commercial sample again achieved rapid pathogen suppression, reducing bacterial counts to below 1.0 log CFU/mL at 20 ppm and eliminating growth by 40 ppm. The isolated Ch-MW60-15 demonstrated a more gradual but consistent inhibition trend.

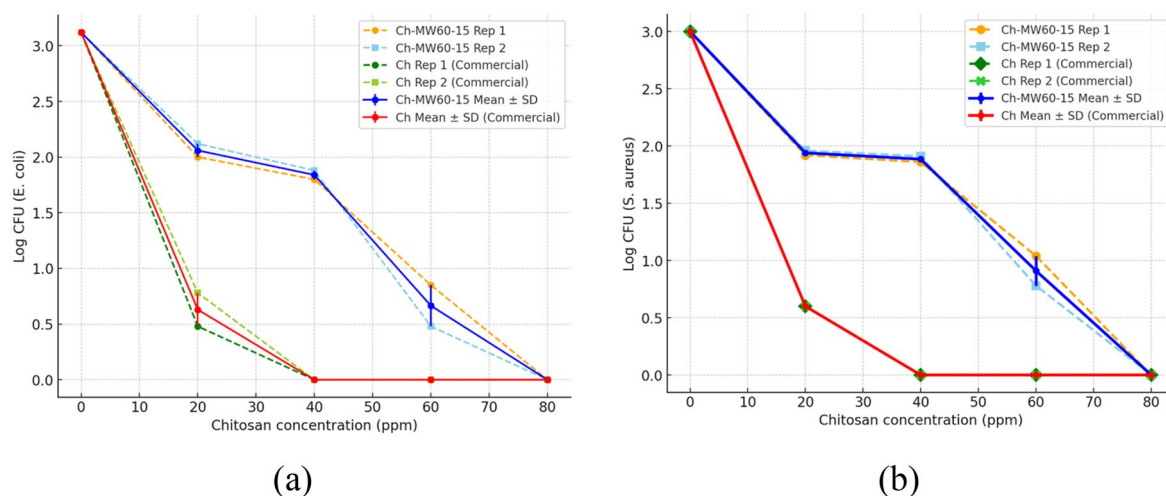


Figure 12. Logarithmic bacterial counts (log CFU/mL) of *Escherichia coli* (a) and *Staphylococcus aureus* (b) as a function of chitosan concentration, illustrating the dose-dependent antibacterial activity of microwave-optimized chitosan, with significant reductions in bacterial viability observed at higher concentrations.

The results show that different species respond to antibacterial treatment in different ways. Ch-MW60-15 showed comparable performance to commercial chitosan against *Escherichia coli* at 20 ppm but required higher amounts to reach the same level of *Staphylococcus aureus* inhibition. The green isolation process produces structural and surface morphological differences in Ch-MW60-15 which control how bacteria respond to the material. The 80 ppm test results show that sustainably derived chitosan can function as a medical-grade antimicrobial agent because it works against both pathogens.

4. Discussion

The rising problem of bacterial resistance against standard antibiotic treatments has increased research studies that aim to discover lasting antibacterial materials which possess natural antimicrobial abilities. The biopolymer chitosan which comes from chitin shows potential as a material for various medical uses including wound dressings and scaffolds and drug delivery systems because of its biocompatibility and biodegradability and its established antibacterial properties (Li & Wu, 2019; Bashir et al., 2022; Zhao et al., 2018). The extraction method used to produce chitosan directly impacts its performance. The traditional methods that use strong alkaline substances and extended heating periods create situations which lead to inconsistent results in chitosan quality. The current methods face problems with sustainability and reproducibility which create a need for developing extraction methods that use environmentally friendly approaches and provide better process control (Qian et al., 2017; Demir, 2023). The research study investigated all chemical structures and macromolecular characteristics and crystal structures and elemental contents and morphological details and particle features and antibacterial effectiveness of green-isolated chitosan from *Pinctada maxima* shells (Figure 1).

The chemical structure and macromolecular properties of the samples show that the three isolation methods and NaOH concentration and microwave-assisted deacetylation method determine the quality of the chitosan product. The FTIR spectra show that the sequential processing steps together with microwave-assisted deacetylation process create structural changes in chitosan which comes from *Pinctada maxima* shells. The samples contain all essential functional groups which identify chitosan and demonstrate that the chitin-to-chitosan process has been completed (Figure 2, Dong et al., 2023; Rahayu et al., 2025). The various treatment methods create differences in absorption band intensity and band sharpness which demonstrate that processing conditions produce different structural outcomes. The chitosan which results from demineralization-first processing (Ch-Cv40-M) reaches a higher DD value of 81.20% compared to the chitosan which results from deproteinization-first processing (Ch-Cv40-P, 75.56%) (Figure 4). The results support the research conducted by Nurmaulida

et al. (2023), which proved that when minerals get removed from the substrate at the beginning it creates better access for alkali deacetylation which results in stronger structural integrity.

Microwave irradiation increases these effects because Ch-MW60-15 achieves a higher DD ratio of 95.37% compared to the standard treatment groups shown in Figure 4. The study results show that microwave treatment enhances chitosan quality through faster molecular vibrations and reaction kinetics according to previous research (Cheng et al., 2020). The NaOH concentration determines chitosan production because greater NaOH levels improve acetyl group cleavage efficiency. The chitosan sample prepared using 60% NaOH exhibits the strongest amine absorption and the highest DD (95.37%) which demonstrates how hydroxide ions facilitate acetyl group removal according to research (Foster et al., 2015). The study results match those who established that increasing deacetylation parameters leads to better functional performance results in chitosan (Tokatlı and Demirdöven, 2018).

The length of time that microwave radiation is applied determines how functional groups in materials will behave. The FTIR spectra show that the O–H stretching band undergoes continuous shifts which reach their maximum strength in Ch-MW60-20 because free hydroxyl groups increase when acetyl groups are removed. The amine band shift from 1630 cm^{-1} in Ch-MW60-0 to 1655 cm^{-1} in Ch-MW60-15 corresponds to the highest DD which proves that optimized microwave treatment creates multiple free amine groups from the material (Triunfo et al., 2022). The C–H stretching vibration maintains its original position while the bending vibrations move to higher wavenumbers which indicates that acetyl groups have been completely removed. This pattern supports the findings of Cheng et al. who found that microwave irradiation enables better deacetylation because it makes polymer chain reactive sites more accessible.

The DD values show systematic changes which extend between 83.29% and 95.37% because microwave exposure time needs to be precisely controlled to maintain structural integrity. The optimal treatment duration, 15 min (Ch-MW60-15), yielded the highest DD, whereas prolonged exposure, 20 min, reduced the DD to 91.15%, which occurred because excessive irradiation caused chain scission and partial degradation of the material (Figure 6). The study results show agreement research who found that microwave treatment improves reaction efficiency but excessive exposure to microwaves results in both polymer stability loss and product quality deterioration (Hwang et al., 2002; Weska et al., 2007).

The structural changes which have occurred result in different macromolecular properties which can be seen in Table 1. The molecular weight decreased from 206.08 kDa to 148.20 kDa and the viscosity declined from 3.17 cP L/g to 2.45 cP L/g because increased DD levels. The development of this opposite trend shows how deacetylation process breaks glycosidic bonds (Potivas and Laokuldilok, 2014). The combination of lower molecular weight and higher DD values leads to improved solubility and bioavailability and antibacterial properties of chitosan which makes it increasingly useful for medical purposes according to two research studies (Pokhrel et al., 2016; Buanasari et al., 2021). The study shows that Ch-MW60-15 reaches its maximum purity level through two processes which include its protein content reduction from 0.70% and its moisture content increase to 1.70%. The study shows that optimized microwave exposure improves material structure while controlling macromolecular properties.

Chitosan chemical processing parameters determine both its molecular characteristics and crystalline structure along with elemental makeup. Microwave-assisted deacetylation creates structural changes in chitosan which crystallographic assessment demonstrates (Figure 7). The XRD patterns show that chitosan exhibits a semicrystalline structure which contains distinct reflections that occur between $2\theta = 10^\circ\text{--}36^\circ$ and supports the orthorhombic structure that previous research has established. The microwave exposure time directly impacts peak intensity and sharpness because irradiation alters both crystal packing and long-range ordering properties. The different treatments lead to various quantitative results which show changes in crystallinity and crystallite dimensions and dislocation density and micro-strain measurements. The untreated chitosan sample has a 44.44% crystallinity index and a 698.35 nm crystallite size according to (Ch-MW60-0) results. The short microwave exposure (Ch-MW60-5) duration of 5 minutes leads to a crystallinity increase of 56.67% and a crystallite size growth to 847.67 nm which shows early molecular reorganization. The 10 minutes of extended exposure (Ch-MW60-10) leads to a crystallite size reduction to 592.17 nm and a crystallinity decline

to 54.58% because the excessive energy input caused partial crystalline order disruption (Podgorbunskikh et al., 2022).

The 15 minutes treatment (Ch-MW60-15) provides optimal structural integrity because it generates the highest crystallinity (60.89%) and produces a crystallite size of 747.14 nm while the reduced dislocation density shows enhanced molecular rearrangement and chain packing efficiency (Cheng et al., 2020). The 20 minutes microwave treatment (Ch-MW60-20) results in decreased crystallinity (40.50%) and reduced crystallite size (602.54 nm) which indicates that an amorphous transformation occurred because of chain scission and increased molecular mobility (Podgorbunskikh et al., 2022). The study found that intermediate microwave durations achieve peak crystallinity while excessive exposure leads to material amorphization which supports previous research showing that controlled irradiation improves polymer order while overexposure leads to material degradation.

The complementary EDX analysis supports the crystallographic findings by showing how microwave-assisted purification changes material composition. The elemental profiles show a progressive increase in carbon content from 16.38% in the untreated sample to 64.43% in the sample treated for 20 minutes which demonstrates how the organic backbone became more visible after acetyl groups were removed during the deacetylation process (Elfadl et al., 2023). The study shows that nitrogen levels remain constant while oxygen levels drop because acetyl groups were removed from the sample. The amine functions needed for biological activity remain intact according to the research. The microwave treatment process successfully removes most remaining mineral components because it causes a major drop in both Ca and Mg levels which includes a reduction of Ca from 22.12% to 2.91% and Mg from 0.09% to approximately 0.19–0.24%. The study demonstrates that microwave-assisted deacetylation process decreases mineral content which results in better elemental purity (Michailidou et al., 2020).

The process of elemental purity enhancement leads to the development of better morphological characteristics. The process of microwave-assisted deacetylation achieves two outcomes by removing remaining minerals from the material and generating uniform particle distribution patterns which create consistent surface structure designs. Microwave-assisted deacetylation enables the production of chitosan materials through property control, which includes improvements to surface structure and particle dimension distribution (Figures 9 and MolluscaBase, 2026). The research results show that chitosan nanoparticles made from *Pinctada maxima* shells display different structural characteristics based on the length of microwave exposure which they undergone. The treatment results of five minutes (Ch-MW60-5) show a uniform particle distribution pattern which shows a particle size distribution between 100 and 250 nanometers. Previous research showed that microwave irradiation changes chitosan crystallinity and physical properties, which results in better biomedical applications through microwave irradiation (Cheng et al., 2020; Kurniawidi et al., 2024).

The extension of particle exposure time to 10 minutes (Ch-MW60-10) results in the particle size distribution reaching its maximum value of 1000 nanometers while showing an average particle size of 349.83 nanometers which has a measurement error of 8.03 nanometers. The microwave treatment duration leads to secondary particle aggregation which requires optimized treatment times to maintain the desired particle qualities. The observed morphological alterations match previous studies which documented the structural alterations of chitosan during different treatment methods (Chandha et al., 2022; Kurniawidi et al., 2024).

The sample with the longest treatment duration of 15 minutes (Ch-MW60-15) shows the highest level of particle shape improvement because it displays an uncommon interconnected structure that contains porous layers and uniformly distributed small particles. The average particle size measures 273.98 nanometers with a standard deviation of 10.49 nanometers and shows a distribution pattern that extends between 200 and 400 nanometers. The new architectural design benefits biomedical fields because it uses its enhanced biological substrate interactions to increase both solubility and bioactivity (Nurmaulida et al., 2023; Cheng et al., 2020). The treatment time of 20 minutes (Ch-MW60-20) brings about undesirable particle shapes, which demonstrate how chitosan materializes after receiving excessive treatment.

In conclusion, the FTIR, viscosity and proximate analysis, and XRD and EDX results collectively demonstrate that the optimized protocol-demineralization-first isolation using 60% NaOH followed

by 15 min microwave irradiation-produces chitosan with superior structural and macromolecular attributes. The resulting sample, Ch-MW60-15, has the highest DD, low molecular weight, favorable crystallinity, and significantly diminished mineral contamination, thereby ensuring purity and stability. Complementary FESEM and particle size distribution analyses further confirm that Ch-MW60-15 exhibits a uniform, interconnected, porous-layered morphology with homogeneous particles, a structural novelty for *Pinctada maxima* derived chitosan. The integrated characterization of Ch-MW60-15 demonstrates that the material cannot be regarded as purely organic bulk chitosan. The results demonstrate two separate structural interpretations because the organic component contains a deacetylated chitosan native structure while the rest of the material presents a detectable amount of CaCO_3 content. The ^1H NMR spectrum recorded in D_2O shows main peaks in the anomeric/carbohydrate region ($\delta \approx 4.65\text{--}4.86$ ppm), which match the chitosan polysaccharide structure. The isolated sample lacks the typical acetyl methyl resonance which appears at $\delta \approx 1.9\text{--}2.1$ ppm because of its higher deacetylation level when compared to standard chitosan. The chitosan ^1H NMR assignments show the pattern of acetyl and backbone signals which experts use to determine DD and DDA values (Rahayu et al., 2025; Yu et al., 2023). Furthermore, the spectrum does not exhibit additional diagnostic resonances such as the $-\text{CH}_2\text{--COO}^-$ signals reported for carboxymethylated chitosan in the $\delta \approx 3.2\text{--}3.5$ and $4.2\text{--}4.5$ ppm regions suggesting that the organic component is not a covalently grafted derivative but rather unsubstituted, high-DD native chitosan (Zajac et al., 2023; Rasifudi et al., 2024). Therefore, the NMR results confirm the chemical identity of the organic matrix while indicating minimal covalent modification.

The whole material testing results show that it contains considerable amounts of inorganic mineral material. The FTIR spectrum shows typical chitosan bands which include O–H/N–H stretching C–H vibrations amide/ NH_2 region and polysaccharide skeletal bands. The XRD pattern shows sharp intense reflections which occur between 20 and 40° (2θ) that indicate crystalline CaCO_3 instead of the broad semi-crystalline features which pure chitosan shows at $\sim 10^\circ$ and $\sim 20^\circ$. The established CaCO_3 phase-analysis references show distinct diagnostic reflections which identify calcite/aragonite/vaterite while they mainly report the calcite reflection that occurs near $2\theta \approx 29.4^\circ$ which helps assign mineral phases in CaCO_3 -containing systems. The EDX analysis shows calcium at percent level which proves the presence of remaining inorganic material that matches the incomplete demineralization of shell-derived chitosan (Mathaba & Daramola, 2020; Selvaraj et al., 2023). While EDX alone does not define the crystallographic phase, its combination with diffraction and vibrational methods represents a standard and reliable approach for identifying inorganic components in polysaccharide mineral composites (Hayati et al., 2022; Dinculescu et al., 2024). Collectively, these findings indicate that Ch-MW60-15 is best described as a high-DD native chitosan containing residual CaCO_3 , i.e. a CaCO_3 -containing chitosan (chitosan– CaCO_3 composite), consistent with literature describing hybrid or composite chitosan–mineral systems where NMR validates the organic component and solid-state methods confirm the inorganic phase. These improvements in chemical structure, crystallinity, elemental purity, and morphology are directly relevant to antibacterial performance, as they enhance solubility, bioactivity, and electrostatic interactions with bacterial membranes. Thus, the optimized chitosan fulfills the structural and compositional criteria of biomedical applications and establishes a strong foundation for its competitive antibacterial efficacy, underscoring its potential as a sustainable biomaterial for biomedicine (Nurmaulida et al., 2023; Chandha et al., 2022; Al-Gethami & Al-Qasbi, 2021).

The antibacterial activity of chitosan derived from *Pinctada maxima* shells exhibits a distinct species-dependent inhibition profile against *Escherichia coli* and *Staphylococcus aureus* as reflected in both the experimental data and structural analyses (Tables 2 and 3; Figure 10). For *Escherichia coli*, the sample obtained using optimized microwave-assisted treatment (Ch-MW60-15) demonstrates a robust, dose-dependent inhibitory effect. For *Escherichia coli*, substantial inhibition is already evident at 20 ppm, as reflected by the marked decrease in colony number compared with the negative control. Further reductions at 40 and 60 ppm demonstrate enhanced bactericidal efficiency, culminating in complete growth suppression at 80 ppm. This rapid inhibition is consistent with the structural

characteristics of Gram-negative bacteria, which possess a relatively thinner peptidoglycan layer. Such structural features facilitate electrostatic interactions between the protonated amino groups of chitosan and negatively charged bacterial membranes, leading to membrane destabilization and cell death (Kaur et al., 2025; Alli et al., 2022; Gomathy et al., 2021; Elhag et al., 2025).

The increasing concentrations of *Staphylococcus aureus* lead to a slower decline in its ability to form colonies. The test shows that 20 ppm begins to show inhibition but the intermediate concentrations between 20 and 60 ppm show residual colonies which complete inhibition only occurs at 80 ppm. This slower response aligns with the structural characteristics of Gram-positive bacteria, particularly their thicker peptidoglycan layer, which can limit chitosan penetration and delay membrane disruption (Gomathy et al., 2021; Elhag et al., 2025). The optimized chitosan maintains its power to kill bacteria from both bacterial types which results in no colonies emerging at 80 ppm.

The broth turbidity controls presented alongside the agar plates further validate these findings. The negative control tubes display visible turbidity due to active bacterial growth, whereas reduced turbidity is observed in treated samples as concentration increases, and the blank control remains clear, confirming experimental sterility. The antibacterial evaluation results acquire improved reliability and reproducibility through the consistent results obtained from both broth microdilution and plate count assays.

The data show a strong antibacterial effect which depends on the concentration of the tested substances. The enhanced performance of Ch-MW60-15 can be attributed to its optimized physico-chemical characteristics, including a high degree of deacetylation (95.37%), reduced molecular weight, improved crystallinity, and a uniform porous morphology (200–400 nm), which collectively enhance electrostatic interactions with bacterial membranes. The research demonstrates that chitosan derived sustainably from *Pinctada maxima* shells can function as a medical-grade antimicrobial biomaterial with high effectiveness (Alli et al., 2022; Elhag et al., 2025).

At 20 ppm, bacterial counts decrease from 3.12 log CFU/mL to approximately 2.0 log CFU/mL, consistent with the pathogen suppression observed in commercial chitosan. Further reductions occur at 40 and 60 ppm, reaching 1.80 and 0.48–0.85 log CFU/mL, respectively, with complete inhibition occurring at 80 ppm. These results align with prior studies that identified similar minimum inhibitory concentrations for chitosan against *Escherichia coli*, confirming its potent antibacterial potential (Kabir et al., 2020; Li et al., 2020).

Ch-MW60-15 shows a slower effectiveness against *Staphylococcus aureus* compared to other test substances. The system starts with a 3.0 log CFU/mL bacterial count which decreases to approximately 1.9 log CFU/mL at 20 ppm and then further drops to 1.0 log CFU/mL at 60 ppm before reaching total bacterial elimination at 80 ppm. The slower response compared with that of *Escherichia coli* is consistent with the structural differences of Gram-positive bacteria, particularly their thick peptidoglycan layer, which hinders penetration (Li et al., 2020; Kusnadi et al., 2022). The antibacterial action of chitosan mainly results from electrostatic forces between its protonated amino groups and the negatively charged bacterial membranes which ultimately lead to membrane destruction (Lin et al., 2023; Ma et al., 2023).

The superior antibacterial effect of Ch-MW60-15 can be directly ascribed to its unique morphology. FESEM analysis reveals a uniform, interconnected, porous-layered architecture with a fine particle distribution (200–400 nm), which substantially increases the surface area and strengthens electrostatic interactions with bacterial cells. This morphology is critical for enhancing antimicrobial activity, as larger, more homogeneous surface areas promote stronger binding and membrane disruption (Qiu et al., 2022; Kosimaningrum et al., 2020). Similar outcomes have been reported for physically and chemically modified chitosan, further supporting the role of morphology in expanding its antibacterial spectrum and utility in biomedical applications, particularly wound dressings and scaffolds (Nanda et al., 2024; Wulandari et al., 2022).

The optimized green isolation protocol produces a high DD value of 95.37% which results in improved crystallinity and decreased residual mineral content to achieve chitosan with higher purity and bioactivity. The antibacterial power of the substance depends on its physicochemical properties which include high DD and low molecular weight of 148.20 kDa and low viscosity of 2.45 cP L/g and 60.89% crystallinity (Putri et al., 2021; Amor et al., 2024). The combination of free amine groups

and enhanced solubility and structural uniformity creates stronger connections with bacterial membranes which results in improved antibacterial effectiveness. The Ch-MW60-15 chitosan product presents a valid option against *Escherichia coli*. It shows slower kinetic rates but maintains similar *Staphylococcus aureus* inhibition effects to commercial chitosan at 80 ppm.

These antibacterial performance results underscore the species-dependent response of Gram-negative and Gram-positive bacteria to chitosan while highlighting the importance of optimized physicochemical properties—chemical structure, crystallinity, elemental purity, and morphology—in modulating efficacy. The integration of these parameter positions Ch-MW60-15 as a sustainable, effective antimicrobial agent. These findings confirm the potential use of green-isolated chitosan from seafood waste as a medical-grade material for biomedical applications, particularly wound dressings, scaffolds, and drug delivery systems, enabling significant advancement toward sustainable healthcare solutions (Li et al., 2020; Amor et al., 2024).

5. Conclusions

This study demonstrates the successful development of a green isolation strategy for chitosan derived from *Pinctada maxima* shells, optimized through microwave-assisted deacetylation. The sample produced via the optimized protocol (Ch-MW60-15) yielded chitosan with a high DD (95.37%), low molecular weight (148.20 kDa), low viscosity (2.45 cP L/g), and enhanced crystallinity (60.89%). FESEM and particle size analysis confirmed a porous-layered morphology with a uniform particle distribution in the 200–400 nm range, a hitherto unreported structural feature for *Pinctada maxima*-derived chitosan. Based on the ^1H NMR data, Ch-MW60-15 exhibits the characteristic chitosan polysaccharide resonances and lacks a prominent acetyl methyl signal near ~ 2 ppm, indicating a high-DD native chitosan backbone. However, XRD and SEM–EDX confirm that a significant CaCO_3 fraction remains, so the material is best classified as a chitosan– CaCO_3 composite rather than purely organic bulk chitosan; such composites are often advantageous for biomedical use because CaCO_3 can enhance mechanical reinforcement, buffering capacity, and mineral-driven bioactivity (e.g., osteoconductive support), while reduced trace minerals (e.g., Mg) improves purity and minimizes surface masking. These optimized structural and macromolecular attributes translated into competitive antibacterial performance. Against *Escherichia coli*, bacterial counts decreased from 3.12 log CFU/mL (0 ppm) to ~ 2.0 log CFU/mL (20 ppm), with complete inhibition at 80 ppm. For *Staphylococcus aureus*, inhibition was more gradual, with counts reduced from 3.0 log CFU/mL (0 ppm) to ~ 1.0 log CFU/mL (60 ppm) before total suppression at 80 ppm. This performance closely aligns with that of commercial chitosan, underscoring the medical-grade potential of sustainably derived materials.

The novelty of this work lies in demonstrating that pearl oyster shell waste can be converted into high-quality chitosan with superior purity, crystallinity, and unique morphology using an environmentally friendly approach. These findings expand the understanding of structure–function relationships in chitosan and highlight its potential use in biomedical applications. Future research should focus on evaluating its in vivo biocompatibility testing (e.g., MTT assay), Cell viability and proliferation analyses, long-term antibacterial efficacy and performance in advanced composite systems to support translational applications.

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Author contributions

CRedit: **Susi Rahayu**: Data curation, Formal analysis, Visualization, Writing – original draft; **Dionysius J. D. H. Santjojo**: Formal analysis, Supervision; **Rahadi Wirawan**: Investigation, Supervision; **Masruroh M.Si**: Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the author(s) used ChatGPT to improve language clarity and grammar. After using this tool, the author(s) reviewed and edited the content as necessary and take(s) full responsibility for the content of the published article.

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No potential conflict of interest was reported by the author(s).

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Data availability statement

Data are available from the corresponding author upon reasonable request.

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