COMBINED MICROWAVE-ASSISTED SUBCRITICAL LIQUID EXTRACTION OF CHITOSAN FROM CRAB SHELL WASTE

Ramyah Segaran[1, a], Rozita Omar[1, b] *, Yuni Kusumastuti[2, c], Razif Harun[1, d], Siti Mazlina Mustafa Kamal[3, e]

[1] Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[a] - ORCID: 0000-0002-3875-8218; [b] - ORCID: 0000-0002-3875-8218;
[d] - ORCID: 0000-0002-9913-568X

[2] Department of Chemical Engineering, Universitas Gadjah Mada, Bulaksumur Yogyakarta 55281, Indonesia
[c] - ORCID: 0000-0002-0142-6754

[3] Department of Food and Process Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[e] - ORCID: 0000-0002-4893-5839

** corresponding author: rozitaom@upm.edu.my*

Abstract

Current chitosan extraction methods require a highly concentrated alkaline solvent that is costly and environmentally unfriendly. This study aimed to assess the potential of using subcritical liquid deproteination to produce amorphous chitin to reduce the alkaline concentration during the deacetylation step to obtain high-quality chitosan. A combination of microwave-assisted demineralisation, subcritical liquid (SL) deproteination, and conventional deacetylation was employed for chitosan production. Distilled water and 2% and 4% sodium hydroxide (NaOH) were used at subcritical liquid treatment temperatures of 100 to 250°C. Meanwhile, deacetylation using 20%-50% NaOH was attempted on the deproteinised chitin. 1.8 M hydrochloric acid at a 1:10 solid-to-liquid solvent ratio produced chitin with acceptable quality during microwave-assisted demineralisation. Demineralised chitin subjected to SL treatment at 150°C and 4% NaOH had the best protein removal (84.6%). Chitosan with a high degree of deacetylation (80.68%) was obtained at a lower alkalinity of 30% NaOH, derived from SL-deproteinised chitin (4% NaOH at 100°C). The crystallinity of chitin after SL treatment was reduced significantly from 72.4% to 59.4%, which allows easier access for the solvent to hydrolyse the acetamide bond. This study confirms that good quality chitosan can be produced by utilising SL treatment of demineralised chitin at a significantly short time and lower deacetylation solvent concentration.

Keywords: *Chitosan, microwave-assisted, subcritical liquid deproteination, deacetylation*

Received: 10.06.2022
Accepted: 19.07.2022

1. Introduction

The conventional chitosan extraction process widely used in the industry involves conventional heating and chemical treatment. The three-step process requires an acidic treatment for the demineralisation step, a dilute alkaline treatment for deproteination, and a highly concentrated alkaline treatment for the deacetylation steps. The last step requires harsh treatment of up to 50% sodium hydroxide (NaOH) or potassium hydroxide (KOH) to obtain high-quality chitosan with a high degree of deacetylation (DD). A large amount of corrosive alkaline waste is produced, resulting in costly effluent treatment [1]. Conventional heating is time-consuming; hence, high energy is needed [2].

The biological extraction of chitin is successful in the fermentation process using enzymes and microorganisms. A lengthy fermentation time (60 h) using *Lactobacillus plantarum* achieved a maximum chitin yield from crab shell waste [3]. Still, high alkalinity was required for chitin deacetylation. The high cost of enzymes and microbe maintenance limits the application of this approach on a large industrial scale [4].

Apriyanti *et al.* [5] and Knidri *et al.* [6, 7] found that the ash and protein content of chitin was reduced severely within minutes by microwave heating compared with hours in conventional heating [6, 8]. Microwave-assisted deacetylation succeeded in producing chitosan with a higher molecular weight and DD compared with those from conventional heating [7]. However, 45%-50% NaOH was still required to produce chitosan with >80% DD during microwave-assisted deacetylation, albeit there was a significant time reduction [6]. A technology that could reduce the crystallinity of the chitin matrix is required if a significantly lower concentration of NaOH is proposed.

Subcritical liquid (SL) treatment is an advanced technology where the solvent is heated to an elevated temperature of 100-374°C without reaching critical points, increasing the kinetics of the extraction process while applying high pressure to maintain the solvent in its liquid state. Under these conditions, the properties of water (viscosity, ion products, and dielectric constant) change significantly, making it possible to act as an acidic or alkaline catalyst [9]. These properties indicate that liquid at subcritical conditions can facilitate many reactions. Nakamura *et al.* [10] compared the extraction of chitin from crab shell waste using subcritical water and conventional methods. Protein removal was successful within 10 min at a temperature between 260 and 320°C, much shorter than the 36 h required for a conventional method. A green solvent (water) can be used successfully for the same process in only a fraction of the time utilising SL extraction.

The governing parameter of SL treatment is the reaction temperature, as a higher temperature might decompose the chitin structure and result in different products. Nakamura *et al.* [10] found that subcritical water treatment at 320-350°C caused part of the chitin to decompose to oligosaccharides, glucosamine, and erythrose. Deacetylation was possible at 250°C with clear production of acetic acid, a deacetylation product [11]. However, beyond 20 min deamination products became prominent, indicating the start of the hydrolysis reaction. At ≥300°C, both deacetylation and hydrolysis reactions were evident even after 5 min. Aida *et al.* [12] studied the dissolution of mechanically milled chitin in high-temperature water and showed that at 220-400°C, other products such as 5-Hydroxymethylfurfural (HMF) and gas are formed from hydrolysis. The unwanted product increases the time increases. Hence, determining a suitable reaction temperature for SL treatment is a significant parameter to preserve the chitin from hydrolysing or decomposing due to high temperature.

This study investigated the effect of demineralisation parameters using the microwave-assisted SL treatment parameters during deproteination for low alkaline concentrations during deacetylation.

2. Material and Methods

2.1 Raw Materials

The raw material used for the study was dried crab (*Portunus pelagicus*) shell waste originating from Sulawesi Island, Indonesia, obtained from the Department of Chemical Engineering, Universitas Gadjah Mada, Indonesia. The crab shell waste was rinsed with boiling water and dried overnight at 80°C in an oven. It was then ground using a dry blender (Panasonic) and sieved (60 mesh) to obtain a size range between 200 and 250 μm.

2.2 Experimental Procedures

The chitosan extraction process follows three consecutive procedures: demineralisation via microwave-assisted treatment, deproteination using SL treatment, and deacetylation of the produced chitin utilising the conventional method due to the limited sample available.

2.2.1 Demineralisation of Crab Shell Waste via Microwave-assisted Extraction (MAE)

Demineralisation removed calcium carbonate and other minerals. The effect of different hydrochloric acid (HCl, 37%, R&M Chemicals) concentrations (1.5 and 1.8 M) and solid-to-liquid solvent ratios (1:5, 1:7.5, and 1:10) were investigated to obtain an ash content of <2% [13]. A modified household microwave oven (Samsung, ME711K, 2.45 GHz, 800W output power) was used [14], setting the temperature instead of the power [7]. The sample and solvent mixture was poured into a 250 ml round-bottomed flask and placed in the microwave cavity heated at 40°C for 10 min.

After each experiment, the mixture was filtered using filter paper (Schleicher & Schuell, GF52) with a diameter of 47 mm. The solid was washed with 100 ml of distilled water to remove any residues of solvent, re-filtered, and dried at 80°C in an oven for 2 h. One gram of each dried sample was analysed for ash content. The balance was kept in a sealed Petri dish in a chiller at 4°C. The yield was calculated based on the weight of solid filtered over the initial solid sample based on duplicate samples.

2.2.2 SL Deproteination of Demineralised Chitin

The demineralised chitin obtained from the best conditions was deproteinated using an SL apparatus [15]. Around 0.5 g of each sample alongside distilled water and 2% or 4% NaOH (A.R. Grade, R&M Chemicals) at a 1:1 ratio were placed in stainless steel 316 tube reactors (6 cm^3). The reactor was heated using a salt bath (1:1 ratio of KNO_3 and $NaNO_3$, A.R. Grade, R&M Chemicals). The SL treatment was performed at 100-250°C for 5 min. After each treatment, a similar solid recovered method as in the demineralisation experiment was performed and a similar yield calculation method was applied. The efficacy of the deproteination treatment was screened using a solubility test described in Section 2.3.2. Samples after SL treatment that were insoluble in both water and 1% acetic acid were considered to be chitin and were subjected to the deacetylation step.

2.2.3 Conventional Deacetylation Experiment

Chitin samples passing the solubility test were treated to obtain chitosan. Conventional deacetylation treatment utilising a heating plate was conducted by heating the mixture of the chitin with NaOH (20%, 30%, 40%, 50% [w/v]) at a solid-to-solvent ratio of 1:10 for 10 min at 100°C. These parameters were adapted from Ibrahim *et al.* [8] but using a hotplate instead of a microwave. After the deacetylation experiment, the samples were filtered and dried similarly to the demineralisation step above. The yield was calculated based on the weight of solid filtered over the initial solid sample. The chitosan samples were kept in the chiller at 4°C until further analysis.

2.3 Analytical Analysis

2.3.1 Ash and Protein Contents in Chitin

The ash content represents minerals left in the demineralised chitin. One gram of the chitin was incinerated in a muffle furnace (Velstar) at 550°C for 3 h. The unburnt inorganic material was cooled, weighed, and used to calculate the ash content (%). The protein content of chitin was measured after the deproteination step, with the aim of <2% protein [16]. The filtrate after SL deproteination treatment was analysed for total protein concentration using the Bradford assay protocol. It was diluted with deionised water before the addition of 2 ml of Bradford reagent (RMStain, R&M Chemicals) and measured in a ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu, UV-1800) at 280 nm (P1) [6]. A standard protein curve was prepared using bovine serum albumin (Merck, 7.5%) at five different concentrations. The initial protein concentration contained in the crab shell (P2) was determined using the Kjeldahl method [17]. The value obtained was 13.1%. The protein removal (% deproteination) was calculated using Equation (1):

$$\% \text{ Deproteination} = P1/P2 \times 100\% \quad (1)$$

2.3.2 Solubility of Chitin and Chitosan

Chitin solubility in both distilled water and 1% acetic acid (99%, HmbG Chemicals) at room temperature was used as a screening method to assess whether chitin had been produced after the SL treatment or other products, as the test is faster and easier. The solubility of chitin in both solutions was observed after thorough mixing for 10 min. The chitin that passed the solubility test (insoluble in both solvents) underwent the deacetylation process described in Section 2.2.3. The chitosan samples obtained after deacetylation were assessed again for their solubility as higher solubility is expected to have better quality chitosan qualitatively. Vanitha Priya *et al.* [18] found that the solubility percentage matched the DD of the produced chitosan. One gram of chitosan from the deacetylation process was dissolved in 100 ml of 1% acetic acid and stirred for 30 min until a homogeneous solution was obtained. The solution was then filtered and dried before the weight of the insoluble filtrate was measured. The percentage of solubility was calculated by using Equation (2):

$$\text{Solubility (\%)} = 100 - \frac{\text{Weight of insoluble sample (g)}}{\text{Weight of initial sample (g)}} \times 100\% \quad (2)$$

2.3.3 Crystallinity of Chitin

The crystallinity of the best demineralised chitin and selected deproteinated chitin were analysed using an X-ray diffractometer (Shimadzu Corporation, XRD6000) operated at 40 kV and 40 mA with Cu kα radiation at $\lambda = 1.5406$ A^{o} between 2θ of 5 and 45° [7]. The crystallinity degree of the polymer was determined using the method described by Ioelovich [19]. The integrated intensity of crystalline and non-crystalline scatterings were used to calculate the degree of crystallinity using a peak analyser software OriginPro 8.1 (OriginLab Corporation).

2.3.4 DD of Chitosan

Fourier-transform infrared (FTIR) spectroscopy (Spectrum 100) analysis was used to determine the DD of chitosan. The baselines and the procedures to calculate the degree of deacetylation from the absorbance ratio follow the method described by Takarina and Fanani [20]. The DD was calculated by applying Equation (3) [21]. The different

components representing each chitosan sample's functional groups were also identified and evaluated using the standard reference chitosan [7].

$$\text{Degree of Deacetylation}(\%)=100-\left(\frac{A_{1655}}{A_{3450}}\times 115\right) \quad (3)$$

3. Results and Discussion

3.1 Production of Chitin and Chitosan

3.1.1 Microwave-Assisted Solvent Demineralisation

Table 1 shows the yield and ash content of chitin obtained using different concentrations of HCl and solid-to-solvent ratios. There was a lower ash content (1.7%) at a higher HCl concentration (1.8 M) compared with the ash content of 3.5% at 1.5 M HCl with the same solid-to-solvent ratio of 1:10. As the HCl concentration increases, more inorganic compounds that react with HCl to form salt chloride dissolve in water. Moreover, the ash content in chitin was reduced significantly as the solid-to-solvent ratio was increased. At the same HCl concentration (1.8 M), the ash content decreased as the solid-to-solvent ratio changed from 1:5 to 1:10. An increase in solvent relative to the mass of the solid means that more solvent is available for the solid to react completely. Treatment using 1.8 M HCl and a solid-to-solvent ratio of 1:10 was chosen to prepare the sample for the deproteination step. El Knidri *et al.* [6] found 95% mineral removal from shrimp shells using 2.5 M HCl as a solvent and heating via microwave for 4 min at a power of 650 W. These results show that microwave-assisted extraction is an energy-efficient method to remove minerals. The approach in the current study could remove >98% of minerals using a lower HCl concentration at the same microwave power setting but at a longer time (10 min).

Table 1. Yield and ash content of demineralised chitin at different hydrochloric acid (HCl) concentrations and solid-to-solvent ratios.

HCl concentration [M]	Solid-to-solvent ratio	Yield [%]	Ash content [%]
1.5	1:5.0	65.0	43.5
	1:7.5	45.6	31.3
	1:10	26.3	3.5
1.8	1:5.0	68.8	23.8
	1:7.5	54.4	6.9
	1:10	52.5	1.7

3.1.2 SL Deproteination

The chitin yield and protein removal after the deproteination step using SL treatment are tabulated in Table 2. Higher SL treatment temperatures resulted in a lower yield at all NaOH concentrations. The trend concurs with the increase in the protein removal percentage. For SL treatment using 4% NaOH, the lowest tested temperature (100°C) resulted in a yield and protein removal percentage similar to distilled water at the highest tested temperature (250°C). Meanwhile, increasing the temperature using 4% NaOH resulted in a considerable yield reduction and the highest protein removal, around 80%, with no significant difference between 150 and 250°C. This shows the importance of solvent use to ensure protein bonds in the chitin structure break. Although the SL treatment failed to meet the aim of the protein removal percentage in this study, another study [22] found that chitosan with a high DD (>80%) can be obtained when protein removal was only at 89.5%.

When distilled water was used as the solvent, 66.1% of protein was removed from the chitin at the highest tested temperature (250°C), showing the possibility of protein removal via a green solvent. The findings reported by Espíndola-Cortés *et al.* [23] support this study: they found maximum removal of protein (96.1%) from shrimp cephalothorax waste using subcritical water treatment for 5 min at 260°C. The current study's significantly lower protein removal could be due to the different chitin sources used. At 2% NaOH, there was better protein removal compared with distilled water at the same treatment temperature and time. Due to time limitations, only two temperature settings were tested for 2% NaOH, so its effect at higher temperatures is unavailable. However, based on the experimented values at 100 and 150°C, it is predicted that protein removal at higher temperatures using 2% NaOH would be better than using distilled water but inferior to that of 4% NaOH. The short treatment time (5 min) may be the reason for the low protein removal.

Table 2. Yield of chitin at different sodium hydroxide (NaOH) concentrations and temperatures.

NaOH [%]	Temperature [°C]	Yield [%]	Protein removal [%]
0	100	69.8 ± 3.75	5.37
	150	57.2 ± 2.85	10.0
	200	54.7 ± 3.50	45.6
	250	50.1 ± 2.60	66.1
2	100	65.2 ± 3.70	17.2
	150	55.2 ± 2.00	35.4
4	100	53.6 ± 3.55	63.2
	150	38.4 ± 3.4	84.7
	200	42.8 ± 2.65	78.9
	250	40.6 ± 2.10	81.2

Note. The yield is presented as the average ± standard deviation.

Apriyanti *et al.* [5] used conventional heating to deproteinate chitin and reported that the protein removal from chitin increased from 83.1% to 88.2% as the reaction time increased from 30 min to 2 h at a temperature of 80°C. With microwave-assisted deproteination, El Knidri *et al.* [6] found that as the time increased from 2 to 6 min, protein removal from chitin increased from 80% to 96% using 5% NaOH. This is due to the increased contact time between chitin and NaOH, which allows the degraded protein to form more sodium-proteinate complexes that could be removed.

3.1.3 Solubility of Deproteinated Chitin

All remaining solids after SL deproteination were insoluble in both 1% acetic acid and distilled water, except for the sample treated using 4% NaOH at 250°C, which was slightly soluble. The highest NaOH concentration and temperature tested might have cleaved the bonds of the chitin chain, causing depolymerisation and thus allowing some solubility [10]. Nakamura *et al.* [10] treated crab shell waste using subcritical water treatment and reported that various amino acids and organic acids from protein hydrolysis were recovered at 260 and 320°C within 10 min. Hence, it is imperative to use lower temperatures and a suitable time for SL treatment to ensure the chitin C=C bonds are not broken but proteins are removed and crystallinity is reduced. All the chitin samples that passed the solubility test proceeded to the next step, deacetylation to produce chitosan.

3.1.4 Conventional Heating Deacetylation of Chitin

The removal of acetyl groups from chitin was done conventionally using NaOH as a solvent (20%, 30%, 40%, and 50%) to produce chitosan. The products were then subjected to a solubility test in 1% acetic acid. A product with greater solubility is also expected to have a high DD [18]. As this test is fast, easy, and cost-effective, it was used for screening. Only selected samples were sent for FTIR analysis to evaluate the DD quantitatively. In general, the solubility of chitosan increased gradually as higher concentrations of NaOH were used at all temperatures except 200 and 250°C using 20% NaOH (Table 3).

Table 3. Solubility of chitosan (%) in 1% acetic acid at different operating conditions of deproteination and deacetylation steps.

NaOH [%] used for SL deproteination	Temp. [°C]	20% NaOH	30% NaOH	40% NaOH	50% NaOH
0	100	43.3 ± 1.4	67.9 ± 5.61	72.5 ± 2.5	77.5 ± 2.5
	150	60.2 ± 1.17	67.9 ± 0.88	**83.9 ± 4.9**	**82.8 ± 3.83**
	200	71.7 ± 4.3	53.8 ± 2.19	59.6 ± 2.43	65.1 ± 0.86
	250	44.1 ± 2.12	40.4 ± 2.43	57.8 ± 2.75	72.4 ± 3.55
2	100	61.4 ± 1.15	76.2 ± 0.9	79.8 ± 1.25	**81.8 ± 1.65**
	150	55.4 ± 3.45	62.1 ± 0.95	64.2 ± 2.25	72.3 ± 1.3
4	100	74.2 ± 2.16	**80.2 ± 2.18**	**83.8 ± 3.86**	**86.2 ± 4.19**
	150	51.2 ± 3.24	48.7 ± 2.69	74.3 ± 1.67	78.2 ± 1.76
	200	67.5 ± 5.49	53.7 ± 2.31	68.3 ± 4.33	75.2 ± 1.24

Note. The values are presented as the average ± standard deviation.

Generally, the solubility was also inversely proportional to the deproteination treatment temperature, except for deproteinated samples using distilled water and 4% NaOH and deacetylated using 20% NaOH. This inconsistency in solubility might be due to the high temperature of the deproteination reaction, which alters the structure of chitin. Disruption of the chemical bonds present in chitin might be the reason for these inconsistent solubility results at 200 and 250°C. It can be inferred that a temperature higher than 150°C is not suitable for chitin deproteination because it can degrade the bonds in the chitin chain. The compounds produced from the decomposition at this high temperature are highly soluble in acetic acid and water. It can also be inferred that the NaOH concentration in the deacetylation step affects the solubility more than the temperature.

The primary objective of this project was to obtain chitosan with higher solubility and DD prepared by using a lower NaOH concentration during deacetylation. Solubility >80% was obtained when deproteination was done at 150°C when using distilled water as the solvent and at a lower temperature of 100°C when using 4% NaOH, with the deacetylation solvent of 40% and 50% NaOH. The positive results indicate that achieving higher solubility at lower alkalinity with SL treatment is possible. Only one deproteination condition (4% NaOH at 100°C) produced high solubility (80.2%) at a lower NaOH concentration (30%) during the deacetylation step. A high NaOH concentration is still required for the SL deproteinated chitin samples to be deacetylated. Better comparison with other studies can only be made by comparing the degree of deacetylation data in the next section.

3.2 Analysis of Chitin and Chitosan

3.2.1 Chitosan DD

The DD of selected samples with high (>80%) solubility and lower solubility (as a comparison) were determined using FTIR spectroscopy. Table 4 compares the solubility of chitosan and the calculated DD from FTIR analysis. For some of the samples, there is not a good correlation between the solubility and DD results. Henceforth, the results are discussed based on the DD data analysed using FTIR analysis only.

Table 4. Degree of deacetylation (DD) and solubility of selected chitosan samples.

NaOH [%] used for SL deproteination	Temperature [°C]	NaOH [%] used for deacetylation	Solubility [%]	DD [%]
0	150	40	83.9	57.6
	150	50	82.8	57.2
2	100	20	61.4	56.9
	100	30	76.2	74.4
	150	20	55.4	73.5
	150	30	62.1	78.9
4	100	30	80.2	80.7
	100	40	83.8	83.0

The DD obtained for both deproteinations using water did not result in a high DD (57.6% and 57.2%), implying NaOH addition is crucial during the deproteination step to extract chitin. Both sodium (Na^+) and hydroxide (OH^-) ions are required to reduce the crystallinity of chitin instead of just OH^- present in water. As the NaOH concentration increases, the Na^+ ions increase, and the space between particles becomes closer, allowing a higher frequency of collisions, forming Na-proteinate more frequently [5]. Furthermore, OH^- released from NaOH during the deproteination step might also accelerate the hydrolysis reaction to break down the amide bond during deacetylation, contributing to a higher DD [24].

When using 2% NaOH, a higher SL deproteination temperature (150°C), and deacetylation using 20% or 30% NaOH, DD increased, although it remained <80%. DD >80% was obtained for SL treatment of 4% NaOH at even low temperature of 100°C and deacetylated with 30% or 40% NaOH. Increasing the NaOH concentration during the deproteination and deacetylation steps gradually reduces the acetyl units in chitin and chitosan, producing amino units at a proportional level. Furthermore, when the NaOH concentrations increase, the acetamide group's resistance decreases along the deproteination and deacetylation process, contributing to a higher DD [25]. It can be concluded that the higher deproteination NaOH concentration resulted in a high DD even at low subcritical treatment temperature for a short time (5 min). It is also important to acknowledge that only 10 min was needed for the SL deacetylation treatments to obtain chitosan with a desirable DD. SL treatment during deproteination of chitin successfully reduced the treatment time during deacetylation (high productivity) with a lower concentration of NaOH (30%-40%).

3.2.2 Chitosan Component Analysis

The FTIR results were also used to interpret the components present and lost in the deacetylated chitosan. The significant peaks that characterise chitosan present in the sample were analysed by comparing them with standard chitosan peaks obtained by El

Knidri *et al.* [7]. Four samples with one high, one medium, and two low DD were chosen to compare the absorption peaks and the differences in the intensity of the peaks, as shown in Figure 1. Samples A and B with lower DD values have a sharper peak of the hydroxyl group (O-H) at 3450 cm^{-1}, compared with samples C and D, which have a higher DD. The peaks observed between 3150 and 3650 cm^{-1} also indicate N-H stretching vibration apart from the O-H stretching vibration. The wide band observed in samples C and D at this wavenumber could relate to the overlapping of the stretching vibration of O-H and N-H bonds, which is consistent with a higher DD [26]. The amide II group (NH) intensity at 1590 cm^{-1} is higher than the amide I group (C=O) at 1655 cm^{-1} for samples C and D, indicating greater removal of acetyl groups corresponding to the high DD values. There is an opposite trend for samples A and B, which explains the lower DD due to low acetyl removal from these samples. It can be summarised that the lower the intensity of the amide I group (C=O), the higher the DD [27].

There is a sharp peak at 1375-1380 cm^{-1} for samples C and D, and a slightly smaller peak for sample A. The band refers to the deformation and vibration of the CH_3 group in the acetamide ($NHCOCH_3$) contained in chitin. A sharp peak infers a higher degree of deformation of the CH_3 groups present in the acetamide. Additionally, there are two sharp peaks around 1750 and about 1220 cm^{-1} for samples A, C, and D, which were deproteinated using 2% and 4% NaOH. These two peaks are absent for sample B – the chitosan derived from deproteination of chitin using water. These peaks are possibly acetyl ester bonds. Gartner *et al.* [28] observed a peak at 1205 cm^{-1} in chitosan due to the C-C vibrations of glucopyranose rings. They also observed a peak at 1775 cm^{-1} in chitosan extracted from crustacean shells (shrimp, crab, and squilla), showing the presence of the acetyl group [29]. Thus, the peaks present in the current study might be due to the residual acetyl groups left in the chitosan after partial deacetylation.

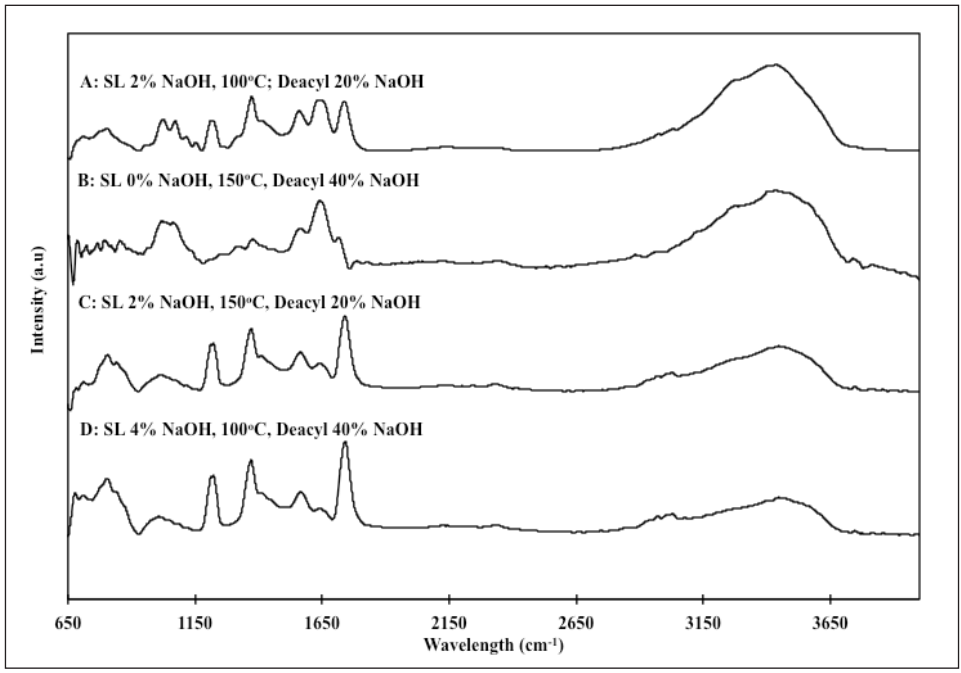

Figure 1. Fourier-transform infrared spectra of selected chitosan samples of low, medium and high degree of deacetylation: 56.9% (A), 57.6% (B), 73.5% (C), and 83.0% (D).

3.2.2 Crystallinity of Chitin

An X-ray diffractogram of the samples is shown in Figure 2; it shows the crystallinity pattern and the crystalline peak of demineralised and selected deproteinated chitin. There are several peaks in the demineralised chitin at 2θ = 20°, 24°, 30°, and 39°. As for deproteinated chitin, there is a significant characteristic peak at 2θ = 20°. The characteristic peak observed in the deproteinated chitin is consistent with the peak observed in the deproteinated crab chitin at 2θ = 19.10° in other studies [7, 30]. As for the peak intensity between the two chitins, the crystalline peak of demineralised chitin is high, with an intensity of 360 at the peak of 2θ = 20°. The deproteinated chitin using 4% NaOH has a lower crystallinity with a relatively low peak with an intensity of 56 at 2θ = 20°. The gradual decrease in crystallinity in both peaks indicates distortion in the chitin's crystal structure, which allows the cleavage of the intra and intermolecular hydrogen bonds [25]. The adsorption ability of chitin increases with the crystallinity reduction as inferred from the graph after SL treatment with NaOH.

The crystallinity degree for demineralised chitin is 72.4%, significantly higher than deproteinated chitin (59.4%). The solvent (4% NaOH) used during the deproteination step gradually eliminated the acetyl groups from the chitin. The gradual decrease in the acetyl groups decreased the structural stability of the chitin, which eventually reduced its crystallinity. There was a slightly higher crystallinity degree (78.4%) in a conventional deproteination of chitin using a 10% NaOH and a significantly longer time (2 h) [7]. A similar outcome when using SL deproteination treatment at only a fraction of the time and lower NaOH concentration shows the superiority of this technique. In this study, NaOH concentration as low as 30% produced chitosan with 80% DD compared with 74.8% DD at 50% NaOH during microwave deacetylation [7]. Those authors used chitin produced from microwave-assisted treatment that had high crystallinity index of 89.7%.

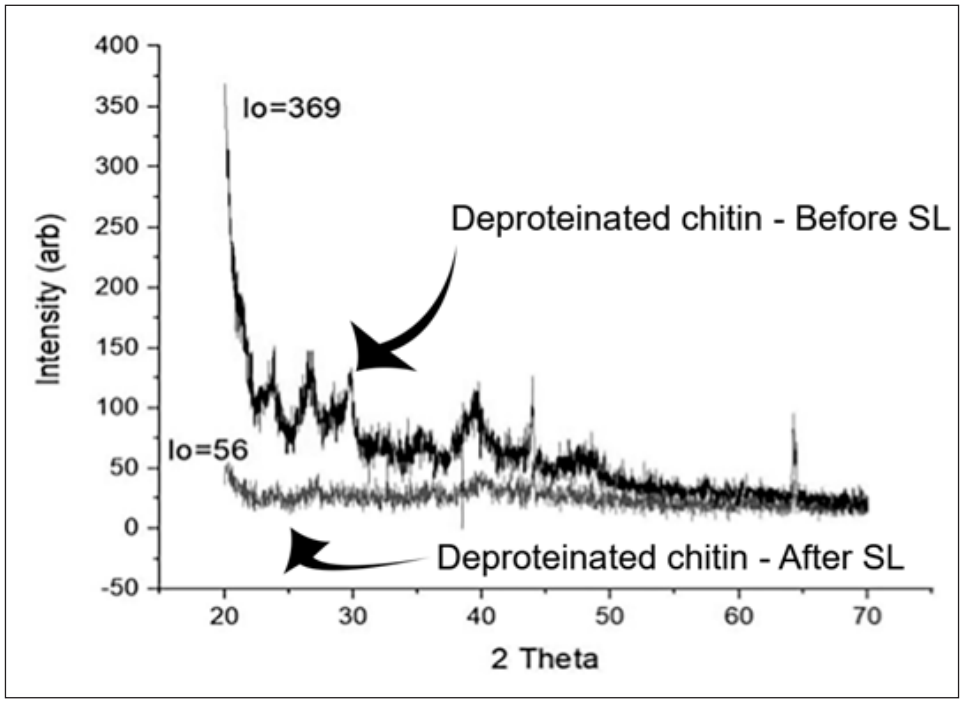

Figure 2. X-ray diffractogram of demineralised and deproteinated chitin.

Hence, SL treatment is better to produce more amorphous chitin for a more accessible and better deacetylation reaction than the conventional and microwave-assisted technique.

4. Conclusions

Microwave-assisted demineralisation of crab shell waste yielded chitin with an ash content <2% using 1.8 M HCl, a 1:10 solid-to-liquid ratio, and a 10 min treatment time. All SL deproteination treatments resulted in insoluble chitin except the highest treatment temperature of 250°C and 4% NaOH, indicating another product was produced. The highest DD (84%) was obtained for chitosan hydrolysed using 40% NaOH from chitin derived using SL deproteination at 4% NaOH and 100°C. A higher crystallinity degree for demineralised chitin at 72.4%, compared with only 59.4% after SL treatment, underscores the efficacy of the treatment. The combined methods of producing chitosan via microwave-assisted demineralisation, SL deproteination, and conventional deacetylation successfully produced good-quality chitosan with a marked reduction in the processing time and lower NaOH required for deacetylation.

5. Acknowledgements

The authors would like to acknowledge SEAMEO for providing financial support under the University Consortium Seed Fund for Collaborative Research Grant. The authors would also like to extend their gratitude to the Universiti Putra Malaysia and Universitas Gadjah Mada for the research facilities and raw materials for completing this study.

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