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Full paper

Characterization and Colorimetric Determination of Chitosan Extracted from Shrimp Shell Waste

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Abstract

Chitosan is a biodegradable biopolymer with diverse agricultural, biomedical, and industrial applications; however, information on chitosan derived from *Caridina nilotica* shrimp shell waste remains limited in East Africa. In this study, chitosan was extracted from Kenyan shrimp shell waste, yielding 30%, and characterized using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), acid–base titration, and a 3,5-dinitrosalicylic acid (DNSA)-based colorimetric assay. FTIR confirmed the characteristic functional groups of chitosan, while the degree of deacetylation was 88.85% by FTIR and 75.11% by titration, reflecting the complementary structural and chemical principles underlying the two methods. The DNSA assay exhibited excellent linearity (R^2 = 0.9963) and provided a rapid, inexpensive, and accessible approach for indirectly assessing chitosan depolymerization. These findings demonstrate that *C. nilotica* shrimp shell waste is a promising source of functional chitosan and that the DNSA assay is a useful complementary tool for chitosan characterization in resource-limited laboratories.

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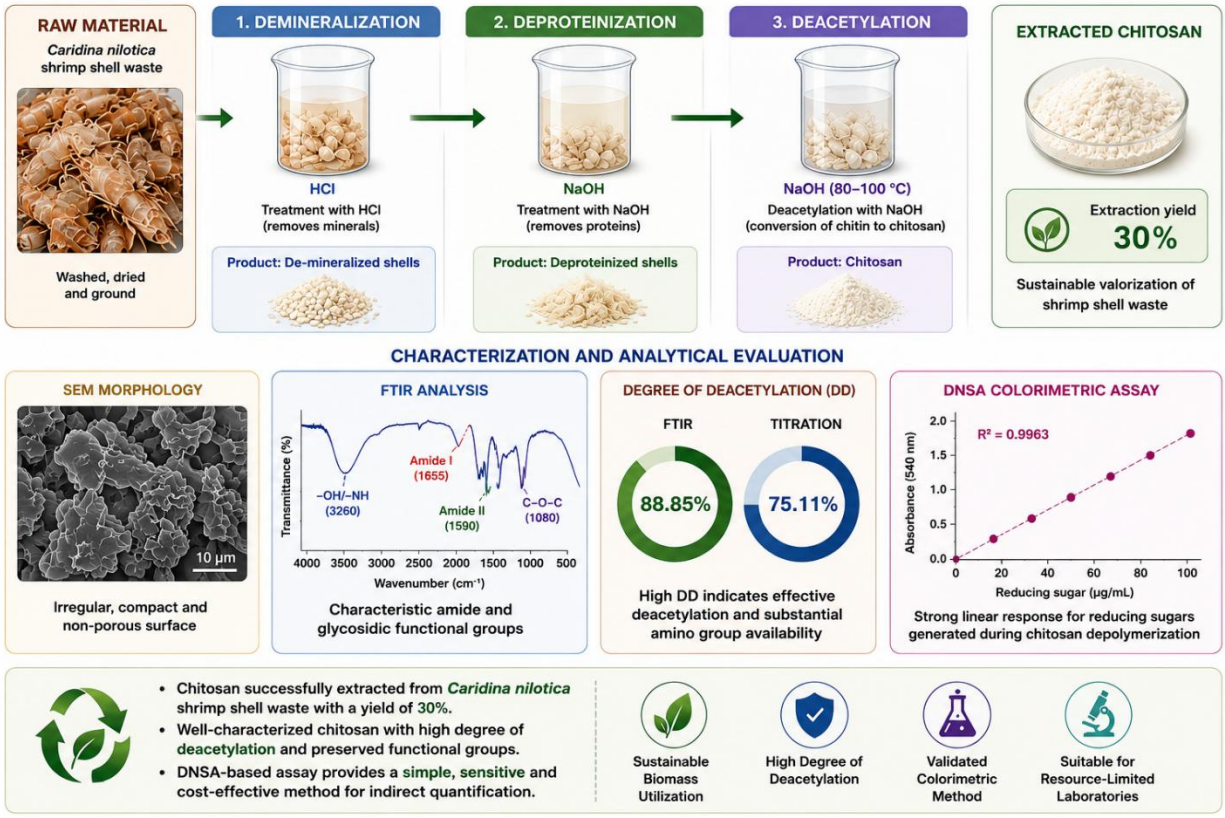

Keywords: Degree of deacetylation, 3,5-dinitrosalicylic acid, Biomass Valorization, Sustainable biomaterials, *C. nilotica*

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1. Introduction

Chitin and its derivative chitosan are linear polysaccharides belonging to the broader class of natural structural biopolymers, which represent an important category of sustainable biomaterials derived from renewable biological resources. Chitin is the second most abundant natural polysaccharide after cellulose [1], [2]. Chitin is widely distributed in nature and is found in the exoskeletons of crustaceans such as shrimp, crabs, and lobsters, as well as in insects, fungal cell walls, algae, and marine zooplankton including corals and jellyfish. Chitosan is obtained through the partial deacetylation of chitin under controlled processing conditions, most commonly via alkaline treatment with sodium hydroxide at elevated temperatures and defined reaction times, although chemical, physical, and enzymatic alternatives have also been reported [2].

Chitosan has attracted considerable scientific and industrial interest due to its unique physicochemical and biological properties. It is biodegradable, biocompatible, non-toxic, and exhibits notable antimicrobial activity [3]. In addition, it possesses functional characteristics such as selective permeability, gel- and film-forming ability,

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metal ion chelation, and high adsorption capacity [4], [5]. These properties underpin its wide range of applications across multiple fields, including medicine, drug delivery systems, tissue engineering, wound healing, agriculture, cosmetics, environmental remediation, water and wastewater treatment, textile processing, and heavy metal adsorption [6]. The versatility of these physicochemical properties has driven increasing interest in chitosan as a sustainable alternative to petroleum-derived polymers across diverse industrial sectors.

The extraction and conversion of chitin to chitosan are strongly influenced by processing parameters, including alkali concentration, reaction temperature, treatment duration, and purification efficiency, which ultimately determine the purity and physicochemical properties of the final product [7]. Although numerous extraction protocols have been reported, considerable variability in raw material composition and processing conditions continues to hinder the production of chitosan with consistent physicochemical properties, emphasizing the need for optimized protocols tailored to individual crustacean species [8], [9]. Various deacetylation approaches have been employed, including chemical alkaline hydrolysis, physical treatments, high-energy techniques, and enzymatic methods [10]. These processes influence major characteristics of chitosan, such as molecular weight, degree of deacetylation, solubility, and functional performance. In addition, the yield and quality of chitin obtained from crustacean sources vary with species, habitat, seasonal conditions, anatomical source, reproductive stage, nutritional status, and age [11], [12]. Consequently, optimization of extraction and deacetylation protocols according to the characteristics of the raw material is essential for producing high-quality chitosan with consistent properties.

Given the increasing demand for sustainable and cost-effective biomaterials, attention has shifted toward the utilization of waste biomass as a source of chitin and chitosan [13]. Shrimp shell waste, in particular, represents an abundant and underutilized resource, especially in coastal regions of Kenya where seafood processing generates significant quantities of by-products [14]. Among the available crustacean resources, *C. nilotica* is abundant in freshwater ecosystems of East Africa but remains largely unexplored as a source of high-quality chitosan. Its utilization could contribute to sustainable waste management while creating value-added biomaterials from an underused local resource.

Despite the growing applications of chitosan, accessible and cost-effective methods for its rapid analysis and characterization remain limited, particularly in resource-limited settings [15], [16]. Existing spectrophotometric methods, including the thiobarbituric acid-based approach reported by Badawy [17], are often affected by reagent instability, pH sensitivity, and analytical interference, which may compromise reproducibility and routine applicability.

In this study, a simple DNSA-based colorimetric assay was developed for the determination of chitosan following sodium nitrite-mediated depolymerization. Unlike thiobarbituric acid-dependent methods, the DNSA approach utilizes a more stable and readily accessible reagent system, providing a rapid and economical alternative for spectrophotometric analysis. In parallel, chitosan was extracted from *C. nilotica* shrimp shell waste sourced in Kenya and systematically characterized to evaluate its physicochemical properties and degree of deacetylation. The study therefore addresses two important gaps: the limited availability of simplified analytical methods for chitosan characterization and the underutilization of locally available shrimp shell biomass as a sustainable source of functional chitosan biomaterials.

2. Results and Discussion

2.1 Extraction Yield of Chitosan

Chitosan was successfully extracted from *C. nilotica* shrimp shell waste with a yield of 30.0% based on the initial dry shell weight. The product was obtained as an off-white flaky powder, characteristic of crustacean-derived chitosan. The yield falls within the range commonly reported for shrimp shell-derived chitosan (20–35%) obtained through conventional chemical extraction methods and is comparable to the 28.4% yield reported by Benhabiles [18] from shrimp shell waste using alkaline deacetylation. Species-specific shell composition and extraction conditions primarily influence variations in yield among studies. The relatively high yield obtained in this study

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demonstrates the potential of locally available shrimp shell waste as a sustainable feedstock for chitosan production.

2.2 Degree of Deacetylation by Acid–Base Titration

The degree of deacetylation (DD) of the extracted chitosan determined by acid–base titration was 75.11%, confirming effective conversion of chitin into chitosan and the presence of a substantial proportion of free amino groups ($–NH_2$). Chitosan with a DD above 70% generally exhibits improved solubility in dilute acidic media together with enhanced adsorption capacity and biological activity, making it suitable for agricultural, environmental, and biomedical applications. The DD obtained in this study is comparable with values reported for shrimp shell-derived chitosan by Dutta and Priyanka (77.04%) [19], Vilar Junior et al. (76.3%) [20], and Boudouaia et al. (88.6%) [21], although considerable variation has been reported in the literature depending on shrimp species, extraction conditions, alkali concentration, and deacetylation time. Similar DD values ranging from approximately 70 to 90% have been reported for chitosan extracted from *Litopenaeus vannamei*, *Penaeus monodon*, and other crustacean species, indicating that the product obtained in this study falls within the range generally regarded as high-quality chitosan.

The DD determined by titration was lower than the value obtained by FTIR analysis (88.85%). This difference is expected because the two techniques evaluate different characteristics of the polymer. Acid–base titration estimates the proportion of protonatable amino groups available in solution and is therefore influenced by polymer solubility, hydrogen bonding, chain aggregation, incomplete protonation, and endpoint determination [22]. Consequently, some amino groups embedded within the polymer matrix may not be fully accessible during titration, leading to lower DD values. In contrast, FTIR estimates DD from characteristic absorbance ratios associated with acetylated and deacetylated functional groups and therefore reflects the overall chemical composition of the polymer rather than only the accessible amino groups. Similar discrepancies between spectroscopic and titrimetric DD measurements have been reported previously and reflect the complementary nature of these analytical approaches rather than analytical inconsistency. Accordingly, the titration results provide an estimate of reactive amino-group availability, whereas FTIR offers a structural assessment of the degree of deacetylation.

2.3 Fourier Transform Infrared (FTIR)

The FTIR spectra of the extracted chitosan and the commercial reference sample exhibited the characteristic absorption bands of chitosan, confirming successful deacetylation of chitin while preserving the fundamental polysaccharide structure (Table 1). The close agreement between the two spectra indicates that the extraction procedure produced chitosan of comparable chemical composition to the commercial material.

A broad and intense absorption band centred at 3448 cm^{-1} was assigned to overlapping O–H and N–H stretching vibrations associated with extensive intermolecular and intramolecular hydrogen bonding within the chitosan matrix [23]. The comparable intensity of this band in both samples suggests similar hydrogen-bonding environments and confirms the hydrophilic nature of the extracted polymer. Weak bands observed at 2311 cm^{-1} and 2034 cm^{-1} are commonly attributed to atmospheric carbon dioxide or overtone vibrations and therefore have limited structural significance.

The characteristic amide I band at 1621 cm^{-1}, corresponding primarily to C=O stretching of residual N-acetyl groups, together with the amide II band at 1564 cm^{-1} arising from N–H bending and C–N stretching vibrations, confirmed that partial deacetylation had occurred while retaining the structural integrity of the polymer backbone [22]. The relatively lower intensity of the amide bands compared with the broad hydroxyl region is consistent with extensive deacetylation and agrees with previous reports for shrimp shell-derived chitosan. Bands located at 1416 cm^{-1} and 1024 cm^{-1} were attributed to CH_2 bending and C–O stretching vibrations of the polysaccharide backbone, respectively (Figure 1). In the fingerprint region, the characteristic β-(1→4)-glycosidic linkage band observed at 926 cm^{-1} further verified preservation of the chitosan backbone following chemical processing. Similar peak positions have been reported for commercially available and crustacean-

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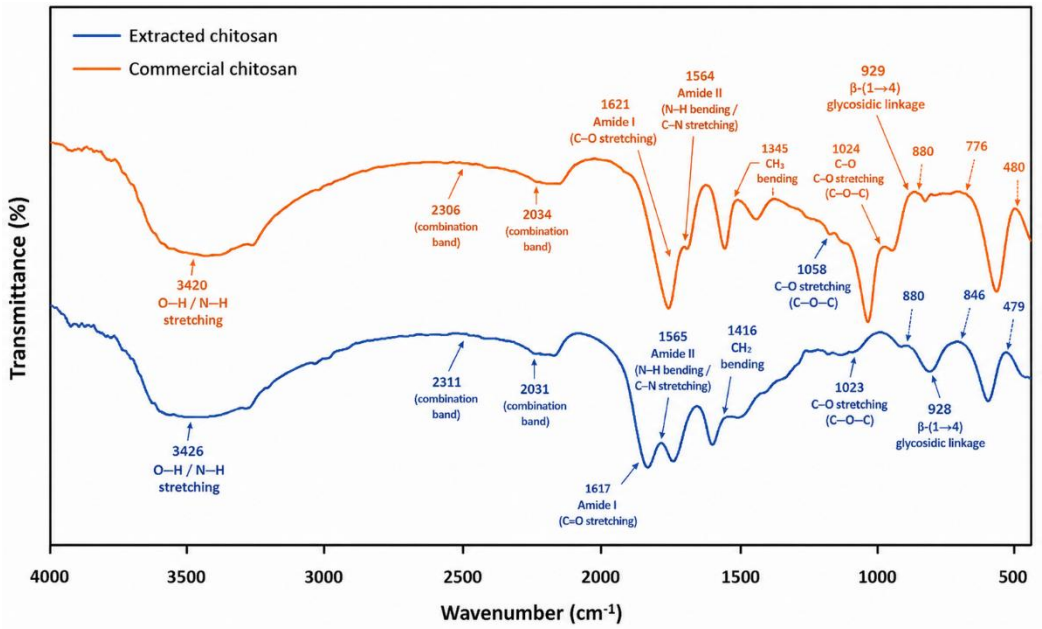
derived chitosan by Nikonenko et al., Moosa et al. [24], [25], and Kimi and Hamdi [23], indicating that the extracted material possesses the expected structural characteristics of chitosan.

The degree of deacetylation estimated from FTIR was 88.85%, whereas the commercial reference exhibited a DD of 92.58%, indicating that the extracted chitosan achieved a high level of deacetylation comparable to commercial-grade material [26]. The higher DD obtained by FTIR compared with acid–base titration reflects the different analytical principles of the two methods. FTIR determines DD from absorbance ratios of characteristic functional groups and therefore represents the overall structural acetylation of the polymer, whereas titration quantifies only protonatable amino groups accessible in solution [22], [27]. Factors such as incomplete dissolution, hydrogen-bonding interactions, and reduced accessibility of amino groups may therefore contribute to lower titrimetric DD values. Consequently, the two techniques provide complementary rather than competing information regarding chitosan quality, and their combined application offers a more comprehensive evaluation of the extracted polymer.

Table 1. Characteristic FTIR absorption bands of extracted chitosan

Wavenumber (cm^{-1})	Relative intensity	Functional group / Assignment	Structural significance
3448	Broad, strong	O–H/N–H stretching	Hydrogen bonding
2311	Weak	Atmospheric CO_2/overtone	No structural significance
2034	Weak	Combination band	No structural significance
1621	Medium	Amide I (C=O stretching)	Residual acetyl groups
1564	Medium	Amide II (N–H bending/C–N stretching)	Degree of deacetylation
1416	Medium	CH_2 bending	Polysaccharide backbone
1024	Strong	C–O stretching	Glycosidic framework
926	Medium	β-(1→4)-glycosidic linkage	Chitosan backbone confirmation

Fig 1. Fourier-transform infrared (FTIR) spectra of extracted chitosan from *C. nilotica* shrimp shell waste and



commercial chitosan reference standard. Major characteristic absorption bands corresponding to O–H/N–H stretching, amide I, amide II, CH_2 bending, C–O stretching, and β-glycosidic linkages are indicated, confirming the characteristic chitosan structure and similarity between the extracted and commercial samples.

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2.4 Scanning Electron Microscopy (SEM)

The SEM micrographs (Figure 2) revealed the morphological characteristics of both the extracted and commercial chitosan samples. Both materials exhibited comparable surface morphologies characterized by irregular, heterogeneous, and largely non-porous structures with randomly distributed granular and fiber-like features, consistent with previous reports on shrimp shell-derived chitosan [28], [29]. The observed morphology is typical of chitosan produced through alkaline deproteinization and deacetylation, processes that disrupt the highly ordered chitin structure and promote the formation of irregular surface features.

Semi-quantitative examination of the SEM micrographs indicated that the extracted chitosan consisted of irregular agglomerates with characteristic dimensions of approximately 5–11 µm, together with smaller fragmented structures generally below 2 µm. The wide size distribution reflects particle aggregation during alkaline extraction and drying, a characteristic commonly reported for shrimp shell-derived chitosan [30], [31]. Because the present SEM analysis was intended primarily for morphological characterization, a detailed particle size distribution was not determined.

The commercial chitosan exhibited a relatively more compact morphology and higher apparent bulk density than the extracted chitosan, likely reflecting differences in raw material source and processing conditions. The irregular surface texture observed in the extracted chitosan may enhance the accessibility of functional amino groups and increase surface interaction sites, which are important for adsorption, binding, and other applications that depend on surface reactivity. Overall, the SEM results support the successful conversion of shrimp shell-derived chitin into chitosan with morphological characteristics comparable to those of the commercial reference sample.

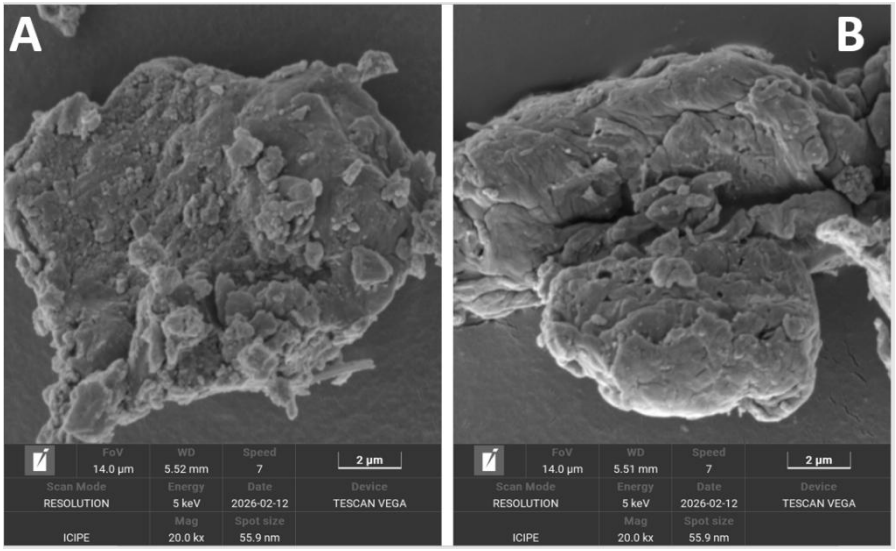

Fig 2. Scanning electron micrographs comparing the surface morphology of (a) chitosan extracted from *C. nilotica* shrimp shell waste with (b) commercial chitosan at different magnifications.

2.5 3,5-Dinitro salicylic acid DNSA Assay

Following incubation, the chitosan samples developed a characteristic orange–red coloration, whereas no visible colour change was observed in the blank, confirming the formation of reducing sugars during depolymerization [32]. Absorbance measured at 540 nm increased progressively with increasing chitosan concentration, ranging from 0.066 to 0.412 after blank correction. This concentration-dependent response indicates enhanced release of reducing sugars resulting from cleavage of glycosidic linkages within the chitosan backbone [33].

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The strong linear relationship between chitosan concentration and absorbance, as evidenced by the glucose calibration curve (R^2 = 0.9963), supports the suitability of the DNSA assay for indirect colorimetric estimation of chitosan depolymerization products (Figure 3). Increasing chitosan concentration produced a corresponding increase in absorbance, reflecting greater release of reducing sugars during depolymerization (Table 2). The ability of the extracted chitosan to generate detectable reducing sugars further reflects the accessibility of reactive glucosamine units and confirms the polymer's functional integrity. These characteristics are relevant to applications involving adsorption, biodegradable biomaterials, and controlled-release systems [34].

Compared with established colorimetric procedures such as the thiobarbituric acid (TBA) method described by Badawy (2012), the DNSA assay offers several practical advantages. It uses inexpensive and widely available reagents, requires only routine UV–visible spectrophotometry, and involves relatively simple sample preparation, making it suitable for laboratories with limited analytical resources [33]. These attributes make the method particularly attractive for preliminary screening and comparative studies of chitosan depolymerization.

Nevertheless, the DNSA assay has inherent limitations. Because the method measures reducing sugars generated after depolymerization, it provides an indirect estimate of chitosan degradation rather than direct determination of chitosan concentration or purity. The assay does not provide information on molecular weight, degree of polymerization, viscosity, crystallinity, or structural heterogeneity, all of which influence the physicochemical properties and functional performance of chitosan [32], [35]. Consequently, DNSA analysis should be regarded as complementary to established characterization techniques such as FTIR, NMR, gel permeation chromatography, X-ray diffraction, and viscometric analysis rather than as a substitute for these methods.

Collectively, the DNSA assay demonstrated excellent analytical linearity while providing a rapid and cost-effective approach for comparative assessment of chitosan depolymerization. When combined with structural characterization techniques, it offers a practical analytical framework for laboratories where access to advanced instrumentation is limited.

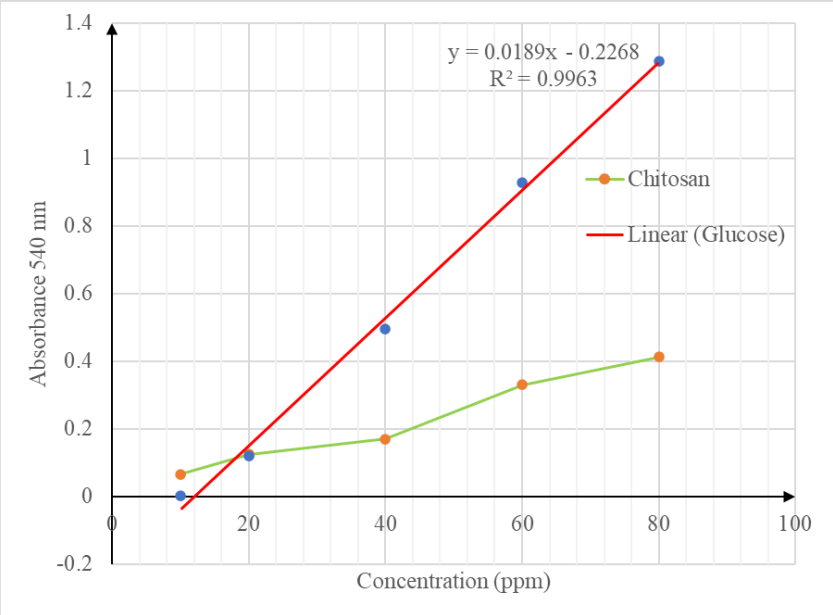

Fig 3. Calibration curves for the DNSA assay showing the relationship between absorbance and concentration for glucose standards and chitosan samples over a concentration range of 10–100 ppm.

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Table 2. Raw absorbance values obtained during the DNSA assay

Chitosan concentration (ppm)	Mean absorbance (540 nm)
10	0.066
20	0.119
40	0.173
60	0.323
80	0.412

3. Materials and Methods

Shrimp shell waste derived from *C. nilotica* was obtained from local seafood vendors in Migori County, Kenya. All chemicals and reagents used in this study were of analytical grade and purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and used as received without further purification.

Fourier-transform infrared (FTIR) spectroscopy was performed using a Bruker Alpha FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) to identify characteristic functional groups and confirm the chemical structure of the extracted chitosan. Surface morphology and microstructural features were examined using a VEGA 3 scanning electron microscope (TESCAN ORSAY HOLDING, Brno, Czech Republic).

3.1 Preparation of Chitosan

Fresh shrimp shells were thoroughly washed under running tap water to remove adhering tissues, fats, and other impurities, rinsed with distilled water, air-dried under shade for 48 h, and subsequently oven-dried at 60 °C for 24 h to remove residual moisture [36]. The dried shells were ground into a fine powder using a clean mortar and pestle.

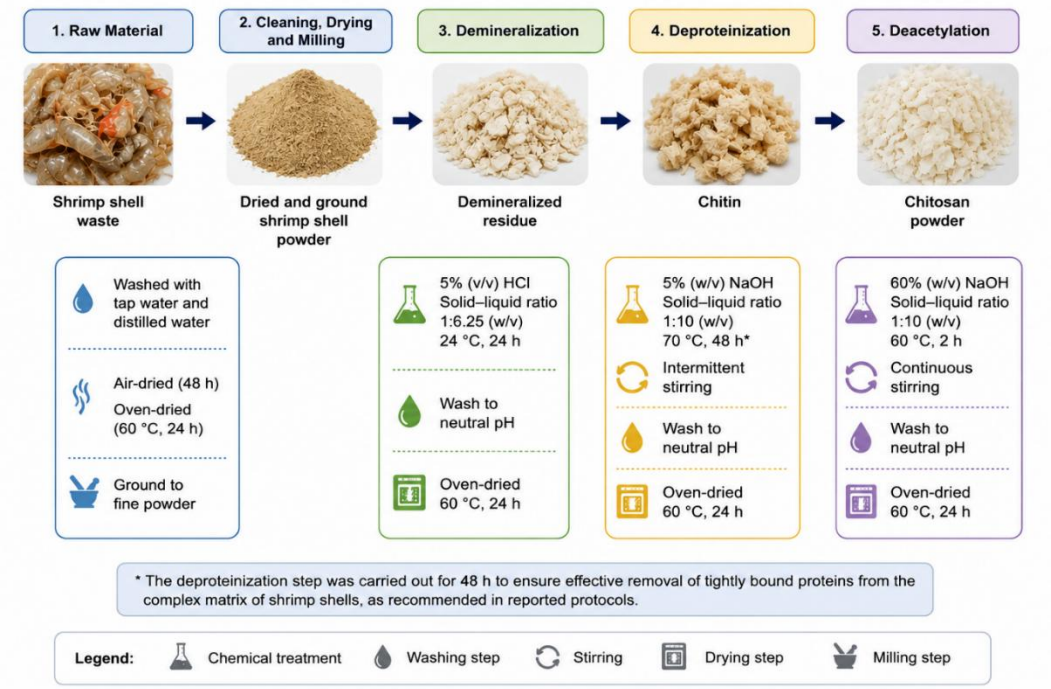

Fig 4. Schematic representation of the extraction and deacetylation process of chitin to chitosan from *C. nilotica* shrimp shell waste.

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Demineralization was performed by treating 40 g of shrimp shell powder with 250 mL of 5% (v/v) HCl at room temperature (24 °C) for 24 h[37]. The residue was washed repeatedly with distilled water until neutral pH and oven-dried at 60 °C for 24 h.

Deproteinization was carried out using 5% (w/v) NaOH at a solid-to-liquid ratio of 1:10 (w/v) and heated at 70 °C for 48 h under intermittent stirring. The extended treatment time was employed to ensure complete removal of tightly bound protein matrices typically associated with crustacean shells, as reported in established chitin extraction protocols[36]. After treatment, the mixture was washed thoroughly with distilled water to remove residual alkali and dried at 60 °C for 24 h to obtain chitin.

Deacetylation was performed using 60% (w/v) NaOH at a 1:10 (w/v) solid-to-liquid ratio and heating at 60 °C for 2 h with continuous stirring [36]. The product was washed to neutral pH and oven-dried at 60 °C for 24 h to obtain chitosan flakes (Figure 4).

3.2. Determination of Degree of Deacetylation (DD) by Acid–Base Titration

The degree of deacetylation (DD) of chitosan was determined by acid–base titration following the method of Dutta [19]. One gram of chitosan sample was dissolved in 1% (v/v) acetic acid, and 30 mL of the solution was used for the analysis. Titration was performed using freshly prepared and standardized 0.1 N NaOH as the titrant. Methyl orange indicator (2–3 drops) was selected due to its sharp and reliable colour transition in the acidic pH range relevant to chitosan protonation–deprotonation behavior. The endpoint was identified by a distinct and persistent colour change from yellow to light orange (Figure 5), corresponding to neutralization of protonated amino groups ($–NH_3^+$). All titrations were carried out in triplicate for each sample to ensure reproducibility.

The DD was calculated using Equation (1), based on the difference in titrant volumes required for neutralization of free amino groups [19].

$$\%\mathrm{DD} = \frac{(\mathrm{V}_1 - \mathrm{V}_2) \times 16}{\mathrm{V}_1 \times 9.94 \times \mathrm{x}} \times 100 \qquad (1)$$

where x is the weight (g) of dried chitosan flakes; V_1 is the volume (mL) of chitosan solution prepared in 0.1 N HCl; and V_2 is the volume (mL) of 0.1 N NaOH consumed during titration. The constant 9.94 represents the theoretical percentage of amino ($–NH_2$) groups in chitosan, derived from the molecular weights of the glucosamine unit (161) and the amino group (16), while 16 corresponds to the gram equivalent weight of the amino group.

Standard precautions, including proper burette calibration, removal of air bubbles, and consistent reading of meniscus levels, were observed to minimize experimental error. Triplicate data were pooled to obtain mean values for DD determination.

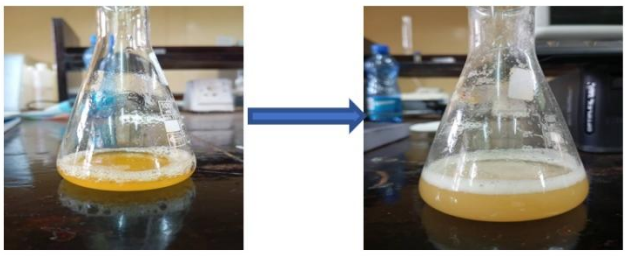

Fig 5. Visual representation of the colour transition during acid–base titration for determination of the degree of deacetylation of chitosan, showing the endpoint change of the methyl orange indicator from yellowish to light orange upon neutralization of protonated amino groups.

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3.3 Fourier Transform Infrared (FTIR) Characterization of Chitosan

Fourier-transform infrared spectroscopy was employed to assess functional group composition and estimate the degree of deacetylation (DD) of the extracted chitosan relative to a commercial chitosan reference standard. FTIR spectra were recorded using a Bruker Alpha 37 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) over the spectral range of 4000–500 cm^{-1} at standard resolution [23].

The fingerprint region (1500–500 cm^{-1}) was specifically examined to confirm the characteristic structural features of chitosan through identification of glycosidic and polysaccharide backbone vibrations. The DD was determined from the absorbance ratio of characteristic amide and amine bands following established procedures [23]. Specifically, absorbance bands at 1320 cm^{-1} (amide III) and 1420 cm^{-1} (CH_2 bending of the protonated amine group) were used in Equation (2), as these bands are sensitive to acetylation level and widely applied for DD estimation in chitosan. Equation (3) was used directly for DD calculation [23].

$$\%\mathrm{DA} = \frac{[(\mathrm{A}_{1320}/\mathrm{A}_{1420}) - 0.3822]}{0.0313} \quad (2)$$

$$\%\mathrm{DD} = 100 - \%\mathrm{DA} \quad (3)$$

where DD is the degree of deacetylation (%), and DA is the degree of acetylation (%).

3.4 Scanning Electron Microscopy (SEM) Characterization of Chitosan

Scanning electron microscopy analysis was performed using a TESCAN VEGA 3 scanning electron microscope (TESCAN ORSAY HOLDING, Brno, Czech Republic). Before analysis, the chitosan sample was dried, mounted on aluminum stubs using carbon tape, and sputter-coated with a thin layer of gold to enhance conductivity. Micrographs were acquired at 20x magnification to evaluate surface morphology, including texture, compactness, porosity, and fibrillar features associated with polymer structure and deacetylation effects. The analysis was used to qualitatively compare structural differences between extracted and commercial chitosan samples.

3.5. 3,5-Dinitro Salicylic Acid (DNSA) Assay Procedure

The 3,5-dinitrosalicylic acid (DNSA) assay was employed to quantify reducing sugars generated following the controlled depolymerization of chitosan, thereby providing an indirect assessment of polymer degradation [32], [38]. Because intact chitosan is a high-molecular-weight polysaccharide with relatively few free reducing ends, it reacts only weakly with DNSA. Controlled depolymerization cleaves the glycosidic backbone, producing lower-molecular-weight oligomers and monomeric reducing sugars containing aldehyde groups capable of reducing DNSA under alkaline conditions. Consequently, the amount of reducing sugars formed is proportional to the extent of chitosan depolymerization. This approach was adopted as a simple and cost-effective alternative for comparative assessment of chitosan degradation using conventional UV–visible spectrophotometry.

A stock solution of extracted chitosan (5 mg mL^{-1}, w/v) was prepared by dissolving the polymer in 1% (v/v) acetic acid under continuous stirring until complete solubilization. Working solutions (1–4 mg mL^{-1}) were prepared by dilution with distilled water. Glucose standards (HiMedia, India) ranging from 10 to 100 ppm were prepared in parallel and used to construct the calibration curve.

For the assay, 1 mL of each chitosan solution, glucose standard, or reagent blank was transferred into separate test tubes. Subsequently, 0.1 mL of freshly prepared 0.5 M sodium nitrite ($NaNO_2$) was added to promote controlled oxidative deamination and partial depolymerization of the chitosan chains, thereby increasing the number of reducing sugar termini available for colorimetric detection. The reaction mixtures were incubated at 80 °C for 45 min in a water bath and then cooled to room temperature [33].

The reaction mixtures were adjusted to pH 4.0 using 1 N HCl before adding 1 mL of freshly prepared DNSA reagent consisting of 0.5 g 3,5-dinitrosalicylic acid, 15 g sodium potassium tartrate, and 10 mL of 2 N NaOH, with the final volume adjusted to 50 mL using distilled water [33]. The mixtures were heated at 75 °C for 15 min to

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facilitate reduction of DNSA by the liberated reducing sugars, forming an orange-red chromophore whose intensity is proportional to the concentration of reducing sugars present.

After cooling to room temperature, absorbance was measured at 540 nm using a UV–Vis spectrophotometer (UV-1600, MRC Laboratory Equipment Ltd., Holon, Israel). Reducing sugar concentrations were determined from the glucose calibration curve according to the method originally described by Miller [39].

4. Conclusions

Chitosan extracted from *C. nilotica* shrimp shell waste exhibited physicochemical characteristics comparable to those of commercial chitosan, confirming the potential of this underutilized biomass as a sustainable source of value-added biopolymers. FTIR and acid–base titration demonstrated a high degree of deacetylation, while SEM revealed the characteristic heterogeneous morphology associated with chemically extracted chitosan. The DNSA colorimetric assay showed excellent linearity and proved to be a rapid, inexpensive, and accessible approach for indirectly assessing chitosan depolymerization, particularly in laboratories with limited analytical infrastructure. However, the DNSA assay should be interpreted within its analytical scope. Because it quantifies reducing sugars released following depolymerization, it does not directly determine chitosan concentration or provide information on molecular weight, viscosity, solubility, crystallinity, or other physicochemical properties that govern material performance. Consequently, the assay is best used as a complementary analytical tool alongside established structural and physicochemical characterization methods. Future studies should incorporate these additional analyses to provide a more comprehensive evaluation of chitosan quality and to further validate the applicability of the DNSA assay across different chitosan sources and processing conditions.

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References

[1] I. Aranaz *et al.*, “Chitosan: An Overview of Its Properties and Applications,” *Polymers*, vol. 13, no. 19, p. 3256, Jan. 2021, doi: 10.3390/polym13193256.

[2] T. Takeuchi, M. Oyama, and T. Hatanaka, “Effect of Chitosan Degradation Products, Glucosamine and Chitosan Oligosaccharide, on Osteoclastic Differentiation,” *BioTech*, vol. 13, no. 1, p. 6, Mar. 2024, doi: 10.3390/biotech13010006.

[3] H. Yadav, R. Malviya, and N. Kaushik, “Chitosan in biomedicine: A comprehensive review of recent developments,” *Carbohydrate Polymer Technologies and Applications*, vol. 8, p. 100551, Dec. 2024, doi: 10.1016/j.carpta.2024.100551.

[4] W. Gonciarz *et al.*, “Chitosan-based formulations for therapeutic applications. A recent overview,” *J Biomed Sci*, vol. 32, p. 62, Jul. 2025, doi: 10.1186/s12929-025-01161-7.

[5] Y. Huang and G. Huang, “Properties of chitosan and its applications as a functional material throughout the plant growth cycle and in sustainable agricultural development,” *Chem. Biol. Technol. Agric.*, vol. 13, no. 1, p. 62, May 2026, doi: 10.1186/s40538-026-00953-9.

[6] S. Sali, M. Thampi, and M. S. Jisha, “Chitosan-based nanocomposites: A sustainable frontier in plant growth regulation and postharvest preservation,” *Physiological and Molecular Plant Pathology*, vol. 141, p. 103026, Jan. 2026, doi: 10.1016/j.pmpp.2025.103026.

[7] I. Thamer, M. Mazurek-Budzyńska, and V. Kumaravel, “Sustainable biopolymer design: extraction of chitin and chitosan using natural deep eutectic solvents with improved antibacterial features,” *Materials & Design*, vol. 259, p. 114775, Nov. 2025, doi: 10.1016/j.matdes.2025.114775.

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i3a02

[8] N.-M. Blebea, C. Pușcașu, R.-A. Vlad, and G. Hancu, "Chitosan-Based Gel Development: Extraction, Gelation Mechanisms, and Biomedical Applications," *Gels*, vol. 11, no. 4, p. 275, Apr. 2025, doi: 10.3390/gels11040275.

[9] M. T. El-Saadony *et al.*, "Chitosan nanoparticles: Green synthesis, biological activities, and sustainable frontiers in targeted drug delivery and cancer nanomedicine – A comprehensive review," *Materials Today Bio*, vol. 35, p. 102358, Dec. 2025, doi: 10.1016/j.mtbio.2025.102358.

[10] Y. Jiang, C. Fu, S. Wu, G. Liu, J. Guo, and Z. Su, "Determination of the Deacetylation Degree of Chitooligosaccharides," *Marine Drugs*, vol. 15, no. 11, p. 332, Oct. 2017, doi: 10.3390/md15110332.

[11] E. Akdaşçi, H. Duman, F. Eker, M. Bechelany, and S. Karav, "Chitosan and Its Nanoparticles: A Multifaceted Approach to Antibacterial Applications," *Nanomaterials*, vol. 15, no. 2, p. 126, Jan. 2025, doi: 10.3390/nano15020126.

[12] S. Jogaiah *et al.*, "Chitosan-metal and metal oxide nanocomposites for active and intelligent food packaging; a comprehensive review of emerging trends and associated challenges," *Carbohydrate Polymers*, vol. 357, p. 123459, Jun. 2025, doi: 10.1016/j.carbpol.2025.123459.

[13] J. Chakravarty and T. A. Edwards, "Innovation from waste with biomass-derived chitin and chitosan as green and sustainable polymer: A review," *Energy Nexus*, vol. 8, p. 100149, Dec. 2022, doi: 10.1016/j.nexus.2022.100149.

[14] E. Zeghdoudi *et al.*, "Valorization of Shrimp Shell Waste: Comparative Phytochemical Composition and Bioactivity of Parapenaeus longirostris and Aristeus antennatus," *ILMU KELAUTAN: Indonesian Journal of Marine Sciences*, vol. 31, pp. 8–14, Mar. 2026, doi: 10.14710/ik.ijms.31.1.8-14.

[15] S. M. Bashir *et al.*, "Chitosan Nanoparticles: A Versatile Platform for Biomedical Applications," *Materials (Basel)*, vol. 15, no. 19, p. 6521, Sep. 2022, doi: 10.3390/ma15196521.

[16] E. Wanyama and J. M. Awika, "Structural modification of Chitosan: Emerging role as a functional ingredient in food systems," *Food Hydrocolloids*, vol. 173, p. 112158, Apr. 2026, doi: 10.1016/j.foodhyd.2025.112158.

[17] M. E. I. Badawy, "A New Rapid and Sensitive Spectrophotometric Method for Determination of a Biopolymer Chitosan," *International Journal of Carbohydrate Chemistry*, vol. 2012, no. 1, p. 139328, 2012, doi: 10.1155/2012/139328.

[18] M. S. Benhabiles, R. Salah, H. Lounici, N. Drouiche, M. F. A. Goosen, and N. Mameri, "Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste," *Food Hydrocolloids*, vol. 29, no. 1, pp. 48–56, Oct. 2012, doi: 10.1016/j.foodhyd.2012.02.013.

[19] J. Dutta and Priyanka, "A facile approach for the determination of degree of deacetylation of chitosan using acid-base titration," *Heliyon*, vol. 8, no. 7, p. e09924, Jul. 2022, doi: 10.1016/j.heliyon.2022.e09924.

[20] J. C. Vilar Junior, D. R. Ribeaux, C. A. Alves da Silva, and G. M. De Campos-Takaki, "Physicochemical and Antibacterial Properties of Chitosan Extracted from Waste Shrimp Shells," *Int J Microbiol*, vol. 2016, p. 5127515, 2016, doi: 10.1155/2016/5127515.

[21] N. Boudouaia, Z. Bengharez, and S. Jellali, "Preparation and characterization of chitosan extracted from shrimp shells waste and chitosan film: application for Eriochrome black T removal from aqueous solutions," *Appl Water Sci*, vol. 9, no. 4, p. 91, May 2019, doi: 10.1007/s13201-019-0967-z.

[22] I. K. Dimzon and T. Knepper, "Degree of Deacetylation of Chitosan by Infrared Spectroscopy and Partial Least Squares.," *International journal of biological macromolecules*, vol. 72, Oct. 2014, doi: 10.1016/j.ijbiomac.2014.09.050.

[23] M. Kimi and M. H. Hamdi, "Direct extraction of chitosan from snail shells by natural deep eutectic solvent," *10.5267/j.ccl*, vol. 12, no. 2, pp. 275–280, 2023, doi: 10.5267/j.ccl.2023.1.001.

[24] A. A. Moosa, A. M. Ridha, and N. A. Kadhim, "Use of Biocomposite Adsorbents for the Removal of Methylene Blue Dye from Aqueous Solution," *American Journal of Materials Science*, vol. 6, no. 5, pp. 135–146, 2016.

[25] N. A. Nikonenko, D. K. Buslov, N. I. Sushko, and R. G. Zhbankov, "Spectroscopic manifestation of stretching vibrations of glycosidic linkage in polysaccharides," *Journal of Molecular Structure*, vol. 752, no. 1–3, pp. 20–24, Oct. 2005, doi: 10.1016/j.molstruc.2005.05.015.

ISSN: 2956-4808
DOI: 10.58332/scirad2026v5i3a02

[26] A. El-Araby, L. Ghadraoui, and F. Errachidi, "Physicochemical Properties and Functional Characteristics of Ecologically Extracted Shrimp Chitosans with Different Organic Acids during Demineralization Step," *Molecules*, vol. 27, p. 8285, Nov. 2022, doi: 10.3390/molecules27238285.

[27] F. Boukhlifi, "Quantitative Analysis by IR: Determination of Chitin/Chitosan DD," 2020. doi: 10.5772/intechopen.89708.

[28] C. Molina-Ramírez, P. Mazo, R. Zuluaga, P. Gañán, and J. Álvarez-Caballero, "Characterization of Chitosan Extracted from Fish Scales of the Colombian Endemic Species Prochilodus magdalenae as a Novel Source for Antibacterial Starch-Based Films," *Polymers*, vol. 13, no. 13, p. 2079, Jun. 2021, doi: 10.3390/polym13132079.

[29] S. Vaezifar, M. Molaei, F. Dibaei, and P. Heydari, "Antibacterial Behavior of Chitosan Nanoparticles Prepared from Shrimp Shells Against Bacteria Causing Skin Infections: Usability in Skin Tissue Engineering," *BioNanoSci.*, vol. 15, no. 3, p. 511, Sep. 2025, doi: 10.1007/s12668-025-02139-3.

[30] H. A. Ahmed, Y. A. El-Maradny, M. A. Shalaby, H. El-Menshawy, and A. E. Abd EL-Wahab, "Isolation and characterization of Chitosan from shrimp shell waste and the sustainable preparation of salicylic acid-loaded Chitosan nanoparticles for antibiofilm applications," *Sci Rep*, vol. 15, p. 19263, Jun. 2025, doi: 10.1038/s41598-025-03355-3.

[31] B. T. Iber *et al.*, "A study on the effects of interfering with the conventional sequential protocol for chemical isolation and characterization of chitosan from biowaste of giant freshwater prawn *Macrobrachium rosenbergii*," *Heliyon*, vol. 9, no. 3, p. e13970, Mar. 2023, doi: 10.1016/j.heliyon.2023.e13970.

[32] A. V. Gusakov, E. G. Kondratyeva, and A. P. Sinitsyn, "Comparison of Two Methods for Assaying Reducing Sugars in the Determination of Carbohydrase Activities," *International Journal of Analytical Chemistry*, vol. 2011, pp. 1–4, 2011, doi: 10.1155/2011/283658.

[33] A. Mojumdar, A. K. Upadhyay, V. Raina, and L. Ray, "A simple and rapid colorimetric method for the estimation of chitosan produced by microbial degradation of chitin waste," *Journal of Microbiological Methods*, vol. 158, pp. 66–70, Mar. 2019, doi: 10.1016/j.mimet.2019.02.001.

[34] M. Rinaudo, "Chitin and chitosan: Properties and applications," *Progress in Polymer Science*, vol. 31, no. 7, pp. 603–632, Jul. 2006, doi: 10.1016/j.progpolymsci.2006.06.001.

[35] D. B. Rivers, S. J. Gracheck, L. C. Woodford, and G. H. Emert, "Limitations of the DNS assay for reducing sugars from saccharified lignocellulosics," *Biotech & Bioengineering*, vol. 26, no. 7, pp. 800–802, Jul. 1984, doi: 10.1002/bit.260260727.

[36] U. L. Usman, N. B. Singh, B. K. Allam, and S. Banerjee, "Biogenic Synthesis of Zinc Oxide/Chitosan Nanocomposite using *Callistemon citrinus* (Bottle Brush) for Photocatalytic Degradation of Methylene Blue," *Macromolecular Symposia*, vol. 407, no. 1, p. 2100357, Feb. 2023, doi: 10.1002/masy.202100357.

[37] T. K. Varun *et al.*, "Extraction of chitosan and its oligomers from shrimp shell waste, their characterization and antimicrobial effect," *Vet World*, vol. 10, no. 2, pp. 170–175, Feb. 2017, doi: 10.14202/vetworld.2017.170-175.

[38] N. N. Deshavath, G. Mukherjee, V. V. Goud, V. D. Veeranki, and C. V. Sastri, "Pitfalls in the 3, 5-dinitrosalicylic acid (DNS) assay for the reducing sugars: Interference of furfural and 5-hydroxymethylfurfural," *Int J Biol Macromol*, vol. 156, pp. 180–185, Aug. 2020, doi: 10.1016/j.ijbiomac.2020.04.045.

[39] G. L. Miller, "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar," *Anal. Chem.*, vol. 31, no. 3, pp. 426–428, Mar. 1959, doi: 10.1021/ac60147a030.

ISSN: 2956-4808
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