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Review Article

Valorization of Shrimp Shell Waste into Functional Chitosan: Recent Advances, Analytical Challenges, and Future Perspectives

Sadiq Bishir^{1,2*} , Rose Chepchirchir Ramkat^{1,3} , Jacqueline Kubochi Makatiani^{1,3} , Njira Njira Pili¹ 

¹ Department of Biological Sciences, School of Sciences and Aerospace Studies, Moi University, Eldoret, Kenya

² Department of Biological Sciences, Umaru Musa Yar'adua University, Nigeria

³ Africa Center of Excellence II in Phytochemicals Textiles and Renewable Energy (ACEII-PTRE), Moi University, Eldoret, Kenya

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ABSTRACT

The increasing accumulation of shrimp shell waste from seafood processing presents both an environmental challenge and an opportunity for sustainable biomass valorization within the circular bioeconomy. Chitosan, a biodegradable and biocompatible polysaccharide obtained through chitin deacetylation, has emerged as a versatile biomaterial with broad applications in biomedicine, agriculture, environmental remediation, food packaging, and advanced functional materials. This review critically evaluates recent advances in the production of chitosan from shrimp shell waste, emphasizing extraction technologies, quality determinants, structural characterization, and analytical evaluation. Conventional chemical extraction is critically compared with emerging green approaches, including microwave-, ultrasonic-, autoclave-, enzymatic-, fermentation-, and deep eutectic solvent-assisted processes, highlighting their effects on yield, degree of deacetylation, molecular weight, scalability, and environmental sustainability. Current characterization and analytical methods are comparatively assessed, with particular emphasis on the complementary role of 3,5-dinitrosalicylic acid (DNSA)-based assays for reducing-end quantification during chitosan depolymerization. The review further discusses advances in functionalized chitosan derivatives, nanocomposites, industrial translation, and sustainable production strategies while identifying standardization of extraction and analytical protocols as a critical requirement for improving reproducibility and facilitating commercial-scale production. Collectively, this review provides an integrated perspective on current progress, remaining challenges, and future research priorities for developing high-quality chitosan from shrimp shell waste.

*CORRESPONDING AUTHOR

Sadiq Bishir; Department of Biological Sciences, School of Sciences and Aerospace Studies, Moi University, Kenya; Email: sadiqbishir@gmail.com

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1. Introduction

The growing demand for sustainable materials has accelerated interest in renewable biopolymers capable of replacing petroleum-derived products in biomedical, agricultural, environmental, and industrial applications. Within the framework of the circular bioeconomy, the valorization of agro-industrial and marine processing residues has emerged as an effective strategy for reducing environmental burdens while generating high-value products [1,2]. Among the various biomass resources available, shrimp shell waste represents one of the most abundant and underutilized marine by-products worldwide. The expansion of aquaculture and seafood processing industries has led to the generation of millions of tons of shrimp shell residues annually, accounting for approximately 50–70% of the total shrimp biomass processed for human consumption [1-3]. Improper disposal of these residues through landfilling, open dumping, or incineration contributes to environmental pollution, odor generation, greenhouse gas emissions, and the loss of potentially valuable biomolecules.

Shrimp shells are complex biocomposites primarily composed of chitin, proteins, calcium carbonate, lipids, and pigments such as astaxanthin [4,5]. Among these constituents, chitin is the most economically important because it serves as the precursor for chitosan, a versatile polysaccharide with remarkable physicochemical and biological properties. Chitin is the second most abundant natural polysaccharide after cellulose and occurs extensively in crustacean exoskeletons, insect cuticles, fungal cell walls, and certain marine organisms [3]. Through partial or extensive deacetylation, chitin is converted into chitosan, a linear cationic copolymer consisting of β -(1 $\rightarrow$ 4)-linked D-glucosamine and N-acetyl-D-glucosamine units. Unlike chitin, chitosan exhibits solubility in dilute acidic media and possesses reactive amino groups that confer unique functional properties including antimicrobial activity, biodegradability, biocompatibility, film-forming ability, metal-chelating capacity, and mucoadhesiveness [6].

The physicochemical characteristics of chitosan are strongly influenced by structural parameters such as degree of deacetylation (DD), molecular weight, crystallinity, and chain conformation. These parameters determine the polymer's solubility, viscosity, mechanical properties, biological activity, and application performance [7]. Consequently, the optimization of extraction processes and accurate characterization of the resulting chitosan are critical for ensuring product quality and application-specific functionality. However, considerable variability exists among published studies due to differences in shrimp species, extraction conditions, analytical methods, and reporting standards. Such inconsistencies complicate comparisons across studies and hinder the development of universally accepted quality criteria for shrimp shell-derived chitosan.

Traditional chitosan production relies predominantly on chemical extraction involving acid demineralization, alkaline deproteinization, and concentrated alkali-mediated deacetylation. While these methods remain effective and industrially scalable, they are often associated with high chemical consumption, energy-intensive processing, and the generation of environmentally undesirable effluents [2]. Consequently, growing attention has been directed toward environmentally benign alternatives including enzymatic processing, microbial fermentation, microwave-assisted extraction, deep eutectic solvent systems, and other green extraction technologies. These emerging approaches seek to improve sustainability while maintaining or enhancing extraction efficiency and product quality [8].

Beyond extraction, reliable characterization and quantification of chitosan remain essential for both research and industrial quality control. Numerous analytical techniques have been employed to evaluate chitosan structure and properties, including Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), scanning electron microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), elemental analysis, and molecular weight determination methods [7]. Similarly, several approaches have been developed for determining chitosan concentration and degree of deacetylation, including acid–base titration, spectrophotometric methods, chromatographic techniques, enzymatic assays, and colorimetric approaches. Nevertheless, substantial differences in sensitivity, specificity, cost, and operational complexity continue to limit standardization and inter-study comparability.

Particular interest has recently emerged in simple spectrophotometric methods for chitosan analysis that are suitable for resource-limited laboratories. Among these, the 3,5-dinitrosalicylic acid (DNSA) assay has gained attention as a rapid and inexpensive approach for quantifying reducing ends generated during chitosan depolymerization. Although DNSA-based methods are not intended to replace structural characterization techniques such as FTIR or NMR, they provide valuable information regarding depolymerization processes, enzymatic hydrolysis, and reducing sugar generation, thereby complementing conventional analytical platforms [9]. However, the strengths, limitations, and future potential of DNSA-based approaches within chitosan research remain insufficiently discussed in the current literature.

Given the rapid expansion of chitosan-related research and the growing emphasis on sustainable biomass valorization, a comprehensive and critical synthesis of recent advances is warranted. This review therefore examines shrimp shell waste as a renewable resource for chitosan production, critically evaluates conventional and emerging extraction technologies, discusses factors influencing chitosan yield and quality, and provides an in-depth assessment of structural characterization and quantitative analytical methodologies. Particular emphasis is placed on degree of deacetylation determination and DNSA-based analytical approaches. Furthermore, emerging applications, current challenges, and future research opportunities are critically discussed within the broader context of sustainable materials development and circular bioeconomy principles.

2. Shrimp Shell Waste as a Renewable Source of Chitin and Chitosan

2.1. Global Generation and Composition of Shrimp Shell Waste

The rapid growth of the global aquaculture and seafood processing industries has led to a substantial increase in crustacean-derived waste, particularly shrimp shell residues. Shrimp constitute one of the most commercially important seafood commodities worldwide, with annual production exceeding several million tonnes. During processing, approximately 50–70% of the shrimp biomass is discarded as waste, comprising heads, shells, tails, and other exoskeletal materials [1,2]. Consequently, shrimp shell waste represents a significant and continuously available biomass resource with considerable potential for value-added utilization.

Shrimp shells are natural biocomposite materials primarily composed of chitin, proteins, minerals, pigments, and minor quantities of lipids. The relative proportions of these constituents vary depending on species, habitat, age, diet, season, and processing conditions [10,11]. Typically, shrimp shells contain approximately 20–30% chitin, 30–40% proteins, and 30–50% minerals, predominantly calcium carbonate, together with carotenoid pigments such as astaxanthin that contribute to shell coloration [4,5].

From a resource recovery perspective, each component possesses potential economic value. Proteins recovered during deproteinization can be utilized in animal feed formulations, while calcium carbonate obtained during demineralization has potential applications in agriculture, pharmaceuticals, and industrial processes. However, chitin remains the most strategically important component due to its conversion into chitosan and other high-value derivatives [3]. The integrated recovery of these fractions aligns closely with modern biorefinery concepts aimed at maximizing resource utilization while minimizing waste generation.

The composition of shrimp shells is not constant across species. For example, shells from *Litopenaeus vannamei*, *Penaeus monodon*, and *Parapenaeus longirostris* exhibit differences in chitin content, mineral composition, and structural organization, which can significantly influence extraction efficiency and the physicochemical properties of the resulting chitosan [12,13]. Such variability underscores the importance of considering raw material characteristics when designing extraction protocols and evaluating process performance.

2.2. Environmental and Economic Significance

The accumulation of shrimp shell waste presents both environmental challenges and economic opportunities. In many regions, shell residues are disposed of through landfilling, open dumping, or incineration, practices

that contribute to environmental pollution and public health concerns. The decomposition of untreated shell waste generates unpleasant odors, attracts insects and rodents, and can result in localized contamination of soil and water resources [5]. Furthermore, incineration contributes to greenhouse gas emissions and represents a loss of potentially valuable biological resources.

The environmental burden associated with shell disposal has stimulated increasing interest in sustainable valorization strategies. Rather than treating shrimp shells as waste, contemporary approaches recognize them as renewable feedstocks for the production of biopolymers, bioactive compounds, fertilizers, animal feed additives, and functional materials. Such strategies support circular economy principles by converting low-value residues into economically valuable products while simultaneously reducing environmental impacts [14].

Among the various valorization pathways (Figure 1), chitosan production represents one of the most economically attractive options due to the polymer's broad industrial applicability and growing market demand. The global chitosan market continues to expand, driven by increasing utilization in biomedical engineering, food packaging, wastewater treatment, agriculture, pharmaceuticals, cosmetics, and nanotechnology [15,16]. Consequently, shrimp shell waste serves not only as an environmental liability but also as a strategic resource capable of supporting sustainable industrial development.

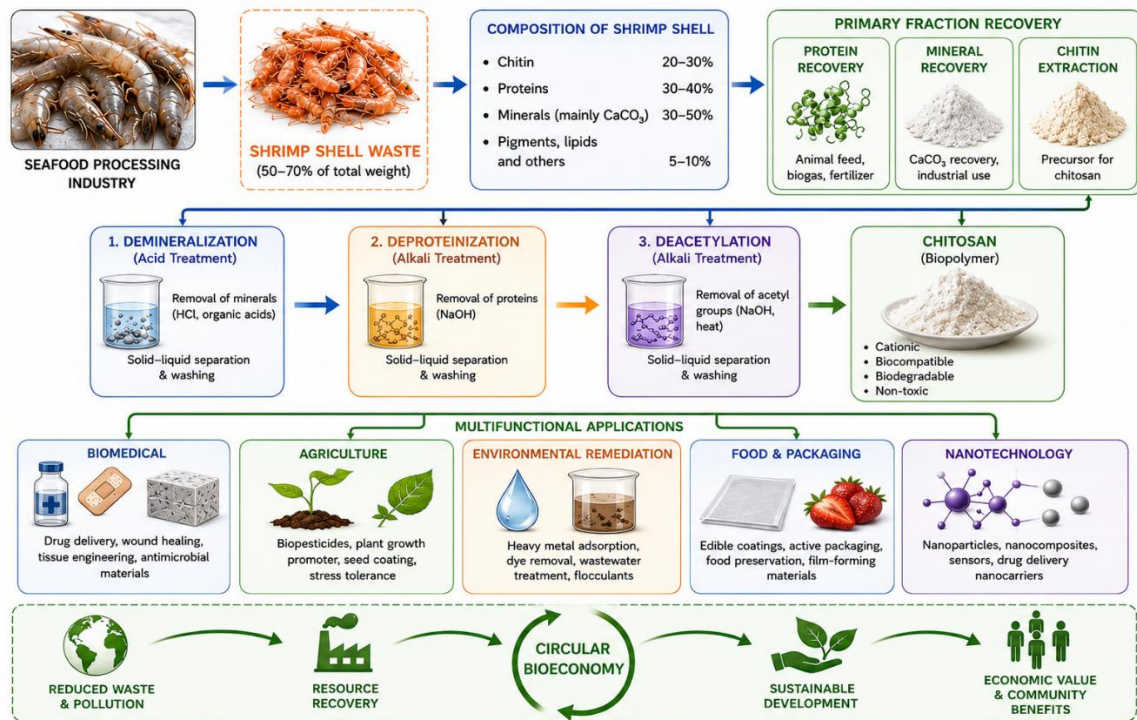


Figure 1. Valorization pathway of shrimp shell waste into functional chitosan within a circular bioeconomy framework.

2.3. Factors Affecting Chitin Recovery

The recovery of chitin from shrimp shells is influenced by a combination of biological, environmental, and processing-related factors. Variability in shell composition directly affects extraction efficiency, chitin purity, and subsequent conversion to chitosan.

Species-related differences are among the most influential factors. Distinct shrimp species possess varying proportions of chitin, proteins, and minerals, resulting in differences in demineralization and deproteinization requirements. Studies have demonstrated that chitin yield and quality can vary considerably even among closely related species subjected to identical extraction procedures [10,11].

Environmental conditions also play important roles. Habitat characteristics, including salinity, temperature, water quality, and nutrient availability, influence shell development and biochemical composition. Seasonal variations can further affect mineralization patterns, pigmentation, and structural organization of the

exoskeleton [4]. Additionally, biological factors such as age, gender, reproductive stage, molting cycle, and nutritional status contribute to observed differences in shell composition.

Post-harvest handling and storage conditions represent another source of variability. Prolonged storage prior to processing may promote microbial degradation and biochemical alterations that affect extraction efficiency [17]. Therefore, standardized collection and storage procedures are essential for maintaining raw material quality and ensuring reproducible extraction outcomes.

Understanding these sources of variability is particularly important when comparing extraction studies reported in the literature. Differences in chitin yield are frequently attributed not only to extraction conditions but also to inherent variations in the raw materials employed. Consequently, meaningful comparisons require careful consideration of both biological and processing factors.

2.4. Shrimp Shell Valorization within the Circular Bioeconomy

The concept of a circular bioeconomy emphasizes the sustainable utilization of biological resources through integrated systems that maximize resource efficiency, minimize waste generation, and promote environmental sustainability. Within this framework, shrimp shell waste represents an ideal candidate for biorefinery-based valorization because of its abundance, renewability, and multifunctional composition.

Traditional linear production systems typically follow a “take–make–dispose” model in which shell residues are discarded after processing. In contrast, circular bioeconomy approaches seek to recover valuable products from each component of the shell matrix. Proteins may be converted into feed ingredients or bioactive peptides, minerals can be recovered for industrial use, pigments may be extracted for nutraceutical applications, and chitin can be transformed into high-value chitosan and its derivatives [1,14].

The integration of chitosan production into seafood processing chains offers several sustainability benefits. First, it reduces the environmental burden associated with waste disposal. Second, it creates additional revenue streams for seafood industries. Third, it decreases dependence on petroleum-derived synthetic materials by providing renewable alternatives. Finally, it supports the development of environmentally friendly technologies in sectors ranging from medicine to agriculture and environmental remediation [1,18].

Recent advances in green extraction technologies further strengthen the sustainability profile of shrimp shell valorization. The adoption of enzymatic processes, biological fermentation, microwave-assisted extraction, and other environmentally benign approaches has the potential to reduce chemical consumption, lower energy requirements, and improve overall process sustainability. However, challenges related to scalability, economic feasibility, and process standardization remain significant barriers to widespread industrial implementation.

Collectively, these considerations demonstrate that shrimp shell waste should no longer be viewed merely as a disposal problem but rather as a strategic renewable resource capable of contributing to sustainable materials production and circular bioeconomy development. The successful conversion of shrimp shell biomass into high-quality chitosan represents a compelling example of waste valorization that combines environmental, economic, and technological benefits.

3. Extraction, Conversion, and Quality Determinants of Chitosan

The extraction of chitosan from shrimp shell waste involves sequential removal of minerals and proteins, followed by deacetylation of chitin to produce chitosan. Although this workflow has remained fundamentally unchanged for decades, considerable advances have been made toward improving extraction efficiency, product quality, environmental sustainability, and process economics. Extraction conditions not only determine chitosan yield but also profoundly influence its degree of deacetylation (DD), molecular weight, crystallinity, and functional properties. Consequently, optimization of extraction protocols remains central to producing chitosan tailored for specific industrial and biomedical applications [1,2].

3.1. Conventional Extraction and Conversion Processes

Conventional chitosan production consists of three sequential steps: demineralization, deproteinization, and deacetylation. Demineralization is typically performed using dilute hydrochloric acid (HCl) to dissolve calcium carbonate and other inorganic salts, thereby increasing the accessibility of chitin within the shell matrix [4]. Acid concentration, reaction time, and solid-to-liquid ratio must be carefully optimized because excessive acid exposure may partially hydrolyze the polymer chains, whereas insufficient treatment leaves residual minerals that compromise product purity [13].

The demineralized material subsequently undergoes deproteinization, most commonly using sodium hydroxide (NaOH), to remove structural proteins tightly associated with chitin fibrils. Treatment conditions strongly influence both protein removal efficiency and polymer integrity. Increasing alkali concentration and reaction temperature generally improves deproteinization but may simultaneously promote partial depolymerization, resulting in reduced molecular weight and altered physicochemical properties [2,11]. Consequently, optimization requires balancing effective protein removal with preservation of polymer structure.

The final and most critical stage is deacetylation (Figure 2), during which acetyl groups are removed from N-acetyl-D-glucosamine residues using concentrated alkali, typically 40–60% NaOH under elevated temperature. This conversion determines the degree of deacetylation, one of the principal quality indicators governing chitosan solubility, charge density, adsorption capacity, and biological activity [6,19]. Higher alkali concentrations and prolonged treatment generally increase DD but also accelerate chain scission, thereby decreasing molecular weight and potentially compromising mechanical performance [1]. Achieving an optimal balance between extensive deacetylation and preservation of polymer integrity therefore remains one of the primary challenges in conventional chitosan production.

Despite its widespread industrial adoption, chemical extraction presents several limitations. Large quantities of concentrated acids and alkalis generate substantial volumes of alkaline and acidic wastewater, increasing treatment costs and environmental burden. In addition, high energy consumption associated with prolonged heating and repeated washing steps reduces overall process sustainability [1,3]. Nevertheless, conventional chemical extraction remains the industrial benchmark because of its high productivity, operational simplicity, and established scalability. Future improvements are therefore likely to focus on reducing chemical consumption and integrating greener extraction strategies rather than replacing chemical processing entirely.

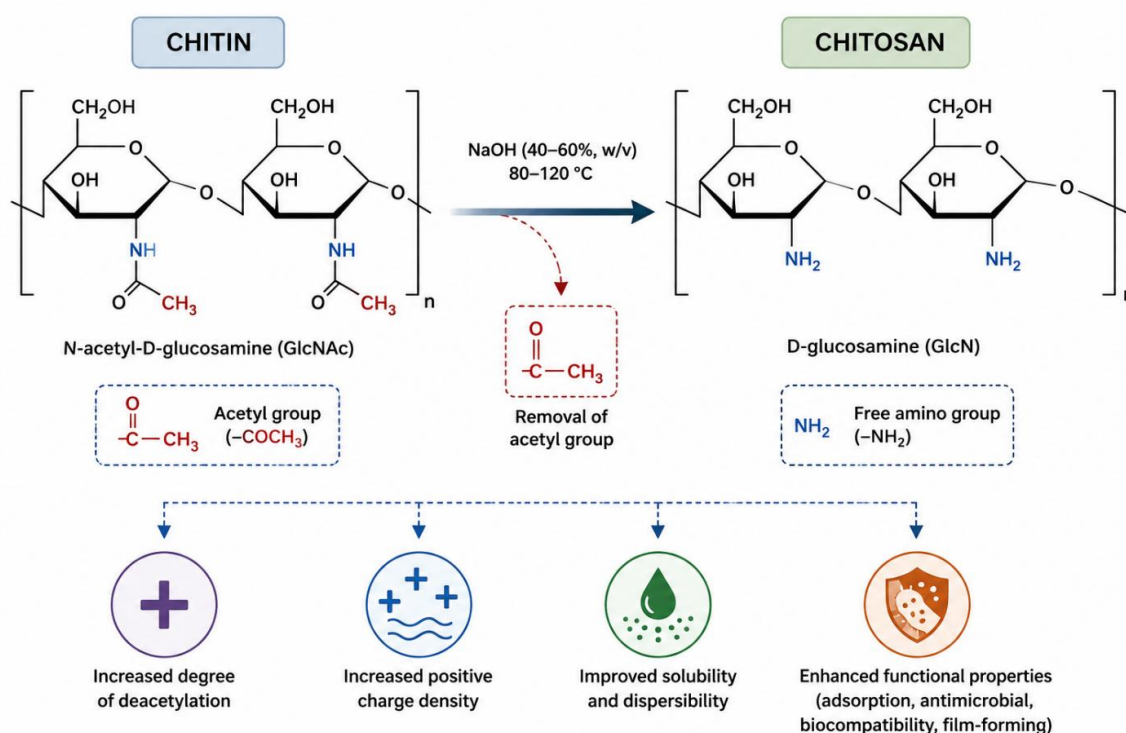


Figure 2. Schematic representation of the alkaline deacetylation of chitin to produce chitosan.

3.2. Emerging Green and Assisted Extraction Technologies

Growing environmental concerns surrounding conventional extraction have stimulated considerable interest in greener and more energy-efficient technologies capable of reducing chemical consumption while maintaining chitosan quality. Among these, microwave-assisted extraction has received particular attention because rapid volumetric heating accelerates demineralization and deacetylation, substantially reducing reaction time and energy requirements compared with conventional heating [13,20]. Several studies (Table 1), have demonstrated that microwave-assisted processing can achieve comparable or higher degrees of deacetylation while better preserving molecular weight owing to shorter exposure times [17].

Autoclave-assisted processing has likewise emerged as a promising alternative by providing uniform temperature and pressure distribution throughout the reaction medium. Compared with conventional heating, autoclave treatment offers improved process control, shorter reaction times, and enhanced reproducibility, making it attractive for pilot-scale production [21]. Similarly, ultrasonic-assisted extraction enhances mass transfer through acoustic cavitation, facilitating penetration of extraction reagents into the shell matrix while reducing chemical requirements and extraction time. However, excessive ultrasonic intensity may promote polymer fragmentation, requiring careful optimization of processing conditions.

Enzymatic extraction represents one of the most environmentally benign approaches because proteolytic enzymes selectively remove proteins under mild operating conditions without extensive degradation of the chitin structure. The resulting chitosan generally exhibits improved molecular integrity and reduced environmental impact compared with chemically extracted materials [3]. Nevertheless, high enzyme costs, relatively slow reaction kinetics, and challenges associated with enzyme recovery continue to limit large-scale industrial implementation.

More recently, deep eutectic solvents (DES) and ionic liquids have attracted growing interest as green extraction media capable of simultaneously dissolving minerals and disrupting protein–polysaccharide interactions while minimizing hazardous chemical waste. Although these technologies remain largely at the laboratory scale, early studies indicate promising extraction efficiency together with improved solvent recyclability and reduced environmental impact [1,14]. Further optimization of solvent recovery and process economics will be necessary before widespread industrial adoption can be achieved.

Overall, no single extraction technology currently satisfies all technical, economic, and environmental requirements. Conventional chemical extraction continues to dominate industrial production because of its maturity and scalability, whereas emerging assisted technologies primarily seek to improve sustainability, reduce chemical consumption, and preserve polymer quality. Future progress will likely depend on integrating complementary extraction strategies rather than replacing conventional processing with a single alternative technology. Hybrid approaches combining chemical treatment with microwave, ultrasonic, enzymatic, or green solvent-assisted processes may therefore represent the most practical pathway toward sustainable industrial production of high-quality chitosan.

Table 1. Representative studies on extraction and characterization of shrimp shell-derived chitosan.

Study	Shrimp Species	Extraction Method	Chitosan Yield (%)	DD (%)	Characterization Techniques
Olafadehan et al. (2021)	<i>Penaeus notialis</i>	Chemical extraction	16.93	89.73	FTIR, SEM
Liyanage et al. (2023)	<i>Litopenaeus vannamei</i>	Chemical extraction	10.15–33.26	82–89	FTIR, XRD
Aberoumand et al. (2025)	<i>Liocarcinus vernalis</i>	Chemical extraction	50.0	99.53	Biochemical characterization
Hosney et al. (2024)	Shrimp biowaste	Optimized chemical extraction	15.1–51.3	89.7–93.8	FTIR, SEM

Çelebi et al. (2026)	Shrimp shell waste	Conventional, microwave and autoclave methods	Variable	67–95	FTIR, molecular weight
Present review	Multiple species	Comparative review	Variable	Variable	Comprehensive assessment

3.3. Factors Influencing Chitosan Yield and Physicochemical Quality

The physicochemical quality of chitosan is governed by a complex interaction between biological characteristics of the raw material and extraction conditions. Shrimp species, habitat, age, nutritional status, seasonal variation, and shell composition all influence the initial proportions of chitin, proteins, and minerals, thereby affecting extraction efficiency and the properties of the final product [4,10]. Consequently, extraction protocols optimized for one shrimp species may not necessarily be applicable to another.

Processing parameters exert an equally important influence on chitosan quality. Demineralization and deproteinization conditions determine polymer purity, whereas alkali concentration, reaction temperature, treatment duration, and solid-to-liquid ratio during deacetylation largely control the DD and molecular weight (MW). Increasing treatment severity generally enhances DD but simultaneously promotes depolymerization, resulting in lower molecular weight and altered crystallinity [12]. Because DD and MW collectively determine viscosity, solubility, adsorption capacity, antimicrobial activity, and mechanical performance, optimization should aim to balance extensive deacetylation with preservation of polymer integrity rather than maximizing a single quality parameter.

Recent optimization studies employing response surface methodology (RSM) have demonstrated that simultaneous adjustment of reaction variables provides better control of chitosan quality than conventional one-factor-at-a-time approaches [2,21]. Future extraction protocols should therefore adopt multi-parameter optimization strategies capable of simultaneously maximizing yield, DD, and molecular weight while minimizing chemical consumption and energy requirements.

3.4. Industrial Scalability and Techno-Economic Considerations

Despite considerable advances in laboratory-scale extraction, translation of chitosan production to industrial scale remains constrained by technical, economic, and environmental challenges. Conventional chemical extraction continues to dominate commercial production because of its simplicity, high throughput, and established processing infrastructure. However, large-scale implementation requires substantial consumption of concentrated acids and alkalis, extensive washing, high water demand, and considerable energy input, all of which increase production costs and generate significant volumes of wastewater requiring treatment [1,3].

Emerging technologies, including microwave-assisted, ultrasonic-assisted, enzymatic, and deep eutectic solvent-based extraction, have demonstrated improvements in extraction efficiency and environmental performance at laboratory scale. Nevertheless, their industrial adoption remains limited by equipment costs, process complexity, solvent recovery, enzyme stability, and insufficient pilot-scale validation [14,17]. Consequently, future commercialization is likely to depend on hybrid extraction systems that combine the robustness of conventional chemical processing with the efficiency of green-assisted technologies.

Techno-economic feasibility has therefore become a key consideration in process development. In addition to maximizing chitosan yield, economically viable production requires minimizing reagent consumption, reducing energy demand, recovering processing chemicals where feasible, and improving process integration. Comparative techno-economic assessment should become a routine component of future extraction studies, enabling objective evaluation of production costs alongside product quality and environmental performance.

3.5. Sustainability Assessment and Future Extraction Strategies

The transition from laboratory-scale production to sustainable industrial manufacturing requires extraction technologies that are environmentally responsible as well as economically competitive. Life cycle assessment (LCA) has increasingly been recognized as an important decision-support tool for evaluating the environmental impacts associated with chitosan production, including chemical consumption, energy use, water demand, greenhouse gas emissions, and waste generation [4,14]. Incorporating LCA into extraction studies enables comparison of alternative processing routes beyond extraction efficiency alone and facilitates selection of technologies with lower overall environmental footprints.

Recent advances in artificial intelligence (AI) and machine learning (ML) also present new opportunities for process optimization. Artificial neural networks, machine learning algorithms, and response surface methodology have been successfully applied to predict chitosan yield and optimize extraction variables, often outperforming conventional empirical optimization approaches [2]. As larger experimental datasets become available, AI-assisted optimization may enable more accurate prediction of process outcomes while reducing experimental cost and development time.

Future progress is also expected through the development of integrated shrimp-shell biorefineries that simultaneously recover multiple high-value products, including proteins, calcium carbonate, pigments such as astaxanthin, chitin, and chitosan, rather than focusing solely on chitosan production [1,5]. Such integrated valorization strategies improve resource efficiency, reduce waste generation, and strengthen the economic viability of shrimp processing residues within a circular bioeconomy framework. Together with green solvents, reagent recycling, renewable energy integration, and carbon footprint reduction strategies, these approaches represent promising directions for establishing more sustainable and commercially competitive chitosan production systems.

Although numerous extraction technologies have been proposed, few have progressed beyond laboratory-scale investigation. Future research should therefore place greater emphasis on standardized process evaluation, pilot-scale validation, techno-economic assessment, and life cycle analysis alongside conventional measurements of yield and degree of deacetylation. Integrating green extraction technologies with AI-assisted process optimization and biorefinery concepts offers a promising pathway toward sustainable industrial production of high-quality chitosan while supporting circular bioeconomy and resource valorization objectives.

4. Characterization and Analytical Evaluation of Chitosan

Characterization is fundamental to establishing the structural integrity, physicochemical quality, and functional performance of chitosan obtained from shrimp shell waste. The physicochemical properties of chitosan, including its DD, molecular weight, crystallinity, morphology, thermal stability, and surface chemistry, are strongly influenced by raw material characteristics and extraction conditions. These properties, in turn, determine its suitability for applications ranging from biomedical engineering and agriculture to environmental remediation and advanced functional materials [1,6,19]. Consequently, comprehensive characterization requires the integration of complementary analytical techniques, as no single method provides sufficient structural and functional information. While spectroscopic techniques primarily elucidate chemical composition and molecular structure, microscopic, diffraction, chromatographic, and thermal methods provide insights into morphology, crystallinity, molecular architecture, and stability. The combined interpretation of these techniques enables a more reliable understanding of structure–property relationships and facilitates quality control during chitosan production. The interrelationship among chitosan structure, characterization techniques, and physicochemical properties is illustrated in Figure 3.

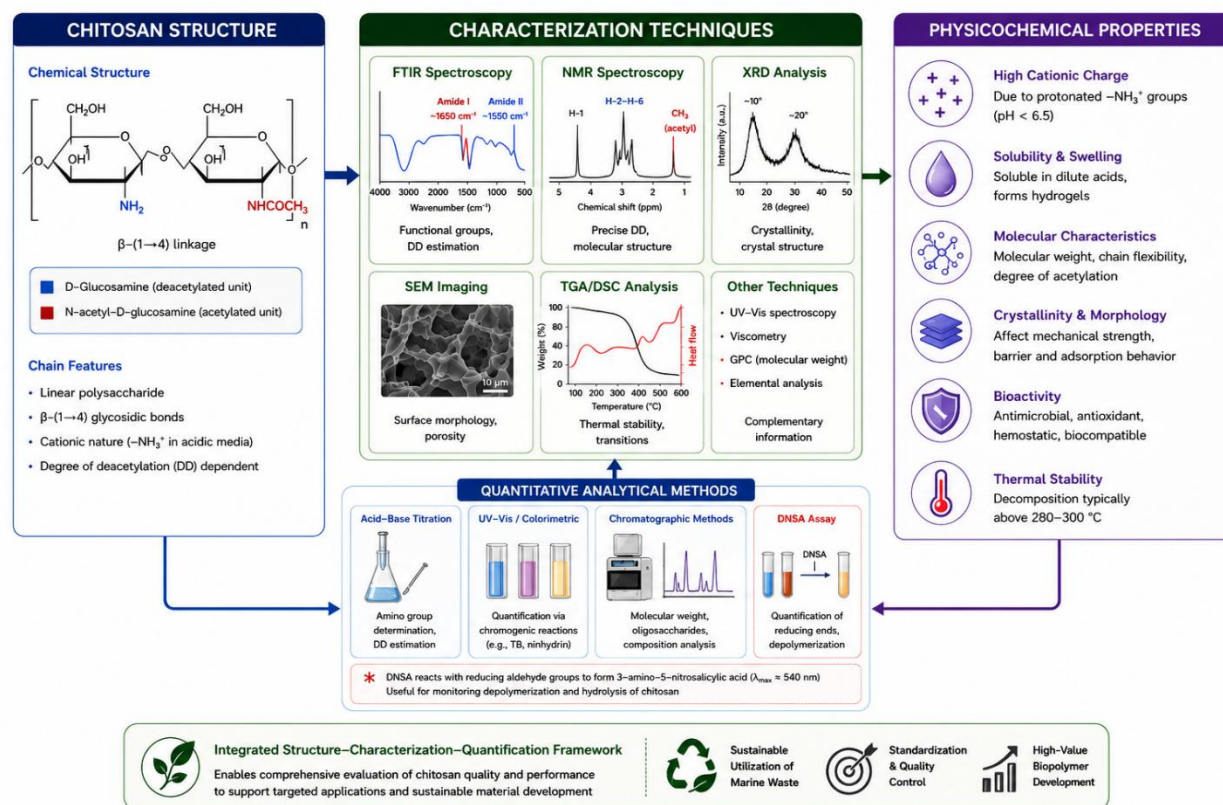


Figure 3. Relationship between chitosan structural attributes, characterization techniques, physicochemical properties, and application domains.

4.1. Structural and Physicochemical Characterization

4.1.1. Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy remains the most widely employed technique for routine characterization of chitosan because of its rapid analysis, minimal sample preparation, non-destructive nature, and relatively low operational cost. The technique provides qualitative and semi-quantitative information on functional groups and is extensively used to verify successful conversion of chitin into chitosan while simultaneously estimating the degree of deacetylation [6,13].

Characteristic FTIR spectra of chitosan exhibit a broad absorption band around 3200–3500 cm^{-1} corresponding to overlapping O–H and N–H stretching vibrations associated with extensive intermolecular hydrogen bonding. Additional diagnostic bands include the amide I band (approximately 1650 cm^{-1}) arising primarily from C=O stretching, the amide II band (1550–1580 cm^{-1}) attributed to N–H bending and C–N stretching, and the amide III band (~1320 cm^{-1}), which collectively provide information on residual acetyl groups and the extent of deacetylation [13]. Bands within the fingerprint region (1500–500 cm^{-1}), particularly those associated with C–O–C glycosidic stretching and β -(1 $\rightarrow$ 4)-glycosidic linkages, further confirm preservation of the polysaccharide backbone [6].

Beyond functional group identification, FTIR has become one of the most frequently applied techniques for estimating DD because it offers a rapid alternative to more sophisticated analytical methods. Various absorbance ratio equations based on amide and hydroxyl or amine bands have been proposed, although differences in baseline correction, peak selection, and calculation procedures often result in variability among reported DD values [1]. Consequently, FTIR-derived DD values should generally be regarded as semi-quantitative estimates rather than absolute measurements. Nevertheless, its accessibility and reproducibility

make FTIR particularly valuable for routine quality control and comparative evaluation of extraction protocols, especially in laboratories where advanced spectroscopic instrumentation is unavailable.

Despite these advantages, FTIR has inherent limitations. Overlapping absorption bands, sensitivity to sample preparation, moisture content, and spectral baseline correction may affect analytical accuracy. Furthermore, FTIR provides indirect estimation of acetylation and cannot fully resolve subtle structural heterogeneity or molecular sequence distribution. Therefore, complementary analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy are often recommended when highly accurate structural characterization is required [2].

4.1.2. Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) provides high-resolution visualization of chitosan surface morphology and microstructural organization. Although SEM does not directly determine chemical composition, it offers valuable information regarding particle architecture, porosity, fibrillar organization, surface roughness, and structural homogeneity, all of which influence the functional performance of chitosan materials [12,22].

Morphological characteristics vary considerably depending on the biological source of chitin and extraction conditions. Chitosan produced through conventional alkaline extraction generally exhibits irregular, heterogeneous, compact, and partially fibrous surfaces, whereas microwave-assisted and enzymatic extraction methods often preserve a more porous and interconnected microstructure due to reduced polymer degradation [13,17]. Increased alkali concentration and prolonged deacetylation may further alter surface morphology by promoting fibril disruption and partial depolymerization, leading to denser particle structures [4].

These morphological differences have important functional implications. Rough and porous surfaces generally provide larger specific surface areas and increased accessibility of amino groups, thereby enhancing adsorption efficiency, antimicrobial interactions, and immobilization of bioactive compounds. Conversely, smoother and more compact morphologies may improve mechanical strength and dimensional stability, making them advantageous for film formation and packaging applications [16,22]. Consequently, SEM observations should be interpreted alongside physicochemical characterization to establish meaningful structure–function relationships rather than merely describing surface appearance.

4.1.3. X-ray Diffraction (XRD)

X-ray diffraction is widely employed to evaluate the crystalline organization of chitosan and to assess structural changes induced during demineralization, deproteinization, and deacetylation. Native chitin exhibits a highly ordered crystalline structure stabilized by extensive intermolecular hydrogen bonding, whereas conversion to chitosan generally reduces crystallinity owing to the removal of acetyl groups and disruption of molecular packing [6,19].

Typical chitosan diffractograms display characteristic reflections near 2θ values of approximately 10° and 20° , although peak intensity and crystallinity index vary considerably according to raw material source, extraction conditions, and molecular weight [12,13]. Chemical extraction using concentrated alkali frequently decreases crystallinity because partial depolymerization and deacetylation disrupt the ordered arrangement of polymer chains. Conversely, milder extraction approaches such as microwave-assisted or enzymatic treatments may better preserve crystalline domains while maintaining desirable physicochemical properties [4].

Crystallinity strongly influences functional characteristics including solubility, swelling behavior, mechanical strength, biodegradation rate, and adsorption performance. Highly crystalline chitosan generally exhibits superior structural stability but reduced solubility and accessibility of reactive amino groups. In contrast, lower crystallinity enhances water uptake and chemical reactivity but may compromise mechanical integrity [23]. Therefore, XRD should be interpreted together with FTIR, molecular weight analysis, and thermal characterization to provide a comprehensive understanding of chitosan quality.

4.1.4. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance spectroscopy represents the most accurate technique for elucidating the molecular structure of chitosan and determining its degree of deacetylation. Unlike FTIR, which estimates acetylation indirectly through absorbance ratios, NMR directly quantifies the relative abundance of acetylated and deacetylated glucosamine residues, thereby providing highly reliable structural information [24,25].

^1H NMR is most commonly employed to determine DD through integration of signals corresponding to the methyl protons of acetyl groups relative to glucosamine ring protons. In contrast, ^{13}C NMR provides additional information regarding carbon skeleton organization, substitution patterns, and molecular conformation. These capabilities make NMR particularly valuable for research requiring precise structural characterization or validation of alternative analytical methods [24].

Despite its superior analytical accuracy, routine application of NMR remains limited by the requirement for expensive instrumentation, high-purity solvents, and specialized technical expertise. Sample solubility may also restrict analysis of highly crystalline or high-molecular-weight chitosan. Consequently, NMR is generally regarded as the reference method for detailed structural investigations, whereas FTIR remains the preferred technique for routine characterization because of its lower cost and greater accessibility.

4.1.5. Thermal Analysis

Thermal characterization provides valuable information regarding the stability, degradation behavior, and processing characteristics of chitosan. Thermogravimetric analysis (TGA) evaluates mass loss associated with moisture evaporation and polymer decomposition, whereas differential scanning calorimetry (DSC) examines thermal transitions such as dehydration, crystallization, and degradation [12,23].

Typical thermograms of chitosan exhibit an initial weight loss below approximately 120°C due to evaporation of adsorbed water, followed by a major degradation event between 250 and 350°C corresponding to depolymerization and decomposition of the polysaccharide backbone [6]. Variations in thermal stability are strongly influenced by DD, molecular weight, crystallinity, and residual mineral content. Chitosan with higher DD and greater structural homogeneity generally exhibits improved thermal stability owing to stronger intermolecular interactions [16].

Although thermal analysis does not directly determine molecular structure, it provides complementary information that is essential for evaluating processing behavior and application potential. When combined with FTIR, XRD, and NMR, thermal techniques contribute to a comprehensive assessment of chitosan quality and functional performance.

4.2. Determination of Degree of Deacetylation

The degree of deacetylation (DD) is one of the most important quality indicators of chitosan because it determines the proportion of free amino groups available along the polymer chain and consequently influences solubility, cationic charge density, adsorption capacity, biodegradability, antimicrobial activity, and chemical reactivity [19]. Chitosan intended for biomedical and environmental applications generally requires DD values above 70%, while higher DD often enhances protonation and functional performance under acidic conditions [26].

Several analytical techniques have been developed for DD determination, each differing in analytical principle, accuracy, instrumentation requirements, and suitability for routine analysis. FTIR remains the most widely adopted method because it is rapid, non-destructive, and inexpensive. However, FTIR-derived DD values depend on the selection of absorbance bands, baseline correction, and calibration equations, leading to considerable variability among studies [26,27]. Acid–base titration estimates DD through quantification of accessible amino groups and is widely used because of its simplicity and low cost. Nevertheless, incomplete

protonation, polymer aggregation, and limited accessibility of amino groups may result in underestimation of DD compared with spectroscopic techniques [25].

NMR spectroscopy is widely regarded as the reference method because it directly quantifies acetylated and deacetylated monomer units with high precision. However, the high cost of instrumentation and greater analytical complexity restrict its routine use in many laboratories [24]. Consequently, no single method can be considered universally optimal. The choice of analytical technique should therefore be guided by the required level of accuracy, instrument availability, and intended application. Future efforts should focus on developing standardized analytical protocols and certified reference materials to improve inter-laboratory comparability and facilitate industrial quality assurance.

4.3. Molecular Weight Determination

Molecular weight (MW) is a critical physicochemical parameter that strongly influences the viscosity, solubility, mechanical strength, biodegradability, film-forming ability, and biological activity of chitosan. Together with the degree of deacetylation, MW largely determines the suitability of chitosan for specific applications. High-molecular-weight chitosan generally exhibits superior mechanical properties and film integrity, whereas low-molecular-weight chitosan and chitooligosaccharides possess enhanced solubility, bioavailability, and antimicrobial activity [6,19]. Consequently, reliable determination of molecular weight is essential for both quality control and application-oriented product development.

Several analytical techniques are available for molecular weight determination, each differing in analytical complexity, accuracy, and instrumentation requirements. Gel permeation chromatography (GPC), also referred to as size exclusion chromatography (SEC), is the most widely accepted method because it separates polymer molecules according to hydrodynamic volume, allowing determination of molecular weight distribution and polydispersity index [24]. When coupled with multi-angle light scattering (MALS), GPC provides highly accurate absolute molecular weight measurements without reliance on calibration standards. However, the high cost of instrumentation, specialized operation, and stringent sample preparation limit its routine application, particularly in resource-limited laboratories.

Viscometric methods remain popular alternatives because they are comparatively inexpensive and straightforward. These approaches estimate molecular weight from intrinsic viscosity using the Mark–Houwink equation, which relates polymer viscosity to molecular size. Although less precise than chromatographic methods, viscometry is widely employed for routine quality assessment and comparative evaluation of extraction conditions [26]. Other techniques, including dynamic light scattering (DLS) and mass spectrometry, have also been explored, particularly for low-molecular-weight chitosan and chitooligosaccharides, although their application remains comparatively limited.

Selection of an appropriate molecular weight determination method should therefore balance analytical accuracy, instrument availability, and the intended application. While chromatographic techniques remain the reference standard for detailed characterization, viscometric methods provide practical alternatives for routine laboratory analysis. Future studies should prioritize standardized molecular weight determination protocols to improve comparability among studies and facilitate industrial quality assurance.

4.4. Quantitative Analytical Methods for Chitosan Evaluation

Accurate quantification of chitosan and assessment of its physicochemical properties are essential for quality control, process optimization, and application development. Analytical methods differ considerably in their underlying principles, analytical performance, instrumentation requirements, and suitability for routine implementation. Consequently, method selection should be guided by the analytical objective rather than relying on a single universal approach.

Acid–base titration remains one of the simplest and most widely applied methods for estimating the degree of deacetylation through quantification of protonated amino groups. The method is inexpensive, requires

minimal instrumentation, and is well suited for routine laboratory analysis. However, its accuracy may be influenced by incomplete protonation, polymer aggregation, and limited accessibility of amino groups, which can lead to underestimation of DD relative to spectroscopic methods [25,26].

FTIR spectroscopy provides a rapid and non-destructive alternative by estimating DD from characteristic absorbance ratios. Its widespread use stems from ease of operation and minimal sample preparation; however, results depend on spectral quality, peak selection, and calculation method, limiting inter-study comparability [24,27]. In contrast, NMR spectroscopy directly quantifies acetylated and deacetylated residues, providing the highest analytical accuracy. Despite this advantage, high equipment costs and greater technical requirements restrict its routine use outside specialized laboratories.

Chromatographic techniques, particularly GPC/SEC and high-performance liquid chromatography (HPLC), provide detailed information on molecular weight distribution, polymer degradation, and oligomer composition. These methods exhibit excellent analytical sensitivity and reproducibility but require sophisticated instrumentation and highly trained personnel, limiting their accessibility in many developing regions [24].

Colorimetric and spectrophotometric methods represent practical alternatives where rapid, low-cost analysis is required. Assays based on dyes or reducing sugar determination require relatively simple instrumentation and are particularly attractive for routine screening. Nevertheless, these approaches generally provide indirect measurements and should be interpreted alongside complementary structural characterization techniques. Among these methods, the 3,5-dinitrosalicylic acid (DNSA) assay has attracted increasing attention because of its simplicity, affordability, and suitability for monitoring reducing sugars generated during chitosan depolymerization.

Overall, no single analytical technique comprehensively characterizes chitosan. Rather, the integration of complementary methods offers the most reliable strategy for evaluating polymer quality. Spectroscopic techniques are particularly valuable for structural confirmation, chromatographic methods provide detailed molecular characterization, whereas colorimetric assays facilitate rapid routine analysis. Continued efforts toward harmonization of analytical protocols and validation procedures will improve inter-laboratory reproducibility and support industrial standardization of chitosan products.

4.5. DNSA-Based Analytical Approaches for Reducing-End Quantification

The 3,5-dinitrosalicylic acid (DNSA) assay is a well-established colorimetric method for quantifying reducing sugars released during polysaccharide hydrolysis. Although originally developed for carbohydrate analysis [28], the method has gained increasing attention in chitosan research because it enables indirect monitoring of polymer depolymerization through quantification of reducing ends generated following cleavage of β -(1 $\rightarrow$ 4)-glycosidic linkages [9,29]. Unlike structural characterization techniques such as FTIR, XRD, or NMR, DNSA does not characterize the polymer itself but instead evaluates degradation products, making it a complementary analytical tool for studies involving chitosan hydrolysis, enzymatic degradation, and molecular modification.

4.5.1. Principle and Analytical Basis

The DNSA assay is based on the reduction of 3,5-dinitrosalicylic acid under alkaline conditions by free aldehyde groups present at the reducing ends of carbohydrate chains. During heating, DNSA is reduced to 3-amino-5-nitrosalicylic acid, producing an orange-red chromophore whose absorbance is typically measured at 540 nm [28]. The intensity of colour formation is directly proportional to the concentration of reducing sugars generated during depolymerization, allowing indirect quantification of glycosidic bond cleavage.

In chitosan analysis, reducing ends are produced following chemical, thermal, or enzymatic cleavage of the polymer backbone. Consequently, the DNSA assay is particularly useful for evaluating depolymerization efficiency, monitoring enzymatic hydrolysis, comparing degradation treatments, and estimating the extent of molecular breakdown. Because native chitosan contains relatively few reducing ends, the assay is not intended

for direct quantification of intact chitosan but rather for assessment of degradation products generated during controlled hydrolysis [29,30]. The fundamental reaction mechanism and its application in chitosan depolymerization studies are illustrated in Figure 4.

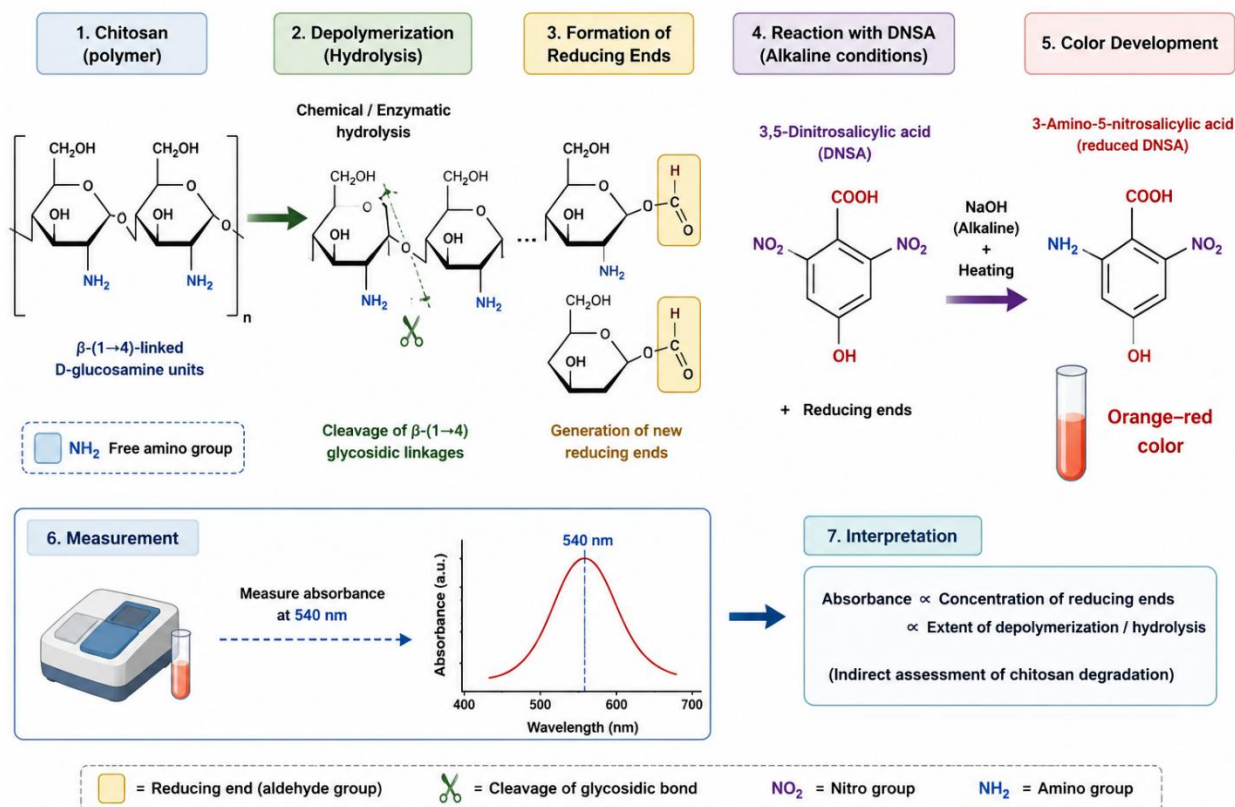


Figure 4. Principle of the DNSA assay for reducing-end quantification during chitosan depolymerization.

4.5.2. Advantages and Analytical Applications

Compared with chromatographic and spectroscopic techniques, the DNSA assay offers several practical advantages. It is inexpensive, rapid, requires only a UV–Vis spectrophotometer, and involves relatively simple sample preparation. These characteristics make it particularly suitable for routine laboratory analysis and for research environments with limited access to advanced analytical instrumentation.

The assay has been widely applied to monitor enzymatic saccharification, evaluate hydrolysis kinetics, compare depolymerization strategies, and estimate reducing sugar production during polysaccharide degradation [28,29]. In chitosan research, DNSA has also been used to assess the efficiency of oxidative, acid-catalysed, and enzymatic depolymerization processes, providing a rapid means of comparing treatment conditions before undertaking more sophisticated structural analyses [9]. Owing to its simplicity and relatively high throughput, the method is particularly valuable during preliminary process optimization and routine quality assessment.

4.5.3. Limitations and Analytical Considerations

Despite its practical advantages, the DNSA assay has important analytical limitations that should be recognized. Most notably, the method lacks specificity because DNSA reacts with all reducing sugars rather than exclusively with chitosan-derived products. Consequently, interfering carbohydrates or degradation products present within complex reaction mixtures may contribute to absorbance, potentially leading to overestimation of reducing sugar concentration [28,30].

Furthermore, glucose is commonly employed as the calibration standard because of its commercial availability and well-characterized reducing properties. However, reducing products generated from chitosan

depolymerization primarily consist of glucosamine-containing oligomers rather than free glucose. As a result, the assay provides an indirect estimation of reducing ends rather than an absolute quantification of chitosan degradation products. This limitation should be acknowledged when interpreting quantitative results, particularly where accurate molar determination is required.

Analytical performance is also influenced by reaction temperature, heating duration, reagent composition, pH, and sample matrix. Careful standardization of assay conditions is therefore essential to ensure reproducibility and facilitate comparison among studies. Validation parameters including linearity, precision, accuracy, and detection range should be reported whenever the method is applied for quantitative analysis.

Collectively, the DNSA assay occupies a unique position within the analytical toolbox for chitosan research. Although it cannot provide direct structural information, its simplicity, affordability, and operational efficiency make it a valuable complementary technique for monitoring reducing-end formation during depolymerization. Future research should prioritize method standardization, development of glucosamine-based calibration systems, comprehensive validation against established chromatographic and spectroscopic techniques, and integration with emerging portable analytical technologies to enhance its reliability and industrial applicability.

5. Emerging Applications, Research Challenges, and Future Opportunities

The unique combination of biodegradability, biocompatibility, non-toxicity, film-forming ability, and reactive amino groups has established chitosan as one of the most versatile natural polymers. Its physicochemical properties can be readily tailored through variation in molecular weight, degree of deacetylation, and chemical modification, enabling applications across biomedical engineering, agriculture, environmental remediation, food packaging, and advanced functional materials. Continued advances in extraction technologies and structural characterization have further expanded opportunities for developing application-specific chitosan materials with improved performance. The major application domains and the physicochemical properties underpinning their functionality are summarized in Table 2.

Table 2. Major applications of chitosan and the physicochemical properties underpinning their performance.

Application Area	Functional Property	Relevant Chitosan Characteristics
Drug Delivery	Mucoadhesion, controlled release	High DD, tailored molecular weight
Wound Healing	Antimicrobial activity, hemostasis	High DD, biocompatibility
Tissue Engineering	Cell adhesion, biodegradability	Porous morphology, biocompatibility
Antimicrobial Materials	Membrane disruption	High amino group density
Plant Disease Management	Defense elicitation	High DD, bioactivity
Plant Growth Promotion	Physiological stimulation	Solubility and bioavailability
Water Treatment	Metal adsorption and chelation	High DD, surface area
Flocculation	Charge neutralization	Cationic amino groups
Food Packaging	Film formation and antimicrobial protection	Appropriate molecular weight and flexibility
Nanoparticle Synthesis	Stabilization and capping	Reactive amino and hydroxyl groups
Nanocomposites	Structural reinforcement	Chemical modifiability
Smart Materials	Stimuli responsiveness	Functionalized chitosan derivatives

5.1. Biomedical Applications

Biomedical research represents one of the fastest-growing areas of chitosan utilization owing to its excellent biocompatibility, biodegradability, antimicrobial activity, and mucoadhesive properties. These characteristics have supported its widespread application in wound dressings, tissue engineering, drug delivery systems, and regenerative medicine [12,15].

In drug delivery, chitosan enhances the stability and controlled release of therapeutic agents while improving mucosal adhesion and cellular uptake. Chemical modification and nanoparticle formulation have further expanded its application for targeted delivery of antibiotics, anticancer agents, proteins, and nucleic acids. Similarly, chitosan-based hydrogels and porous scaffolds have demonstrated considerable promise for cartilage, bone, and skin tissue regeneration by promoting cell adhesion, proliferation, and extracellular matrix formation [15]. These advances highlight the importance of tailoring physicochemical properties such as molecular weight and degree of deacetylation to meet specific biomedical requirements.

5.2. Agricultural Applications

Increasing concerns regarding excessive use of synthetic agrochemicals have stimulated growing interest in chitosan as a sustainable agricultural input. Chitosan functions both as a plant biostimulant and an elicitor of plant defense responses, enhancing resistance against fungal, bacterial, and viral pathogens while improving tolerance to abiotic stresses [3].

Recent developments have focused on nano-enabled formulations in which chitosan serves as both an active antimicrobial material and a carrier for fertilizers, pesticides, and plant growth regulators [31]. These systems improve controlled release, increase treatment efficiency, and reduce environmental losses compared with conventional formulations. Nevertheless, further field validation under diverse agroecological conditions remains necessary before widespread commercial adoption.

5.3. Environmental and Industrial Applications

The high density of amino and hydroxyl functional groups enables chitosan to effectively adsorb heavy metals, dyes, pharmaceuticals, and other emerging contaminants, making it an attractive biosorbent for wastewater treatment [16]. Adsorption efficiency can be further enhanced through cross-linking, surface activation, or incorporation into composite materials, thereby improving mechanical stability and regeneration capacity.

Beyond environmental remediation, chitosan has gained increasing importance in food preservation, biodegradable packaging, membrane technology, and antimicrobial coatings. Its film-forming ability, oxygen barrier properties, and biodegradability have supported development of sustainable alternatives to petroleum-derived packaging materials [1,32]. These applications demonstrate how structural modification of chitosan can be used to tailor functionality for specific industrial requirements while supporting the transition toward renewable biomaterials.

5.4. Functionalized Chitosan Derivatives and Advanced Nanocomposites

Chemical modification has substantially expanded the functional versatility of chitosan beyond that achievable with the native polymer. Derivatives such as carboxymethyl chitosan, quaternized chitosan, thiolated chitosan, and graft copolymers exhibit improved solubility, antimicrobial activity, mucoadhesion, and chemical stability, thereby extending applications in drug delivery, tissue engineering, water treatment, and biosensing [1,3].

Similarly, chitosan-based nanocomposites have emerged as an important research frontier. Incorporation of metallic nanoparticles, magnetic nanoparticles, graphene-based materials, metal oxides, and bioactive ceramics enhances mechanical strength, adsorption capacity, antimicrobial performance, electrical conductivity, and catalytic activity while retaining the intrinsic biocompatibility of chitosan [27]. These multifunctional materials are increasingly being investigated for advanced biomedical devices, smart food packaging, environmental sensors, and water purification systems. Despite promising laboratory-scale results, improved control of nanoparticle dispersion, long-term stability, and biosafety remains essential for successful commercialization.

5.5. Current Research Challenges and Future Opportunities

Despite significant advances in chitosan research, several challenges continue to limit broader industrial implementation. Considerable variability in extraction protocols, molecular weight, and degree of deacetylation often results in inconsistent physicochemical properties, complicating comparison among studies and restricting product standardization. Likewise, differences in analytical methodologies continue to produce variability in reported characterization data, highlighting the need for harmonized analytical protocols and validated reference materials.

Future research should increasingly focus on translating laboratory-scale advances into standardized manufacturing technologies capable of producing reproducible, application-specific chitosan. Greater integration of green extraction methods, advanced polymer modification, multifunctional nanocomposites, and standardized analytical workflows will facilitate production of higher-value chitosan materials. In parallel, stronger collaboration between academia and industry will be essential to accelerate commercialization while ensuring product quality, regulatory compliance, and environmental sustainability. Such interdisciplinary efforts will strengthen the role of shrimp shell-derived chitosan as a sustainable biomaterial supporting circular bioeconomy and next-generation functional materials.

6. Conclusions

The valorization of shrimp shell waste into functional chitosan represents a sustainable approach that simultaneously addresses environmental challenges associated with seafood processing residues and the growing demand for renewable biomaterials. Advances in extraction technologies, structural characterization, and analytical evaluation have significantly improved our understanding of the relationships between processing conditions, physicochemical properties, and functional performance. While conventional chemical extraction remains the dominant production route, emerging green technologies offer promising opportunities to enhance sustainability and reduce environmental impacts.

The diverse applications of chitosan in biomedicine, agriculture, environmental remediation, food packaging, and advanced functional materials highlight its versatility and industrial relevance. However, challenges related to process standardization, variability in physicochemical properties, analytical inconsistencies, and large-scale implementation continue to limit broader commercialization. Future research should focus on developing standardized extraction and characterization protocols, integrating green and scalable production technologies, and advancing data-driven optimization strategies. Such efforts will be essential for realizing the full potential of shrimp shell-derived chitosan as a key biomaterial supporting circular bioeconomy and sustainable materials development.

Author's Contributions

S.B.: Conceptualization, Methodology, Literature Review, Data Curation, Visualization, and Project Administration. **R.C.R.:** Supervision, Critical Review and Editing of Manuscript, Methodological Guidance, Validation, and Resources. **J.K.M.:** Critical Review and Editing of Manuscript. **N.N.P.:** Review of the final draft of the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that there is no commercial or financial conflict of interest that could be perceived as influencing the content of this article.

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