Research Article

High quality, low molecular weight shrimp and crab chitosans obtained by short-time holistic high-power microwave technology

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Abstract

The study sought to investigate the impact of a holistic high-power microwave technology during all stages of the extraction on the quality, time of extraction, and degree of deacetylation (DD) of shrimp chitosan (SC) and crab chitosan (KC). The demineralization and deproteinization stages took 7 and 8 min, at 750 and 875 W, respectively. The deacetylation process was conducted at two powers, 875 W and 1250 W, for 10, 15, and 20 min. It only took 25 min at 875 W to successfully prepare chitosan with a high DD and 30 min to reach the maximum DD. The highest DDs by the potentiometric titration method, FTIR, and ^{1}H NMR of SC were 86.6%, 86.7%, and 83%, compared to 83.8%, 82.7%, and 80% for KC, respectively. Extracted SC had 79% solubility, 14.125 kDa, a 46.57% crystallinity index, 705.40% WBC, and 434.60% FBC, against 74.5%, 16.982 kDa, 74.14%, 689.82%, and 413.20% for KC, respectively. The study proved that 30 min of holistic high-power microwave at 875 W produced low-molecular-weight chitosan with relatively high deacetylation and low content of viscosity, crystallinity, and protein residue. The technique can provide a feasible alternative to the commercial production of low-molecular-weight chitosan in less time and energy.

Article Highlights

- High-power (875 W) microwave irradiation at 15 min-deacetylation produced Chitosan's maximum deacetylation degree (DD).
- Holistic microwave irradiation (MW) can successfully produce high DD chitosan, and low molecular weight in 30 min.
- The chemical structure of MW-extracted chitosan was confirmed by FTIR and ^{1}H NMR and low crystallinity by X-ray.

1 Introduction

Generally, optimizing the preparation conditions of biologically active macromolecules is a useful approach to getting the intended product under the least drastic and most favorable conditions for good yield while taking into consideration all the influencing factors [1, 2]. Since a variety of processing agents affect chitosan's physical and chemical properties, it appears that producing highly deacetylated chitosan in the shortest possible time by a simple and economical method on a commercial scale is a hard task.

Chitosan is a linear amino-polysaccharide derived from the alkaline deacetylation of biopolymer-chitin at high temperatures. During deacetylation, N-acetyl-D-glucosamine units are transformed into D-glucosamine units, including free amino groups that have a positive ionic charge. The free amino groups turn chitosan into a cationic form, with possibly valuable antiviral or antibacterial activities like cationic proteins, being reported as antiviral [3, 4] and antibacterial activities [5, 6]. The positive ionic charge of chitosan allows it to chemically bind to fats, and bile acids, and target the bacterial negative cytoplasmic bacterial membrane. This property is the major factor that makes it a versatile tool for a wide range of applications [7, 8]. Additionally, low molecular weight chitosan has been proven to possess high biological activity, allowing it to be employed in many applications such as pharmaceuticals, medical fields, aquaculture, food technology, and water treatment [9].

Several scientists offered various strategies for the production of chitosan through chitin deacetylation; most of these were based on the use of strong solutions of sodium hydroxide at high temperatures, including alkaline high-temperature treatments at high pressures, ultrasound deacetylation, and enzymatic deacetylation. Chitosan was generally developed using traditional heating methods that required a great deal of time and energy. Many authors are currently dealing with chitin and chitosan extraction time problems [10].

Chitosan extraction takes a lot of time and takes a lot of energy by using conventional methods. Microwave technology is considered to be a great alternative to conventional methods because it allows for faster reactions, shorter reaction times, reduction of energy consumption, and fewer side reactions. Microwave irradiation is gaining traction as a renewable energy source capable of completing chemical transformations in minutes rather than hours or days [10, 11]. Mostly, microwave irradiation was used in the final phase of the chitosan extraction process, converting chitin to chitosan [12]. In addition, the previous studies extracted high molecular chitosan using a low microwave power at the deacetylation step. To get low molecular chitosan the previous methods needed to undergo a subsequent step (depolymerization), extending the time of preparation, and raising the total costs. This study assumes that using a holistic high-power microwave technique to extract chitosan at all stages of the extraction process will result in high-quality chitosan with a high degree of deacetylation (DD) in a short amount of time.

Therefore, this work aimed to obtain chitosan of good quality in a very short time using holistic high-power microwave technology. Developing the process of chitosan extraction via using a holistic high-power microwave technology may lead to new products with probably low molecular weight and upgraded physicochemical properties. Thus, it is possible to obtain highly solubilized chitosan in the least amount of time and at the lowest cost without using the depolymerization stage.

2 Materials and methods

2.1 Raw materials

The shrimp shells (*Metapenaeus monoceros* (Fabricius)) and crab exoskeletons (*Portunus pelagicus*) were collected from seafood restaurants in Zagazig, El-Sharkiya Governorate, Egypt. The shrimp shells were collected during the same week while crab exoskeletons samples were collected during the same day. All samples were frozen until further processing. After removing the residual meat, shells were cleaned with tap water and subsequently distilled water, then left to dry at 65 °C to a constant weight. The dried material was ground and sifted through a 250 μm sieve, then stored in a freezer until use.

2.2 Chemical reagents

All the chemicals and solvents utilized in this investigation were purchased at the highest analytical grade or extra purity. Hydrochloric acid (HCl) and acetic acid (CH₃COOH) were purchased from SD Fine-Chem Limited, India. Sodium Hydroxide pellets (NaOH), Acetone (CH₃COCH₃), Ethyl alcohol (C₂H₅OH 95%), and commercial chitosan (75%) were purchased from Loba Chemie Pvt. Ltd. for High-Grade Laboratory Reagents and Fine Chemicals Mumbai, India.

2.3 Preparation of chitosan by microwave technique

Chitosan is typically produced by acetyl group removal from chitin. The holistic microwave-assisted preparation was carried out in a domestic microwave oven (Smart-Torbito SM-2300MS, China), equipped with a turntable and a power range of 125–1250W. The following sequences were maintained during the extraction of chitosan, as shown in Fig. 1.

2.3.1 Extraction of Chitin

2.3.1.1 Demineralization Dried shrimp and crab shell powder were first treated with 1N HCl in a ratio of 1:10 (solid: solvent) for 7 min at 750 W. This ratio was derived from surveying the different methods mentioned by El Knidri et al. [10]. The resultant fraction was then filtered, rinsed with distilled water until it achieved a neutral pH,

Fig.1 Schematic diagram for synthesis of chitosan by microwave technology

and dried to a constant weight at 65 °C. During the demineralization process, minerals (calcium carbonate and calcium chloride) are removed.

2.3.1.2 Deproteinization To remove protein, demineralized shrimp and crab shells were deproteinized using an alkaline treatment with 1N NaOH at a ratio of 15 ml of solvent for each gram of the sample at 875 W for 8 min. This ratio was derived from surveying the different methods mentioned by El Knidri et al. [10]. The residue was filtrated, washed thoroughly with distilled water until the pH was neutral, and then dried at 65 °C to a constant weight. The resulting material is known as chitin.

2.3.1.3 Decolorization The decolorization stage was carried out under condensation, similar to the Soxhlet process for lipid extraction, until the color was eliminated, and a bleached product was obtained. Decolorization was performed by immersing chitin in acetone at a ratio of 15 ml of solvent for each gram of the sample for 2 h in a water bath at the boiling point 57 °C [13] under condensation, followed by stirring at 150 rpm for 1 h to remove oils and pigments. To further bleach and eliminate impurities, chitin was submerged in ethyl alcohol 95% at a ratio of 15 ml of solvent for each gram of the sample for one hour in a water bath at the boiling point 78.4 °C [13] under condensation, followed by one hour of stirring at 150 rpm.

2.3.2 Deacetylation of chitin to chitosan

The decolorized chitin was deacetylated by treating it with 12.5 N NaOH at a ratio of 15 ml of solvent for each gram of the sample at different microwave powers (875W and 1250W) for different irradiation times (10, 15, and 20 min). The alkali fraction was isolated by filtration and washed thoroughly with distilled water until the pH became neutral. Chitosan was obtained and dried to a consistent weight at 55 °C. The DD of chitosan was determined using the potentiometric titration technique for all shrimp and crab samples. The extracted chitosan from shrimp and crab was denoted as SC and KC, respectively, while the commercial chitosan was denoted as CC.

2.4 Chemical analysis of raw materials and chitin

2.4.1 Moisture content (%)

The moisture content of the shells and chitin was determined by the gravimetric method according to [14]. The water mass loss was calculated by drying the sample to a constant weight in an air oven at 105 °C for 24 h. The water mass was determined with the following equation as the
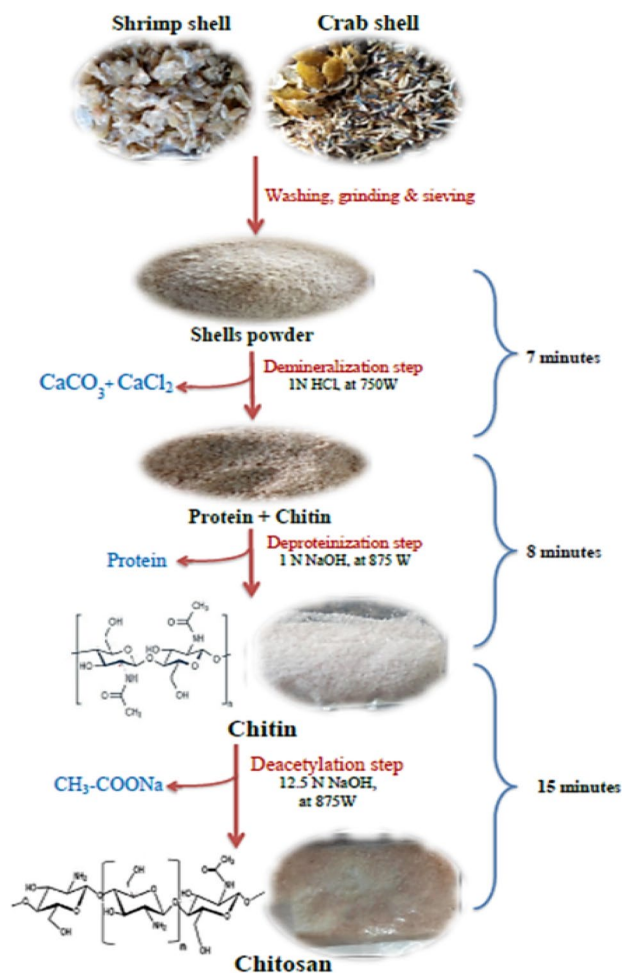
difference between the weight of the wet and oven-dried samples per gram.

$$\text{Moisture content \%} = \left(\frac{(w1 - w2)}{w1} \right) \times 100 \qquad (1)$$

where, W1 and W2 are the weights of wet and oven-dried samples, respectively.

2.4.2 Ash content (%)

The ash content was determined by sample incineration (0.5 g) at 650 °C for 4 h in a muffle furnace according to [14]. The percentage of ash value was calculated from the ratio between the weight of the residue and that of the sample weight using the following equation:

$$\text{Ash \%} = \left(\frac{(w2)}{w1} \right) \times 100 \qquad (2)$$

where, W1 and W2 are the weights (in grams) of the initial sample of chitosan and residue, respectively.

2.4.3 Determination of total protein (%)

The total nitrogen was determined by using the micro Kjeldahl method according to [14]. The protein percentage revealed in this study was calculated by subtracting chitin nitrogen from total nitrogen before and after the deproteinization step and multiplying it by 6.25 [15].

2.5 Determination of chitosan degree of deacetylation (DD) by potentiometric titration method

A weighed amount (0.2 g) of chitosan was dissolved in 0.1 N HCL (20 cm^3) and deionized water (25 cm^3). After 30 min of

NH$^+$, respectilvely), were recorded. The DD% was calculated using the equation below [16].

$$DD\% = 2.03 \times \left(\frac{(V_2 - V_1)}{M + 0.0042(V_2 - V_1)} \right) \qquad (3)$$

where, M denotes the sample weight. 2.03 is the coefficient derived from the chitin monomer unit molecular weight, while 0.0042 is the coefficient derived from the difference between chitin and chitosan unit molecular weights.

2.6 Characterization of the chitosan with the highest DD

The SC and KC with the highest DD (86.6 and 83.8% respectively) and CC were chosen for the following analyses.

2.6.1 Solubility

In a beaker, 0.2 g of sample was solved in 1% acetic acid (20 ml) for 30 min on a magnetic stirrer at 250 rpm. After 30 min, the solution was filtered onto filter paper and the insoluble fraction was cleaned with distilled water and dried to a constant weight. Chitosan solubility was calculated by the following equation [17].

$$\text{Solubility\%} = 100 - \left(\frac{\text{Weight of insoluble fraction} \times 100}{\text{Initial weight of sample}} \right) \qquad (4)$$

2.6.2 Water binding capacity (WBC)

A weighed sample (0.5 g) and 10 ml distilled water were placed in a centrifuge tube (50 ml), agitated for 1 min, then shaken for 30 min, and left at room temperature for 12 h. The samples were centrifuged for 25 min at 232×g. After decanting the supernatant, the tubes were weighed again, and WBC was determined using the following equation [18].

$$\text{WBC \%} = \frac{(\text{Final weigh to tube}) - (\text{Initial weigh to tube} + \text{chitosan})}{\text{Sample weight}} \times 100 \qquad (5)$$

stirring, another 25 cm^3 of deionized water was added, and the stirring was repeated for another 30 min. The solution was titrated against gradual additions (0.1 ml) of 0.1 N NaOH, allowing it to equilibrate, and recording the pH. This process continued until the deflection points, V1 and V2 (the neuralisation points with 0.1 N NaOH of the free H$^+$ and protonated

2.6.3 Fat binding capacity (FBC)

A representative sample (0.5 g) of chitosan was mixed with 10 ml olive oil in a 50 ml centrifuge tube for 1 min, held at room temperature for 12 h, then shaken for 30 min. The samples were centrifuged at 232×g. After discarding the

supernatant, the tube was re-weighted. The following equation was used to calculate FBC [19].

$$FBC\% = \frac{(Final\ weigh\ to\ tube) - (Initial\ weigh\ to\ tube + chitosan)}{Sample\ weight} \times 100 \qquad (6)$$

2.6.4 Intrinsic viscosity determination

Chitosan samples were dissolved in 1% acetic acid at three different concentrations (0.06, 0.08, and 0.1 g/dl). An Ostwald viscometer measured the flow time for each concentration at room temperature. The intrinsic viscosity was defined by capillary viscometry based on the flow time (t_0) of the same volume of solvent or chitosan solution (t) and calculated from the following equations:

Specific viscosity (η_{sp}) was defined by Eq. (7):

$$\eta_{sp} = (t - t_0)/t_0 \qquad (7)$$

The reduced viscosity (η_{red}) was obtained by dividing the η_{sp} to the chitosan concentration (C) in g/dL, Eq. (8):

$$\eta_{red} = \eta_{sp}/C \qquad (8)$$

The inherent viscosity (η_{inh}), was defined by Eq. (9):

$$\eta_{inh} = \ln \eta_{red}/C \qquad (9)$$

The reduced viscosity and inherent viscosity were plotted versus the chitosan concentration to determine the intrinsic viscosity [20].

2.6.5 Determination of the average molecular weight

By using the Mark–Houwink–Sakurada equation (MHS) Eq. (10), the molecular weight of chitosan was estimated from its intrinsic viscosity ([η]).

$$[\eta] = K \cdot M^a \qquad (10)$$

where $k = 1.81 \times 10^{-3}$ ml/g and a = 0.93 depending on the kind of solvent and temperature [21].

2.6.6 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded for chitosans using an FTIR spectrophotometer (4100 Jasco-Japan) with a frequency range of 400–4000 cm^{-1}. The DD% of chitosan could be calculated using the following equation [22].

$$DD\% = 118.883 - \left(40.1647 \times \frac{A1655}{A3450}\right) \qquad (11)$$

where A1655 is the absorbance of the amide band at 1655 cm^{-1}. A3450 is the absorbance of the O–H band at 3450 cm^{-1}. The factor (40.1647) is the ratio of A1655/A3450 for completely N-acetylated chitosan. The number 118.883 was presented as a baseline.

2.6.7 Proton nuclear magnetic resonance spectroscopy (^{1}H NMR)

^{1}H NMR (Joil spectrometer, USA) (500 MHz) was used to examine chitosan samples (10 mg) dissolved in 1 ml CD$_3$COOD/D$_2$0 (2% v/v) solution. The DD% of chitosan samples was determined using the equation below [23].

$$DD\% = 100 - \left(\frac{2xA_{CH3}}{A_{H2-H6}}\right) \times 100 \qquad (12)$$

where $A_{CH3} = CH_3$ area in -NHCOCH$_3$ group, AH2-H6 = areas of H2, H3, H4, H5 and H6.

2.6.8 X-ray powder diffraction (XRD)

The chitosan crystallinity was determined by XRD analysis. A PANalyticalX`Pert PRO X-ray machine (Netherland) was used to perform the XRD measurements on powder samples. Cu Kα radiation has been used as an X-ray source (45 kV, 30 mA). The samples were scanned at a 4 min^{-1} scanning rate and 25 °C from 2θ = 5–40°. Using the following equation [24], the crystalline index (CI%) was determined from the ratio of the crystal phase to the total of the crystal phase and amorphous phase.

$$Crystallinity\ index\% = \left(\frac{I_{110} - I_{am}}{I_{110}}\right) \times 100 \qquad (13)$$

where I_{110} represents the highest intensity of the lattice diffraction pattern at 20°. I_{am} represents the intensity of amorphous diffraction at 16°.

2.6.9 Scanning electron microscopy (SEM)

SEM was used to examine the surface morphology of chitosan samples using a High-Resolution Field Emission SEM (Quanta FEG 250-Czechoslovakia). A sputter coater (Edwards S150A-BOC Edwards, UK) was used to coat chitosan samples with gold under a vacuum before the

Table 1 Means of moisture, ash, and protein content of shrimp and crab shells and chitin (%) compared to previous studies

			Moisture content (%)	Ash content (%)	Protein content (%)	References
Shells	Shrimp		11.39 ± 1	35.02 ± 1	38.49 ± 0.89	
	Crab		5.37 ± 1	56.08 ± 1	36.24 ± 0.89	
	LSD at 0.05		2.26	2.27	2.02	
	Previous studies*			53.8	33–40	[26, 28]
				32–38		[28]
Chitin	Microwave irradiation method	Shrimp	8.85 ± 1	0.99 ± 0.01	2.83 ± 1	
		Crab	9.30 ± 1	1.69 ± 0.2	3.11 ± 1	
		LSD at 0.05	0.33	0.62	1.26	
	Conventional temperature method	Shrimp	6.19 ± 1	0.72 ± 0.01	1.91 ± 0.58	
		Crab	5.84 ± 1	0.64 ± 0.01	1.68 ± 0.58	
		LSD at 0.05	1.45	1.22	1.32	
	Previous studies*				12	[33]
					10	[36]

The displayed values report the mean ± SD of three separate experiments

*Results of some previous studies for comparison with the results of the current study

The values in Table 1 show no statistically significant differences ($p \leq 0.05$). This supports the applicability of the existing approach to extracting low-molecular-weight chitosan, whether on shrimp shells, crab shells, or other species. The values also indicated no statistically significant differences ($p \leq 0.05$) between the new approach in the current study, "holistic high-power microwave technology," and the traditional method used in the preliminary study. This gives an advantage to the new approach; the new technique has a consensus time of 15 min for demineralization and deproteination versus 9 h for the conventional heating method. This will greatly reduce the preparatory costs, and the new technique will be highly cost-effective

examination to improve contrast. At various magnifications and locations, images of the sample surface were captured.

2.7 Statistical analysis

All experiments were carried out in triplicate, except for FTIR, ^{1}H NMR, XRD, and SEM analysis, which were performed on the SC and KC with the highest DD (86.6 and 83.8%, respectively) and CC. Results were obtained from triplicate (n = 3) independent experiments. The statistical significance of differences between means of data for the chemical analysis—solubility, WBC %, FBC %, intrinsic viscosity, and the average molecular weight—was checked by one-way ANOVA with a significance level of $p \leq 0.05$. The statistical significance of differences between means of data for the degree of deacetylation (DD) by potentiometric titration method was checked by a two-way ANOVA with a significance level of $p \leq 0.05$.

3 Results and discussion

3.1 Chemical analysis of raw materials

The proximate chemical composition of fresh shrimp and crab wastes is presented in Table 1. The values in Table 1 report the mean ± SD of three separate experiments. The moisture content of the shrimp and crab shells was 11.39% and 5.37%, respectively. Chemical analysis revealed that the waste of the shrimp shell retained more water than the waste of the crab shell, in agreement with the results reported by Olafadehan et al. [25]. The crab waste was found to have high ash content (56.08%) compared with the shrimp waste, which was 35.02%. Hamdi et al. [26] found 53.8% ash in blue crab shells, and Zhang et al. [27] obtained an ash content in shrimp shells of more than 30%. The crude protein obtained showed that the shrimp and crab shell waste were rich in this component at 38.49% and 36.24%, respectively. Rødde et al. [28] found that the protein content varied between 33 and 40%, and the ash content varied between 32 and 38% of the dry weight of the shrimp shells. The composition difference may be due to the used species.

3.2 Chemical analysis of chitin

Eliminating all organic and mineral content in the raw materials is the purpose of any extraction process. The produced chitin quality depends on the extraction conditions such as acid and alkali concentrations and the reaction time with samples [29, 30]. To judge whether there are any differences in moisture, ash, and protein content between shrimp and crab shells and chitin prepared under the used method. The ingredients were measured in the produced chitin. The results are shown in Table 1. The values in Table 1 report the mean ± SD of three separate experiments. The moisture content of the extracted chitin from shrimp and crab shells was 8.85% and 9.30%, respectively. The high moisture content may be due to the remaining water in the sample, which was utilized during the deproteinization step, as previously stated by da Silva et al. [31]. The content of ash and protein indicates the effectiveness of the demineralization and deproteinization stages. Compared to the precursor, high reductions in the ash and protein content of extracted chitin were observed. After 7 min of microwave irradiation (MW), the ash content dropped to around 0.99% for shrimp chitin and 1.69% for crab chitin. Samar et al. [32] obtained similar results.

Under the selected conditions of demineralization (high MW power (750 W) and 1N HCl), the demineralization of shrimp chitin reached 99.1% in 7 min against 98% in 10 min in a similar study using lower microwave power and HCl 2.5 M [33]. The fact that Chitin is tightly linked to proteins necessitates a more rigorous treatment to eliminate all associated proteins [34]. Deproteination at room temperature should be done at 4% NaOH for 21 h to get a low protein content [35]. In this study, only 8 min of microwave technique was enough to minimize the protein residue in shrimp chitin to 2.83% and crab chitin to 3.11%. These percentages were lower than those obtained by Olafadehan et al. [25], using the traditional heat technique. The deproteinization extent in this study using 875 W and 1 N NaOH for 8 min. reached 97.2 and 96.9%, for shrimp and crab chitins, respectively, excelling the level (88%) obtained by Knidri et al. [33] for shrimp chitin, using MW technique at 650 W and 5% NaOH in 6 min. Marei et al. [36] used the traditional heating approach to achieve maximum deproteinization at 90 °C for 8 h, with a percentage of removal of only 90%. Microwave irradiation produces effective internal heating by direct coupling of microwave energy with the molecules of biomass, which explains the mechanism by which microwave technology reduces reaction time. The high-frequency microwave energizes the starting materials (i.e., amorphous carbon and other carbon sources) to a in a short time. The energized mineral particles alongside the energized water molecules will help extract minerals in a short time. The same concept applies for deproteinization and deacetylation. The heating mechanism of microwave irradiation can be explained by two main processes, namely, dipolar polarization and ionic conduction. When the dipolar molecules or ion molecules of the samples are irradiated by microwave, they are sensitive to the electric field and lead to the alignment of the dipolar or ions with the electric field by rotation. During this process, the dipolar molecules or ion molecules are influenced by oscillation and molecules friction, resulting in the rapid heating of the samples under microwave irradiation with a fast-processing time [37]. Moreover, there is evidence that microwave irradiation can improve the regioselectivity and stereoselectivity aspects of vital importance in synthesizing bioactive compounds [38].

Preliminary studies were done to obtain chitin using the conventional heating method and the results are shown in Table 1. It took us 3 h in the demineralization step and 6 h in the deproteinization step to reach a removal ratio close to the ratio that we obtained for both shrimp and crab chitin, which took only 15 min by microwave technology in this study. Thus, in this study, holistic high-power microwave technology assisted in reducing the time it takes, as well as chemicals, with a high percentage of demineralization and deproteinization to obtain pure chitin. Finally, the new technique has a consensus time of 15 min. for demineralization and deproteination versus 9 h for the conventional heating method. This will greatly reduce the preparatory costs, and the new technique will be highly cost-effective.

During the preliminary study, we estimated the extracted yield percentage (%) based on the dry weight throughout the extraction processes. Crustacean shells consist mainly of minerals, protein, and chitin. Using the holistic high-power microwave technology used in the current study, we found that the yield percentage (%) of shrimp shells was 42% minerals, 28% protein, and 30% chitin, against 73%, 12%, and 15% for crab crustaceans, respectively. In general, crustacean shells consist of 30–40% proteins, 30–50% minerals, and 20–30% chitin, but these percentages vary depending on the source, or even the species, from which chitin is isolated. For example, *Crangon crangon* shrimp waste consists of 10–38% proteins, 31–44% minerals, and 24–46% chitin [10]. This demonstrates that using the holistic high-power microwave technology in this study, enabled the obtention of precise results comparable to previous studies, requiring longer time and higher temperatures. The 15 min-deacetylation process led to chitosan production amounting to 25% and 11% of the starting shell waste and 83.3% and 73.3% of chitin of shrimp and crab, respectively.
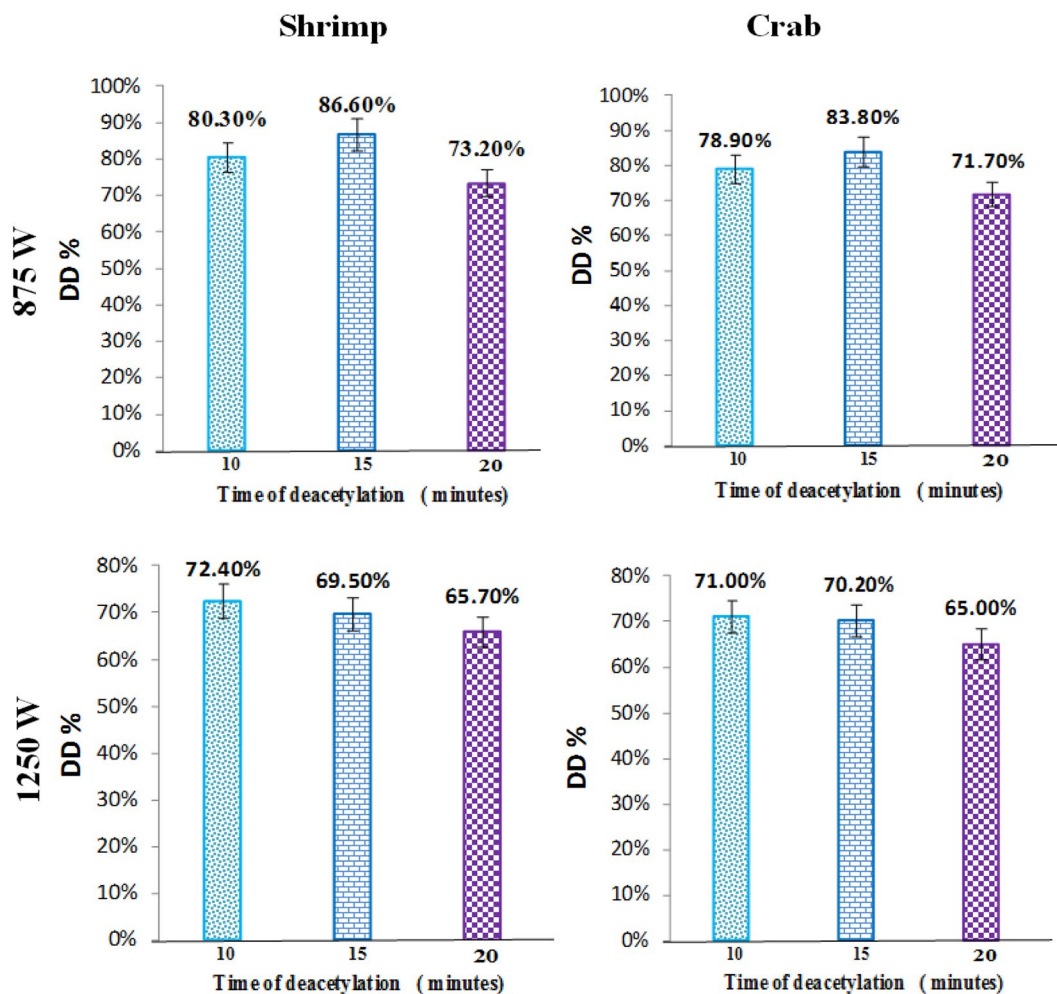
Fig. 2 Means of the degree of deacetylation (DD%) of extracted shrimp and crab chitosan as assayed by the potentiometric titration method. The statistical significance of differences between means of data for (DD) was checked by a two-way ANOVA with a significance level of $p \leq 0.05$. The data is presented as the mean $\pm$ SE. The DD at 875 W for 10, 15, and 20 min were 80.30 $\pm$ 2.3%[b], 86.60 $\pm$ 1.7%[a], and 73.20 $\pm$ 3.2% [c] for shrimp chitosan (SC) against 78.90 $\pm$ 4% [b], 83.80 $\pm$ 0.58% [a], and 71.70 $\pm$ 2.6% [c] for crab chitosan (KC), respectively. The DD at 1250 W for 10, 15, and 20 min were 72.40 $\pm$ 1.5 [c], 69.50 $\pm$ 2.5% [d], and 65.70 $\pm$ 1.5% [e] for SC against 71.00 $\pm$ 3.1% [c], 70.20 $\pm$ 2.1% [c], and 65.00 $\pm$ 2.1%.[d] for KC, respectively. The LSD at 0.05 for Watt, Time, and Compare Means were 3.9, 4.8, and 1.5 for SC against 4.6, 5.7, and 1.8 for KC, respectively. Shrimp chitosan and crab chitosan at 875 W with a 15-min deacetylation time showed a significant difference at the level of significance ($p \leq 0.05$)

3.3 Results of chitosan DD experiment by potentiometric titration method

In this study, high-watt holistic microwave technology for chitosan extraction during demineralization, deproteinization, and deacetylation steps was investigated. This was done to obtain highly deacetylated chitosan in minutes rather than days or even hours, to reduce the effort and energy used by traditional methods. The ash and protein levels in the shells and the resulting chitin were determined to evaluate the effect of microwave power at 750 W and 875 W during demineralization and deproteinization stages for 7 and 8 min, respectively. Section 3. 1. contains the discussion of results. As a result of using microwave-aided extraction, deacetylation time was significantly reduced, as was energy consumption. The impact of 10, 15, and 20-min periods at 875 W and 1250 W microwave irradiation on the chitosan DD during the deacetylation step were investigated. The results are shown in Fig. 2. The values in Fig. 2 report the mean of three separate experiments. The results show that chitosan with a high DD has been extracted at 875 W for 10 min with a DD% of 80.3% and 78.9% for SC and KC, respectively. It confirms that chitosan was successfully prepared in 25 min using comprehensive microwave technology with a high DD of 875 W. Microwave-assisted chitosan extraction was studied only during the deacetylation step of chitin to chitosan [12, 39]. Knidri et al. [33] and El Knidri et al. [40]

studied the influence of microwave irradiation during all stages of chitosan extraction at a maximum power of 650 W. In an experiment, El Knidri et al. [40] obtained chitosan with a DD of 74.79% in 24 min via the microwave irradiation method at 650 W. Hence, the optimal condition of this study (875 W, 25 min) yielded a similar quantity of chitosan but with a higher DD. At 875 W, the maximum DD of chitosan was achieved at 15 min, recording 86.6% and 83.8% for SC and KC, respectively. The maximum DD reached by Knidri et al. [33] was 82.8% at 650 W for 14 min during the deacetylation step. He et al. [41] reported that chitosan with DD between 55 and 70% is considered low; 70–85% means medium; 85–95% means high; and 95–100% means ultrahigh chitosan. Subsequently, results have shown that microwave radiation at 875 W may be employed for chitosan extraction with a high DD as a simple, effective, and speedy heating approach in just 30 min. A decrease in the DD was observed at 875 W for 20 min, which was 73.2% and 71.7% for SC and KC, respectively. The obtained findings may be attributed to the thermal degradation of chitosan or concentration of NaOH due to microwave heating at higher powers or over longer periods. Consistent with Al Sagheer et al. [12], they reported that extending the period after exceeding the optimum time causes chitosan degradation and loss into the reaction solvent. Thus, heating polar solvents forces their constituting particles to rotate with the field and lose energy during collisions. This may be subsequently reflected on the degree deacetylation. The DD at 1250 W for 10, 15, and 20 min were 72.4%, 69.5%, and 65.7% for SC against 71.0%, 70.2%, and 65.0% for KC, respectively. It is clear that not only with the increase in time, the DD decreases, but also with the increase in the power of the microwave, the DD decreases. We note that the DD at 875 W for 10 min is higher than at

1250 W, as well as at 15 and 20 min. This could be due to the effect of the rapid increase in temperature achieved via microwave heating. Whatever, chitosan with a higher DD was obtained by microwave heating for 30 min at 875 W, as opposed to conventional heating methods, which required a much longer time of 7–8 h to prepare chitosan. For example, under deacetylation conditions of 50% NaOH at 100 °C for 8 h [36], have been obtaining lower DD (74%) for shrimp chitosan. As a result of this experience, the SC and KC with the highest DD (86.6 and 83.8%, respectively) and CC were chosen for the rest of the analyses.

When we used the conventional heating method to deacetylate chitin, it took us 4 h of heating at 100 °C in addition to 2 h of shaking on the shaker device to reach a deacetylation point of 83.8% for shrimp chitosan and 84.5% for crab chitosan. While using holistic high-power microwave technology, it took us only 15 min to achieve a similar deacetylation rate at 875 W. Even though the current study and Knidri et al. [33] had the same total extraction time, the current study has a higher percentage of demineralization, deproteinization, and deacetylation than Knidri et al. [33] and the conventional heating method in the study conducted by Marei et al. [36]. On an industrial scale, conventional chemical procedures are considered the most common methods for chitosan recovery from crustacean shells. They use strong acids and bases to achieve the desired results. Moreover, classical chemical treatments are hazardous, energy-consuming, and environmentally unfriendly. A holistic high-power microwave at 875 W for 30 min can be a common chemical reaction technique since it provides an approach for extracting highly clean, economical, simple, effective, and convenient speedy DD chitosan.

Table 2 Means of Solubility, WBC, FBC, Intrinsic viscosity, and average molecular weight, and CI% for CC, SC, and KC compared to previous studies

Chitosan type	Solubility (%)	WBC (%)	FBC (%)	Intrinsic viscosity	Average molecular weight (kDa)	CI%	References
CC*	68.5[b]	673.76[b]	387.82[b]	39.672[a]	44.66[a]	76.34	
SC*	79[a]	705.40[a]	434.60[a]	13.418[c]	14.125[c]	46.57	
KC*	74.5[ab]	689.82[ab]	413.20[ab]	15.987[b]	16.982[b]	74.14	
LSD	1.99	2.00	2.00	1.99	1.99		
Previous studies**	60	660.6 to 745.4	314 to 535		165		[18, 33, 46, 48]
		712.99	326				[36, 47]
		458 to 805	722 to 819				[45, 48]

The data is presented as average values (means). Values with superscripts a,b, and c in the same row have different levels of significance ($p \leq 0.05$). * SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan. **Results of some previous studies for comparison with the results of the current study

3.4 Characterization of the chitosan with the highest DD

3.4.1 Solubility

Chitosan solubility is one of the essential properties that must be understood and regulated [42]. The findings in Table 2 indicate that the obtained chitosan has a higher solubility than commercial chitosan in 1% acetic acid, registering 79% and 74.5% for SC and KC, respectively, against only 68.5% for CC. The obtained solubility ratio may also confirm the degree of deacetylation for shrimp and crab chitosan. The removal of acetyl groups from chitin determines chitosan solubility. Where the presence of the hydrophobic acetyl group may cause insolubility due to more hydrogen bonds between it and the 3,6–OH on the same or adjacent chitin chains by the following bonds —NH···O═C and —OH···O═C. On the other hand, the high removal of the acetyl group leads to the presence of free primary amino groups, and chitosan becomes an electrolyte with a high density of positive charges (R–NH_3^+ groups) in acidic solutions. As a result, chitosan becomes able to dissolve in diluted acidic aqueous solutions. The low molecular weight of the resultant chitosan might have an impact on its solubility. Because an increase in molecular weight results in an increase in molar mass for a given amount of solvent, it is predicted that a polymer will have a smaller solubility range and a lower solubility concentration with the increasing molecular weight. But the lower the molecular weight, the greater the attraction between molecules, and thus the higher the solubility. According to Divya et al. [43], the solubility of chitosan in acetic acid is a sign of its purity. Kumari et al. [18] found that the solubility of chitosan from crab was 60%, with a DD% of 70%, when using 40% KOH at 90 °C for six hours. This confirms that the microwave method used in this study led to better solubility in less time and less energy when compared to conventional heating methods.

3.4.2 WBC analysis

WBC is one of the functional properties of chitosan and expresses the tendency of water to bind to hydrophilic substances [44, 45]. As indicated in Table 2, the WBC of CC, SC, and KC was 673.76%, 705.40%, and 689.82%, respectively. When compared to commercial chitosan, the isolated chitosan from shrimp and crab had a greater WBC. The present findings support those of earlier researchers, who discovered that WBC varied amongst crawfish chitosan samples, ranging from 660.6 to 745.4% [46]. According to Kucukgulmez et al. [47], *Metapenaeusstebbingi* chitosan has a WBC of 712.99%. Furthermore, the study conducted by Cho et al. [48]

submitted that commercial chitosan from shrimp and crab shells had a WBC of 458% to 805%. The differences in results may be explained by Huang and Tsai [49], with hydrostatic pressure that degraded chitosan into far fewer molecules, increasingly its surface area, thus allowing hydrogen bonds to form with water molecules. The contact angles of chitosan were reduced as the DD was raised, implying that chitosan's hydrophilicity had risen.

3.4.3 FBC analysis

Chitosan can be used as a functional food and a nutritional component with higher FBC levels [45]. According
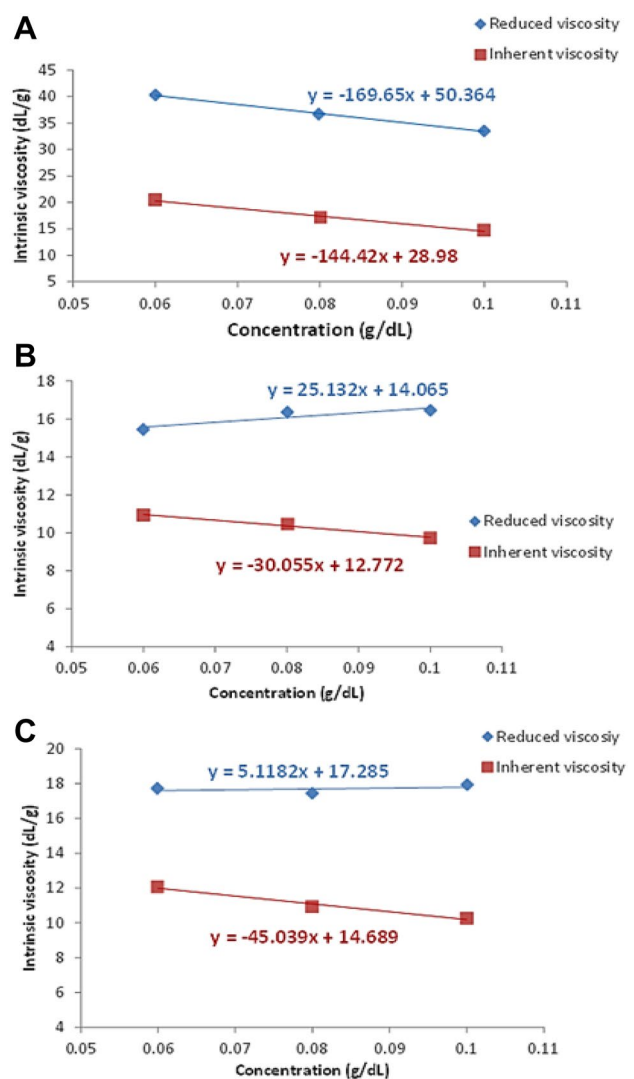

Fig. 3 Evolution of reduced and inherent viscosity as a function of chitosan concentration: **A** CC, **B** SC, and **C** KC (Where, SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan)

to the FBC results, chitosan has a high ability to bind or absorb fat. Chitosan has three types of reactive functional groups: the amino group and primary and secondary hydroxyl groups at the C-2, C-3, and C-6 positions, respectively. It can form stable covalent bonds via several reactions, such as etherification, esterification, and reductive amination reactions. As shown in Table 2, the FBC of CC was 387.82%, whereas the FBC value of SC and KC was 434.60% and 413.20%, respectively. The FBC has a similar pattern to the WBC. It is due to chitosan with a low molecular weight is more effective than chitosan with a high molecular weight at binding fat. This conclusion is consistent with the findings of Huang and Tsai [49]. Chitosan has an FBC ranging from 314 to 535% [48]. Marei et al. [36] reported that the FBC of shrimp chitosan is around 326%, but Rasweefali et al. [45] discovered that the FBC of chitosan samples ranges from 722.00 to 819.17%. The difference in results between this study and earlier studies might be attributed to the type of oil used in the trial. A higher WBC value than FBC can be ascribed to the fact that water has a higher polarity than oil, causing greater water attachment to the chitosan chain's amine and hydroxyl groups [49]. The low molecular weight chitosan produced in this way can be used in many excellent applications, based on its enhanced solubility and functional characteristics when compared to integral chitosan, according to Gonçalves et al. [50].

3.4.4 The intrinsic viscosity and average molecular weight analysis

The data in (Fig. 3) represents the evolution of a linear fit of the reduced and inherent viscosity as a function of chitosan concentration, which was used to calculate the intrinsic viscosity, as presented in Table 2. The intrinsic viscosity decreased as the DD of chitosan increased. The intrinsic viscosity of SC and KC was 13.418 and 15.987, respectively, compared to 39.672 for the CC, corresponding to DD values of 86.6%, 83.8%%, and 74.6%, respectively. This demonstrates how chitosan deacetylation affects the viscosity and flow parameters of the solutions [51].

We obtained the viscosimetric average molecular weight by substituting the intrinsic viscosity value in Eq. (10), which is approximately 14.125 kDa for SC with 86.6% DD and 16.982 kDa for KC with 83.8% DD while increasing to 44.66 kDa for CC with 74.6% DD, as shown in Table 2. These results are similar to those found by El Knidri et al. [40]. The variance in molecular weight is generally influenced by the DD and the different chitosan sources. Furthermore, numerous parameters in the extraction of chitosan, such as concentration of used alkali, reaction
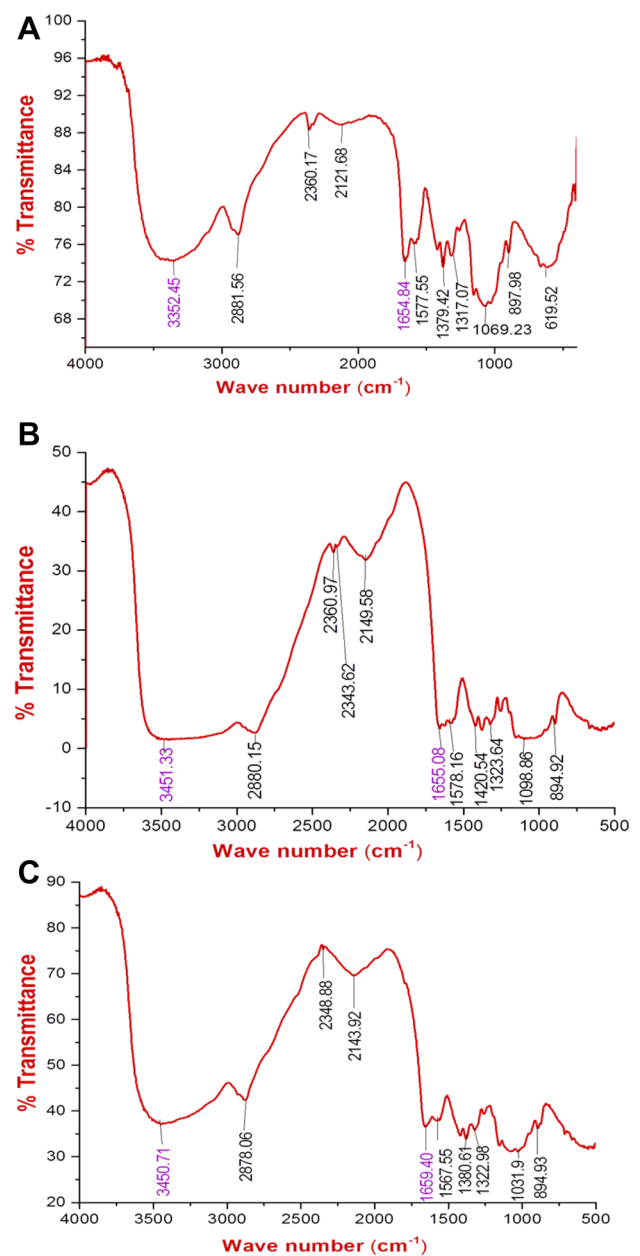

Fig. 4 FTIR spectra of chitosan and their corresponding wavenumber: **A** CC, **B** SC, and **C** KC (Where, SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan)

duration, and prior chitin treatment, may influence the molecular weight of chitosan [40] and particle size [47]. The molecular weight of chitosan extracted with high-power microwave technology (875 W) was at a low rate of 14.125 kDa, which is much lower than that obtained by Knidri et al. [33] with low microwave power (650 W), which was on the order of 165 kDa. This may be due to the effect of the high power of microwave radiation on the chitosan chain. Thus, the holistic high-power microwave technology used here caused the production of low

Table 3 FTIR bands and their corresponding wave numbers of the commercial and extracted chitosan

Functional group	Wavenumber (cm^{-1}) frequency			
	Range of standard wavenumber (cm^{-1})*	CC**	SC**	KC**
Primary amines NH$_2$ and pyranose OH	3450–3455	3352.45	3451.33	3450.71
(C–H) in pyranose ring	2850–2950	2881.56	2880.15	2878.06
Amide I (C=O)	1620–1660	1654.84	1655.08	1659.40
Amide II (NH$_2$)	1590–1610	1577.55	1578.16	1567.55
(CH$_2$) in CH$_2$OH group	1420–1430	1420.96	1420.54	1421.35
(CH$_3$) in NHCOCH$_3$ group	1375–1382	1379.42	1379.85	1380.61
(C–O–C) glycosidic linkage	1155–1165	1154.09	1159.06	1156.51
C–O–C stretching	1070–1075	1069.23	1098.86	1079.69
Pyranose ring	890–900	897.98	894.92	894.93

*The range of standard wavenumbers (cm^{-1}) is similar to that obtained in the studies conducted earlier by Kaya et al. [53] and Kim [55], ** (SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan)

molecular-weight chitosan. El-Sayed et al. [52] reported that the inhibitory activities of molecular weight chitosan between 10 and 100 kDa were as efficient as the antibiotics Flomox and Kluacid against Bacillus cereus. Also, Chang et al. [53] mentioned that the DPPH antioxidant activity was higher in chitosan with a molecular weight of less than 72 kDa with a reduction potential of up to 90% than in chitosan with a molecular weight of 300 kDa. So, the low molecular weight chitosan produced in this study can be considered a possible alternative for antimicrobial agents or additives in pharmaceutical compositions and medical applications.

3.4.5 FTIR spectrum analysis

To comprehensively define the structure of chitosan, the FTIR chitosan spectrum was registered. The FTIR chitosan spectrums are exhibited in Fig. 4. The extent of the absorption peaks of the chitosan derived from the tested species with FTIR yielded ranges similar to those obtained in the studies conducted earlier by Kaya et al. [54] and Kim [55] as presented in Table 3. CC, SC, and KC had given absorption peaks of 3352.45 cm^{-1}, 3451.33 cm^{-1}, and 3450.71 cm^{-1}, respectively, which correspond to the stretching vibrations of the hydroxyl group –OH. While, the distinctive absorption peak, which corresponds to the –CH stretching regions of CC, SC, and KC was at 2881.56 cm^{-1}, 2880.15 cm^{-1}, and 2878.06 cm^{-1}, respectively. SC, KC, and CC absorption peaks in the FTIR spectrum match those achieved by [33, 56, 57]. Amide I (C=O in –NHCOCH$_3$) and amide II (amine -NH$_2$) were ascribed to absorption bands between 1620 and 1660 cm^{-1} and 1590–1610 cm^{-1}, respectively. Whereas the distinctive bands of saccharide structure seem to be at roughly 1155 cm^{-1} to 1165 cm^{-1} (the C–O–C glycosidic linkage), in the chitosan, which is

consistent with Kim [55]. This confirms the preservation of the structure of the obtained product. Yang et al. [58] obtained similar results. Pyranose ring vibrations are observed at 890 cm^{-1} to 900 cm^{-1} for CC, SC, and KC. These results agree with the results found previously by Apriyanti et al. [59] and Boudouaia et al. [60]. A slight FTIR spectra variation between prepared chitosan and commercial chitosan may be due to the different molecular weights of the prepared and standard chitosan; the prepared is generally lower in molecular weight than the standard. This corresponds to Saeed et al. [61]. Mourya et al. [62] and Suryani et al. [63] also indicated that the chemical structure of low molecular weight chitosan is similar to that of native chitosan except for the difference caused by the depolymerization and the decrease of the acetyle group in low molecular weight chitosan compared to native chitosan. The DD% was estimated using the intensity of the amide I band (at 1655 cm^{-1}) and the OH band (at 3450 cm^{-1}), according to Eq. (11). The outcomes are shown in Table 5. In this study, high-power microwave technology induced a higher deacetylated degree (86.7%) than a previous study by Knidri et al. [33] using a lower-power microwave (650 W), which recorded 82.84%.

3.4.6 ^{1}H NMR analysis

The most effective approach to determining the structure of chitosan is ^{1}H NMR [33]. So, the chemical composition of chitosan samples was confirmed by the ^{1}H NMR spectrum, as shown in Fig. 5. The summary of the chemical shifts of all chitosan samples is given in Table 4. The chemical shift of the C$_1$ proton of the glucosamine unit in CC, SC, and KC appears at 4.88, 4.88, and 4.92 ppm, respectively. The chemical shift of the C$_2$ proton appears at roughly around 3.18–3.24 ppm, while the peak from 3.74 to 4.34 ppm is

Fig. 5 Chemical shifts for protons of chitosan that were determined by ¹H NMR: **A** CC, **B** SC, and **C** KC (Where, SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan)
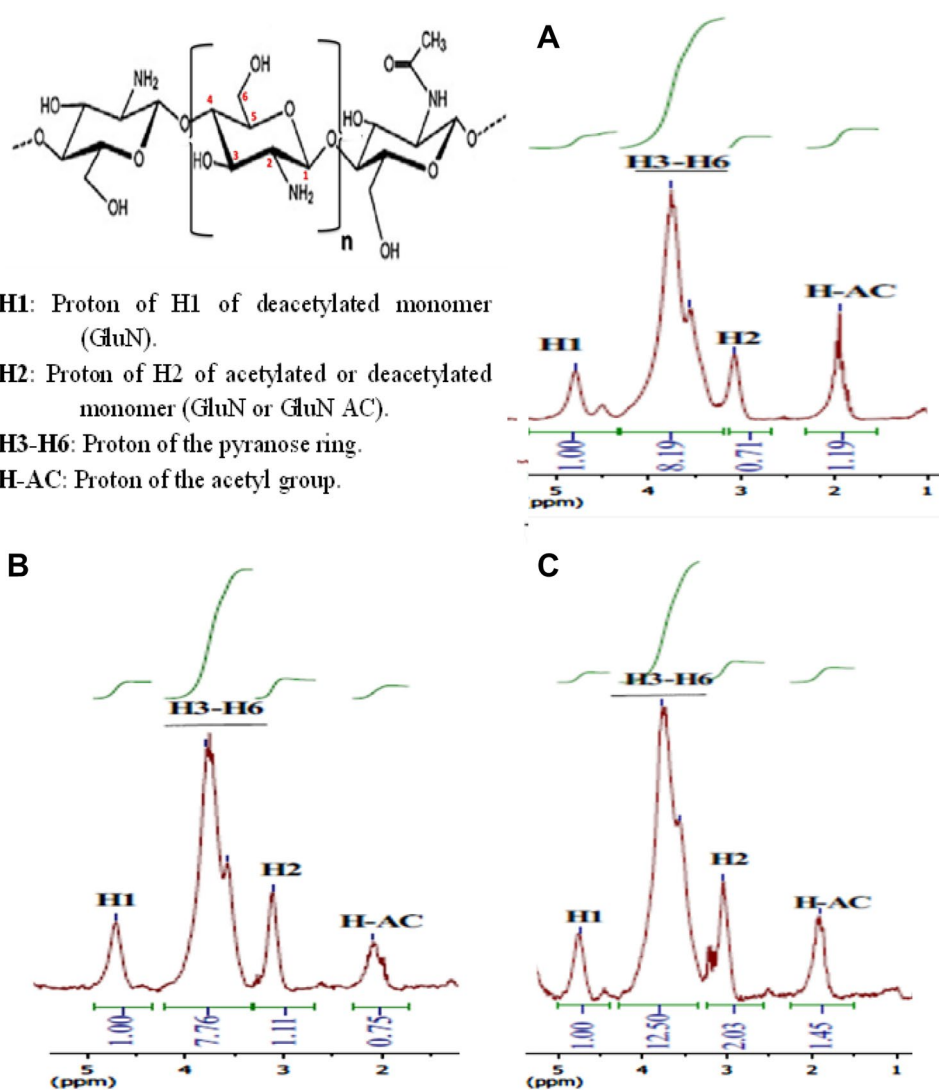

H1: Proton of H1 of deacetylated monomer (GluN).

H2: Proton of H2 of acetylated or deacetylated monomer (GluN or GluN AC).

H3-H6: Proton of the pyranose ring.

H-AC: Proton of the acetyl group.

Table 4 Chemical Shifts for protons in chitosan determined by ¹H NMR

Types of protons		Position (δ, ppm)		
	Ranges of position (δ, ppm)	CC*	SC*	KC*
H_1 (H_1 of GluNH$_2$)	4.85–4.97	4.88	4.88	4.92
H_2 (H_2 of GluNH$_2$)	3.18–3.24	3.22	3.21	3.22
H_3, H_4, H_5, H_6	3.74–4.34	3.84	3.80	3.91
HN-COCH$_3$	1.95–2.09	1.98	2.02	1.97

*SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan

Table 5 DD% for CC, SC, and KC by potentiometric titration method, FTIR, and ¹H NMR

Chitosan type	DD%		
	Potentiometric titration	FTIR	¹H NMR
CC*	74.6%	78.7%	73.2%
SC*	86.6%	86.7%	83%
KC*	83.8%	82.7%	80%

*SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan

Fig. 6 XRD of chitosan: **A** CC, **B** SC, and **C** KC (Where, SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan)
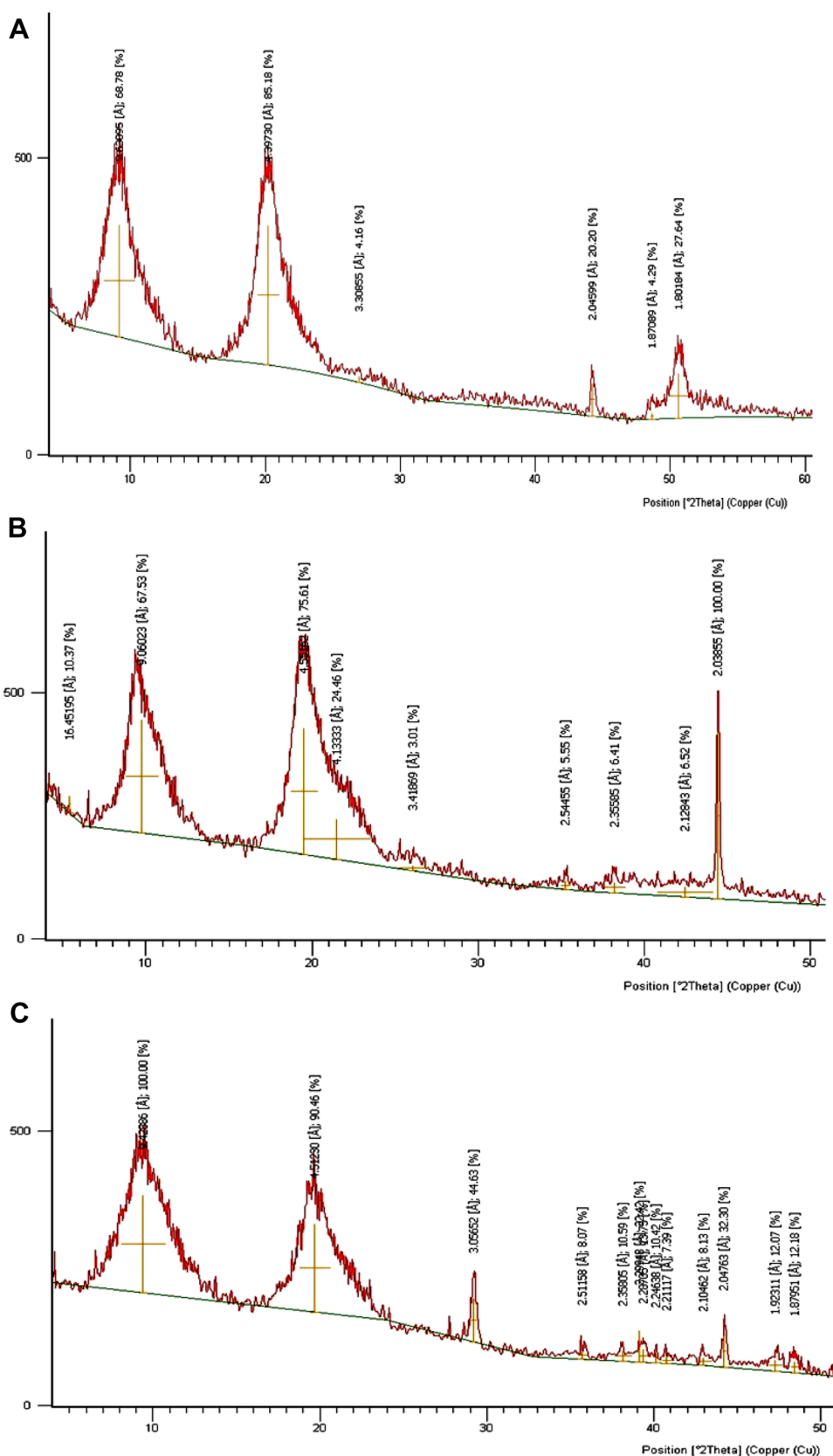
assigned to C_3–C_6 protons of the glucosamine unit and N-acetylglucosamine unit (Protons of the pyranose ring) [55]. The peaks at 1.98, 2.02, and 1.97 ppm for CC, SC, and KC, respectively refer to the methyl protons in the acetamide group. The obtained results are consistent with the results found previously by Kim [55] and Leke-Aladekoba [64]. The area of the methyl group signal (at 1.95–2.09 ppm), and the sum of the H2, H3, H4, H5, and H6 areas (3.18 to 4.34 ppm) were used to compute the DD%, according to Eq. (12). The DD% for SC, KC, and CC samples employed by ^{1}H NMR are presented in Table 5.

3.4.7 XRD analysis

Microwave-irradiated shrimp and crab chitosan diffractograms are depicted in Fig. 6. It demonstrates the existence of two distinctive crystalline peaks at $2\theta = 9.17°$, 9.75°, and 9.37° for CC, SC, and KC, respectively, and at $2\theta = 20.18°$, 19.49°, and 19.66° for CC, SC, and KC, respectively. Gbenebor et al. [65] and Ibitoye et al. [66] found similar findings. Generally, numerous chitosan XRD patterns exhibit two distinct peaks that are often located around $2\theta = 9–11°$ and $2\theta = 19–20°$ [33, 67]. The obtained results concur highly with results given by Jampafuang et al. [68], who found that peaks at roughly $2\theta = 9–11°$ refer to amine I "–NH–CO–CH_3" of chitosan, and peaks at roughly $2\theta = 19–20°$ refer to amine II "–NH_2" of chitosan. According to the obtained findings, chitosan produced using the microwave technique has a lower crystallinity index than commercial chitosan. The X-ray data represents an average of molecules arranged in a periodic crystal lattice. In addition, the technique is not easily applicable to oligosaccharides. Due to the difficulty of obtaining single crystals from oligosaccharides [69], this confirms the preservation of the structure of the obtained chitosan. The CI% for SC and KC was 46.57% and 74.14%, respectively, but

Fig. 7 SEM images of CC (**A** 800x, **B** 24000x), SC (**C** 800x, **D** 24000x), and KC (**E** 800x, **F** 24000x) (Where, SC is the shrimp chitosan, KC is the crab chitosan, and CC the commercial chitosan)
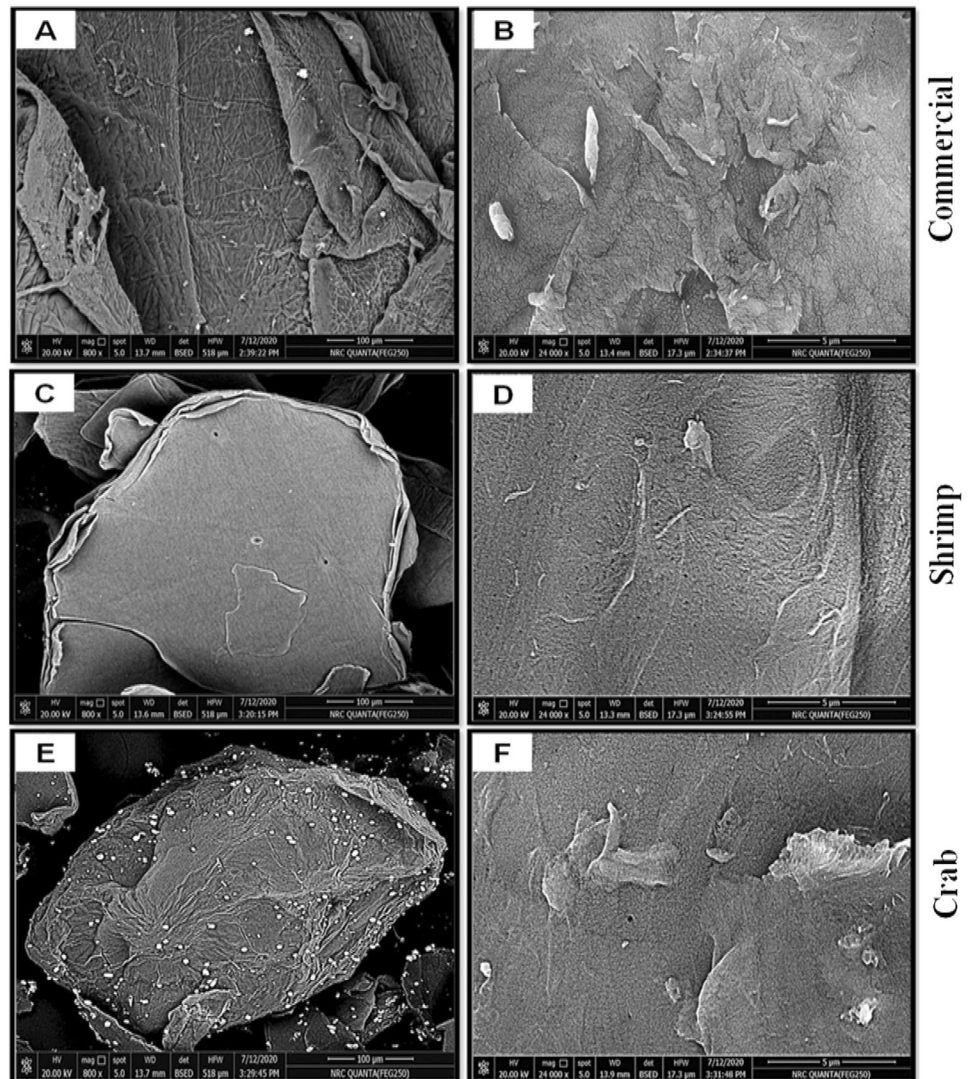
increased to 76.34% in CC. The crystallinity index (CI%) is shown in Table 2. Rasweefali et al. [45] obtained similar results in terms of CI% values. The degree of crystallinity of SC in this study attained a lower value (46.57%) than that obtained by Knidri et al. [33], at (61.20%), probably due to the higher deacetylation degree in the first case. In the same trend, the CI% for the commercial sample CC is greatly higher (76.34%), than the prepared sample, being associated with lower DD (74.6%).

3.4.8 SEM analysis

The surface morphology of the obtained chitosan was examined using SEM. At lower magnification (800X), the commercial and produced chitosan appeared to feature layers of flakes, a lamellar organization, and a dense structure as shown in Fig. 7. The obtained results concur highly with results given by El Knidri et al. [40] and Knidri et al. [33]. While the surface morphologies of commercial and produced chitosan appear to have a smooth surface at higher magnification (24000X). The surface morphology of the obtained SC with a high DD appears smoother than the surface morphology of the obtained KC with a low DD and the surface morphology of the CC appears to have a rough surface. The larger amount of free amino groups (-NH$_2$) in high deacetylated chitosan might explain these results compared to low deacetylated chitosan. The surface roughness in low deacetylated chitosan returns to the presence of monomers containing the acetamide group (acetylated monomer "Glu-NHCOCH$_3$") in the chitosan chain. As the number of free amino groups increases, the surface of chitosan becomes smoother. The obtained results are consistent with the results found previously by Hussain et al. [21] and Yang et al. [58].

4 Conclusions

This study identified the optimal conditions for microwave-assisted extraction of chitosan during the demineralization, deproteinization, and deacetylation steps of shrimp and crab shell waste. Chitosan has been successfully prepared under optimal conditions of high-power microwave technology in 30 min with a higher deacetylated degree (86.7%) at 875 W. The molecular weight and the crystallinity index (CI%) were both relatively lower in the new preparation (SC), recording 14.125 kDa and 46.57% (CI), respectively. The physicochemical features of the produced chitosan measured by X-ray, FTIR, and ^{1}H NMR findings, confirmed the high degree of deacetylation and preserved chemical characteristic structure of chitosan. When compared to a previous study using low-power microwave technology, these results are impressive and produce a new product of chitosan. In the current study, the use of microwave-assisted heating for just 30 min improved chitosan production and properties and reduced energy and time consumption, compared to previous microwave methods and conventional heating methods. As a result, the current study's enhancing chitosan extraction can provide a clean, convenient, appealing, and feasible alternative to commercial production. The preparation process is quick and simple, and the resulting new product is specified with a relatively high deacetylation extent, low molecular weight, low viscosity, low crystallinity, and low protein residue. So, the low molecular weight chitosan produced in this way can be used as a possible alternative antimicrobial agent or additive in pharmaceutical compositions and medical applications. The authors seek to apply this method in an expanded manner to extract chitosan from its different sources in future experimental or application works.

Author contributions MS and AA conceptualized the research concept. AE has done the data curation; Formal analysis and investigation. AA has achieved the Funding acquisition, revised the manuscript, and administered the project. MS has supervised the Investigation and validated the methodology. RS has contributed to the Methodology; Project administration; Resources. AS has contributed to Supervision, validation, and Visualization. AE has written the draft manuscript and MS has written the final manuscript.

Data availability Any data associated with this work are available on request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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