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COMPARATIVE PHYSICOCHEMICAL PROFILING OF LOW- VERSUS HIGH-MOLECULAR-WEIGHT BIOCHITOSAN DERIVED FROM MANGROVE CRAB *(Scylla paramamosain)* SHELL WASTE

H.N. Imtihani[1,✉], N.I. Azkiya[2], and Y. Nailufa[1]
[1]Department of Technology Pharmacy, University of Hang Tuah, Surabaya-60111, Indonesia
[2]Department of Chemical Engineering, Politeknik Negeri Malang, Malang-65141, Indonesia
✉Corresponding author: hilya.nur@hangtuah.ac.id

ABSTRACT
Biochitosan derived from marine biomass has attracted wide interest in pharmaceutical and biomedical applications due to its biocompatibility and functional amino groups. This study aimed to isolate and characterise chitosan from mangrove crab (*Scylla paramamosain*) shell waste and to evaluate the physicochemical differences between high molecular weight chitosan (HMWC) and low molecular weight chitosan (LMWC) for their potential use in cocrystal formulations. Chitosan was isolated through demineralisation, deproteinization, deacetylation, and decalcification, followed by depolymerisation to obtain LMWC. Physicochemical evaluations included moisture content, ash value, degree of deacetylation (DD), ninhydrin test, SEM morphology, SEM-EDX elemental analysis, FTIR spectroscopy, XRD, and Gel Permeation Chromatography (GPC). The results showed that LMWC exhibited superior quality, with lower moisture (3.09%) and ash content (7.41%), and the highest DD (77.76%) compared to HMWC. SEM images confirmed that LMWC had a finer and more porous morphology, while SEM-EDX revealed the dominance of carbon and oxygen with undetected nitrogen due to instrumental limitations. FTIR confirmed the presence of $–NH_2$ and –OH groups, and XRD patterns indicated that LMWC exhibited crystallinity more consistent with standard chitosan than HMWC. GPC further demonstrated a significant reduction in molecular weight from 73,000 Da (HMWC) to 19,000 Da (LMWC), improving solubility and reactivity. Collectively, these findings indicate that LMWC more closely resembles commercial chitosan in structural and functional properties, making it more suitable for pharmaceutical cocrystal formulation.
Keywords: Biochitosan, Low-High Molecular Weight, *Scylla paramamosain*, Characterisation, Cocrystal.
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INTRODUCTION

Chitosan is a natural polysaccharide obtained through the partial deacetylation of chitin, which is abundantly found in the exoskeletons of crustaceans, insects, and fungal cell walls. Crustacean shell residues are estimated to be capable of producing approximately 2,000 tons of chitosan annually, making it the second most abundant natural polymer after cellulose.[1] Structurally, chitosan is a linear copolymer composed of β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units, where the balance between acetylated and deacetylated units strongly determines its physicochemical and functional properties.[2] Because of its biocompatibility, biodegradability, low toxicity, and the presence of reactive amino groups, chitosan has attracted extensive interest for applications in drug delivery, wound healing, tissue engineering, food preservation, and wastewater treatment.[3–5] The performance of chitosan in these applications is closely related to its intrinsic parameters, primarily the degree of deacetylation (DD), molecular weight (MW), and crystallinity. The DD governs the number of free amino groups available for protonation or chemical modification, thus influencing solubility, charge density, and interaction with other molecules.[6] Meanwhile, the MW affects solution viscosity, film-forming ability, and biological interactions. High molecular weight chitosan (HMWC) is known for its structural rigidity and mechanical stability, but its poor solubility in neutral aqueous systems often limits its utility. Conversely, low molecular weight chitosan (LMWC) exhibits superior solubility, bioavailability, and biological activity due to shorter polymer chains and higher accessibility of amino groups.[7–9] Cocrystals are multicomponent crystalline systems formed through non-covalent interactions, typically hydrogen bonds, between an active pharmaceutical ingredient (API) and a suitable conformer.[10] By carefully selecting excipients such as LMWC, the solubility,

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dissolution rate, stability, and bioavailability of poorly soluble APIs can be enhanced without altering their intrinsic pharmacological activity.[11,12] The presence of abundant amino and hydroxyl groups in chitosan makes it a promising coformer, capable of forming strong hydrogen bonds with complementary functional groups in drug molecules or small organic acids such as succinic acid and nicotinamide.[13] Mangrove crab (*Scylla paramamosain*) shell waste represents a sustainable and underutilised source of chitin. Indonesia, with its extensive mangrove ecosystems and seafood industries, produces large quantities of crustacean waste, much of which remains unexploited. Converting this waste into high-value biomaterials such as chitosan not only adds economic value but also reduces environmental burden.[14] However, despite the abundance of research on shrimp and crab-derived chitosan, limited studies have investigated systematic comparisons between HMWC and LMWC derived from *Scylla paramamosain* and their potential in pharmaceutical cocrystal formulations. This research also aligns with the Sustainable Development Goals (SDGs), particularly SDG 12 (Responsible Consumption and Production) and SDG 14 (Life Below Water). The utilisation of mangrove crab shell waste as a raw material for chitosan production promotes the valorisation of marine biomass residues, reducing environmental burdens associated with crustacean waste disposal. Furthermore, the development of low molecular weight chitosan with improved physicochemical properties supports the advancement of sustainable biomaterials for pharmaceutical applications, contributing to environmentally responsible innovation in drug formulation. This study aims to prepare high- and low-molecular-weight chitosan (HMWC and LMWC) from mangrove crab shell waste and to characterise their structural, physicochemical, and thermal properties. Analytical techniques such as SEM, EDX, FTIR, XRD, GPC, and DSC were used to examine morphology, elemental composition, functional groups, crystallinity, molecular weight, and thermal stability. The results were then evaluated to assess the potential of LMWC for pharmaceutical cocrystal applications using succinic acid and nicotinamide as model coformers.

EXPERIMENTAL

Material and Tools

Green mangrove crab (*Scylla paramamosain*) shell waste obtained from Sang Seafood Restaurant (Surabaya, Indonesia) was used as the raw material for chitosan extraction. Hydrochloric acid (HCl), sodium hydroxide (NaOH)-Merck®, ethanol (analytical grade), and distilled water. Sample preparation utilised standard laboratory equipment, including an oven, grinder, 100-mesh sieve, magnetic stirrer with heating, sonicator, centrifuge, and freeze dryer. Analytical characterisation was conducted using SEM and SEM–EDX (FEI Inspect-S50, USA), FTIR spectrometer (Thermo Scientific Nicolet iS10, USA), XRD (PANalytical X'Pert MPD, Netherlands), GPC (Tosoh Bioscience, Japan), and DSC (Mettler Toledo, Switzerland).

General Procedure

Procedure of High Molecular Weight Chitosan (HMWC) Isolation

HMWC is isolated from mangrove crab shells through six steps: pre-treatment, pre-demineralisation, deproteinization, demineralisation, deacetylation, and decalcification. Cleaned crab shells were boiled for 15 minutes, dried at 110 °C, ground, and sieved through a 100-mesh screen. The resulting powder was pre-demineralised using 5% HCl (1:15 w/v) for 1 hour, washed to neutral pH, and dried at ~80 °C. Deproteinization was carried out with 1 M NaOH (1:10 w/v) at 80 °C for 1 hour, followed by washing and drying. Demineralisation was performed with 1 M HCl (1:10 w/v) at 30 °C for 3 hours, then washed and dried. The obtained chitin was deacetylated using 50% NaOH (1:15 w/v) at 75 °C for 3.5 hours under sonication, washed to neutral pH, and dried. Finally, decalcification was conducted by soaking the chitosan in 3% HCl (1:15 w/v) at 50 °C for 1.5 hours, followed by repeated washing with warm water and drying at 55 °C.[15]

Procedure of Low-Molecular-Weight Chitosan (LMWC) Isolation

To obtain LMWC, the HMWC is depolymerised following the procedure[16]: 10 g of HMWC is dissolved in 830 mL of 0.1M HCl at room temperature for 30 minutes. Then, 170 mL concentrated HCl(12M) is added to make a 1% chitosan solution in 2M HCl. The solution is stirred magnetically(750rpm) and heated for a specific time.

After reaction, ethanol is added to precipitate oligochitosan. The precipitate is washed extensively with ethanol, centrifuged to remove residual acid, and freeze-dried, yielding LMWC powder with enhanced reactivity and bonding ability due to shorter chains.

Detection Method

Procedure of Chitosan Basic Physicochemical Evaluation

Chitosan from mangrove crab shells was evaluated at three stages: after deacetylation, after decalcification (high-molecular-weight chitosan, HMWC), and after depolymerisation (low-molecular-weight chitosan, LMWC). Parameters analysed included moisture content, ash content, degree of deacetylation (DD), ninhydrin reaction, and organoleptic properties (aroma, taste, and colour). DD was determined by FTIR spectroscopy using absorbance ratios at 1655 cm^{-1}(amide) and 3450 cm^{-1}(hydroxyl). The ninhydrin test assessed free amino groups based on the intensity of purple colouration. Moisture content was measured with a moisture balance (limit ≤10%), while ash content was obtained by incinerating samples at up to 600°C and weighing the residue.[17–20]

Procedure of Chitosan Advanced Structural and Physicochemical Characterisation

Chitosan samples were characterised using various analytical techniques. Surface morphology was observed by SEM (FEI Inspect-S50, USA) at 20 kV after sonication, drying, and gold coating. Elemental composition was determined by SEM–EDX (EDAX, AMETEK, USA).[21] FTIR spectra were recorded using a Thermo Scientific Nicolet iS10 spectrometer with KBr pellets scanned from 4000–400 cm^{-1}. XRD analysis was conducted with a PANalytical X'Pert MPD diffractometer (40 kV, 20 mA, Cu-Kα radiation) to assess crystallinity. Molecular weight distribution was measured by GPC (Tosoh Bioscience) using a TSKgel column with pullulan standards, eluted at 0.5 mL/min and 30°C in acetate buffer (pH 4.5). Thermal properties were evaluated by DSC (Mettler Toledo) from 30–300°C at a heating rate of 10°C/min.[22]

RESULTS AND DISCUSSION

The results of physicochemical, structural, and thermal characterisation of HMWC and LMWC are presented and discussed together in this section.

Chitosan Basic Physicochemical Evaluation Result

For basic physicochemical evaluation, chitosan was evaluated at three stages of isolation: deacetylation, decalcification (HMWC), and depolymerisation (LMWC), focusing on moisture, ash, degree of deacetylation (DD), ninhydrin reaction, and organoleptic properties. Deacetylated chitosan appeared pinkish-white with 6.15% moisture, but high ash (43.06%) and low DD (68.41%), indicating low quality. HMWC showed slight improvement with 5.77% moisture, 32.00% ash, and DD of 69.86%, but still below standard. LMWC had the best characteristics-cream-white powder with 3.09% moisture, 7.41% ash, and the highest DD (77.76%) with a dark purple ninhydrin result, confirming higher purity and more amino groups. Overall, further processing of LMWC significantly enhanced chitosan quality for pharmaceutical use.[23,24] All results of the basic physicochemical evaluation of chitosan are presented in Table-1.

Table-1: Chitosan Basic Physicochemical Evaluation Result

Parameter	Specification	Deacetylated	HMWC	LMWC
Organoleptic	Flakes to powder	Powder	Powder	Powder, slightly granular
Color	Light brown to white	Pinkish white	Cream white	Cream white
Moisture Content	2–10%	6.15%	5.77%	3.09%
Ash Content	<10%	43.06%	32.00%	7.41%
Ninhydrin Test	Color change to purple	Changed to light purple	Changed to purple	Changed to dark purple
Degree of Deacetylation (DD)	>70%	68.41%	69.86%	77.76%

Based on these results, LMWC exhibited the best quality due to its moisture and ash contents being within specifications, along with the highest DD value, indicating a greater number of free amino groups compared to the other two samples. Therefore, subsequent characterization tests, including SEM, SEM-EDX, FTIR, XRD, GPC, and DSC, were conducted by comparing HMWC and LMWC with standard chitosan.

Chitosan Advanced Structural and Physicochemical Characterisation Result

The morphological evaluation by SEM(Fig.-1) at 500× magnification revealed clear differences among HMWC, standard chitosan, and LMWC. HMWC displayed large, irregular particles with rough surfaces and a tendency to aggregate. In contrast, LMWC showed smaller, fragmented particles with smoother surfaces and more defined porosity. Standard chitosan exhibited intermediate characteristics, with relatively homogeneous medium-sized particles and moderately smooth surfaces. These results indicate that LMWC has a morphology more comparable to standard chitosan than HMWC, reflecting the impact of molecular weight reduction on particle structure and its functional properties, including solubility, molecular interactions, and potential pharmaceutical and biomedical applications.

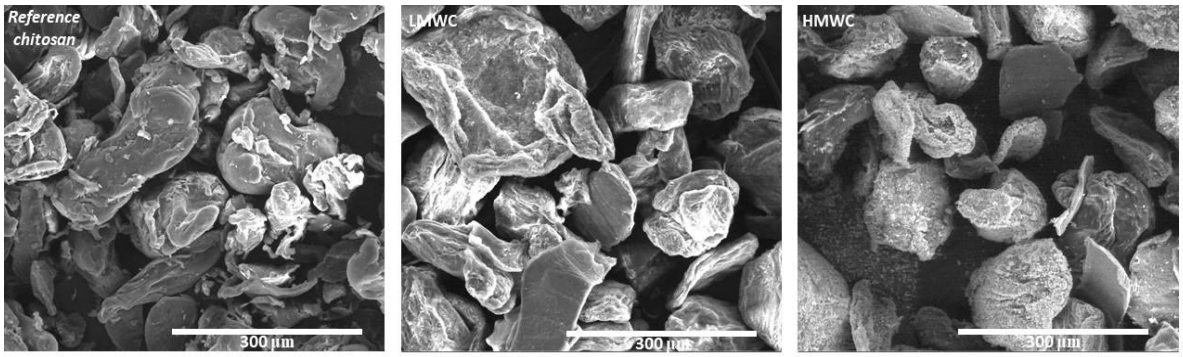

Fig.-1: Morphology of Reference Chitosan, LMWC, and HMWC Observed by SEM At 500× Magnification

In addition to SEM, SEM-EDX analysis was performed to assess the elemental composition of standard chitosan, HMWC, and LMWC (Fig.-2). All samples were dominated by carbon (C) and oxygen (O), consistent with the polysaccharide backbone. Nitrogen (N), representing amino groups (–NH_2), was detected only in standard chitosan (~10%), but was absent in HMWC and LMWC. The absence of N signals does not indicate the lack of amino groups, but rather the limitations of EDX in detecting light elements with low atomic numbers. EDX itself has a detection threshold for nitrogen in polymer films, such that nitrogen below this level may not be quantitatively detected.[25] In addition, factors such as surface distribution of amino groups, the presence of other elements (e.g., Ca, Si, Mg, Al), and reduced relative nitrogen content after chemical modification. Thus, SEM-EDX confirmed the predominance of C and O but was less representative for assessing amino functionality in HMWC and LMWC, particularly when nitrogen levels fall below the instrumental detection limit or decrease due to chemical modification.[26]

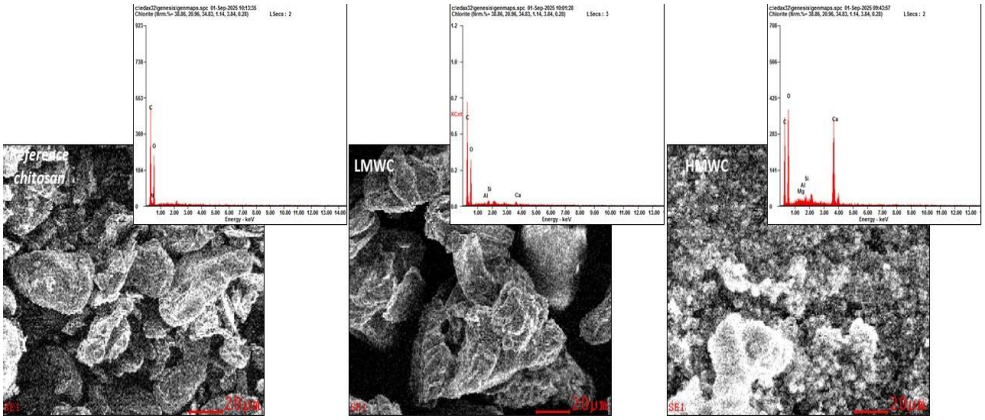

Fig.-2. Morphology and Elemental Composition of Reference Chitosan, LMWC, and HMWC Analysed by SEM-EDX

To complement the analysis, FTIR characterisation was performed to identify the functional groups of chitosan (Fig.-3). All spectra showed a broad band at 3200–3500 cm^{-1} (–OH and –NH_2 stretching), C–H stretching at 2800–2900 cm^{-1}, amide I (C=O) at ~1650 cm^{-1}, amide II (N–H bending) at 1590–1560 cm^{-1}, and C–O–C stretching at 1000–1100 cm^{-1}.[16,27] LMWC exhibited spectra most similar to reference chitosan, particularly at –OH/–NH stretching (~3400 cm^{-1}), C–H (~2900 cm^{-1}), amide I

(~1655 cm^{-1}), and amide II (~1590 cm^{-1}), indicating preservation of key functional groups. In contrast, HMWC showed band broadening and shifts, reflecting a less ordered molecular structure. Combined SEM-EDX and FTIR results provided complementary insights: SEM-EDX confirmed C and O as dominant elements, with nitrogen detected only in reference chitosan, while FTIR verified the presence of amino, hydroxyl, and acetyl groups in both HMWC and LMWC. Thus, the absence of nitrogen in SEM-EDX for HMWC and LMWC is attributed to EDX limitations rather than the loss of amino groups, with FTIR serving as a crucial confirmatory technique.

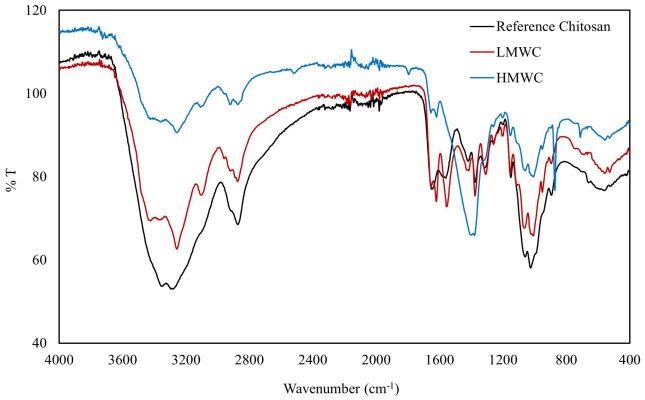

Fig.-3: FTIR Spectra of Reference Chitosan, LMWC, and HMWC

XRD results further confirmed that LMWC exhibited a crystallinity pattern most similar to standard chitosan, which showed two characteristic peaks at 2θ ≈ 10° and 20°. LMWC displayed sharper peaks at the same positions, indicating higher crystallinity but still consistent with the standard. In contrast, HMWC showed a broad, weak amorphous pattern, reflecting lower structural order. Overall, both FTIR and XRD analyses demonstrated that LMWC more closely resembled standard chitosan, making it more representative of the reference material. The results are shown in Fig.-4.

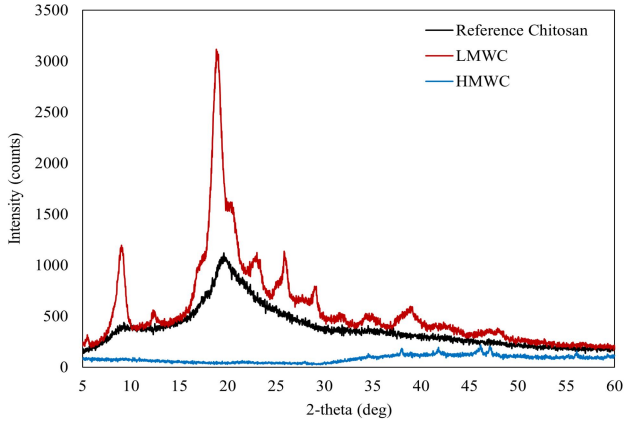

Fig.-4. XRD Spectra of Reference Chitosan, LMWC, and HMWC

FTIR and XRD results were consistent with SEM surface morphology analysis. Both FTIR spectra and XRD diffraction patterns showed that LMWC had characteristics most similar to standard chitosan, in terms of functional groups and crystallinity. This aligned with SEM observations, where LMWC displayed a smoother and more homogeneous surface compared to the rough and irregular morphology of HMWC. These differences can be explained by the greater ability of LMWC to form intramolecular hydrogen bonds, resulting in a more stable molecular structure closer to standard chitosan.[27] In contrast, HMWC, with its higher molecular weight, showed amorphous XRD patterns, broader FTIR bands, and heterogeneous SEM morphology, all indicating lower structural order. Together, these techniques confirmed that LMWC more closely represents standard chitosan in chemical, crystalline, and morphological properties. GPC analysis further supported these findings. Literature reports chitosan molecular weights ranging from 10,000 to 1,000,000 Da. Results from this study showed that HMWC had a molecular weight of ~73,000 Da, while LMWC was significantly lower at ~19,000 Da, confirming successful depolymerisation into shorter polymer chains. The lower molecular weight of LMWC enhanced solubility, diffusivity, and reactivity due to the higher

proportion of free amino groups at the chain ends.[28–30] The lower molecular weight of LMWC enhanced solubility, diffusivity, and reactivity due to the higher proportion of free amino groups at the chain ends. Overall, integrated characterisation demonstrated that LMWC is closer to standard chitosan in purity, optimal DD, homogeneous morphology, semi-amorphous structure, and favourable reactivity. These properties make LMWC more suitable for pharmaceutical cocrystal formulation, as it can interact more effectively with coformers to improve the stability and bioavailability of active compounds. LMWC has an impact on greater benefits in the industrial, biomedical, and environmental fields. In addition, LMWCH has better biodegradability and bioactivity compared to HMWC.[31,32]

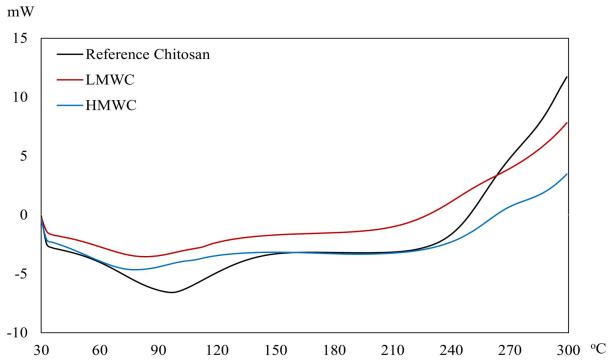

Fig.-5: DSC Thermogram of Reference Chitosan, LMWC, and HMWC

The DSC results are presented in Fig.-5. In standard chitosan, the thermogram shows an endothermic peak at ~96.44°C with an endset at 137.94°C, corresponding to the release of bound water in the polymer matrix. This indicates that standard chitosan is relatively stable in retaining hydrogen-bonded water, with a melting point consistent with reported values around 105°C.[33] LMWC thermogram showed a lower endothermic peak at 83.22°C with an endset at 124.59°C, confirming that it releases bound water more easily due to its higher porosity and lower molecular weight. Whereas, HMWC exhibited the lowest peak at 76.52°C with an endset at 121.70°C, suggesting that although it has a denser structure, water is mainly distributed on the surface and evaporates more easily. Thus, the thermal stability of LMWC lies between standard chitosan and HMWC. Overall, DSC analysis confirms that the thermal properties of chitosan depend on molecular weight and degree of deacetylation. LMWC shows moderate thermal stability but offers better solubility and chain mobility, supporting its use in pharmaceutical applications. The results of this study demonstrate a clear contribution to Sustainable Development Goals (SDGs), particularly SDG 12 (Responsible Consumption and Production) and SDG 14 (Life Below Water). The successful isolation of chitosan from mangrove crab shell waste confirms the effective valorisation of marine by-products into a value-added biopolymer. The physicochemical characterisation revealed that LMWC exhibited improved quality parameters, including lower moisture and ash content, a higher degree of deacetylation, and enhanced structural uniformity, comparable to HMWC. These findings indicate that marine biomass waste can be efficiently converted into functional, pharmaceutical-grade materials, supporting sustainable resource utilisation and reducing environmental burdens associated with crustacean shell disposal.

CONCLUSION

This study prepared and characterised high- and low-molecular-weight chitosan (HMWC and LMWC) from mangrove crab (*Scylla paramamosain*) shell waste through sequential deacetylation and depolymerisation. Physicochemical, spectroscopic, structural, and thermal analyses showed that LMWC exhibited better properties than HMWC, including lower ash and moisture content, a higher degree of deacetylation, more uniform morphology, reduced molecular weight (19,000 Da), and moderate thermal stability. FTIR confirmed higher amino group content, XRD indicated crystallinity similar to standard chitosan, and GPC verified successful depolymerisation. Although nitrogen was not detected by EDX due to instrumental limits, FTIR confirmed the presence of amino groups. Overall, LMWC demonstrated superior solubility, reactivity, and structural similarity to commercial chitosan, making it more suitable for pharmaceutical applications such as cocrystal formation with succinic acid and nicotinamide. This study supports the advancement of Sustainable Development Goals, particularly SDG-12(Responsible Consumption and Production).

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

H.N. Imtihani https://orcid.org/0000-0003-3670-7609
N.I. Azkiya https://orcid.org/0009-0005-4598-7077
Y. Nailufa https://orcid.org/0000-0003-0240-4626

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