Effects of extraction parameters on physicochemical and functional characteristics of chitosan from *Penaeus monodon* shell

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ABSTRACT

Objective: To investigate the effect of extraction parameters with particular interest during the microwave deacetylation process on the characteristics of chitosan produced from *Penaeus monodon* (*P. monodon*) sourced within the coastal region of Lagos, Nigeria for applications of controlled release systems for pharmaceutical industries.

Methods: Chitosan was extracted from shrimp (*P. monodon*) shell and evaluated as a controlled release system for curcumin. Effects of relevant processing parameters on physicochemical and functional characteristics of the extracted chitosan were assessed. The crude chitosan was purified and used to prepare controlled release formulations for curcumin via ionic gelation with tripolyphosphate.

Results: Data from the study showed that increasing time and temperature during deproteinization significantly improved the removal of protein bound to the shell matrix. Also, the ratio of the weight of the deproteinized sample to the volume of HCl used for demineralization influenced the process. During microwave-assisted production of chitosan from chitin, increase in the concentration of the deacetylating medium significantly increased solubility, viscosity and degree of deacetylation, whereas increasing temperature and time during deacetylation of chitin degraded the biopolymer to give low molecular weight chitosan. Optimized extraction and purification process yielded absolutely soluble medium to low molecular weight chitosan. The encapsulation efficiency, loading capacity, percentage yield, release efficiencies in simulated gastric and intestinal fluids of curcumin loaded in the formulations of chitosan from *P. monodon* were compared favorably to encapsulation and release characteristics of the encapsulated curcumin in commercially available chitosan used as the reference.

Conclusions: Valorization of shrimp waste into pharmaceutically graded medium molecular weight chitosan was achieved. The chitosan obtained can be used as a controlled release system for phyto-pharmaceuticals.

1. Introduction

The production of shell waste from shrimp processing industries is on the increase[1]. Globally, 6–8 × 10⁶ metric tons (MT) of shells from crustaceans are produced by the shrimp processing industries in the sea-food agricultural subsector with the majority (about 25%) in Southeast Asia[2]. In Nigeria, the annual shrimp production from the marine and freshwaters is estimated at 2.5–3.5 × 10⁵ MT with 66% export majorly due to economic values and the preference of the populace for the estuarine shrimp *Nematopalaemon hastatus*[3,4]. *Penaeus notialis* (pink shrimp) was the dominant species in Nigeria before its stock dwindles towards the end of the 20th century due to aggressive capture and non-culture nature of the Nigeria shrimp industry. Climate change-induced aquafauna redistribution led to the sudden emergence of *Penaeus monodon* (*P. monodon*) (tiger shrimp), which revived and prevented the industrial shrimp farming in Nigeria from oblivion[5].

Shrimp production and processing in Nigeria generate 1.6 × 10⁴ MT of waste two decades ago[6]. Today, biomass estimation of shrimp waste is higher (10⁵ MT) due to the rapidly growing shrimp processing centres and industries in the country. The waste biomass composed of exoskeleton and cephalothoraxes represents 50%–70% of the waste materials[7]. The disposal of shrimp wastes is becoming an increasingly difficult and challenging menace for
the shrimp production and processing industry, and underutilization of shrimp waste which contains valuable pigments, polysaccharides and proteins has contributed to pollution problems[8,9]. Valorization of this by-product used as alternative protein source in feedstuff for fisheries, poultry or livestock production is yet to be fully exploited[10,11]. In order to alleviate potential adverse health and environmental effects, there is a need to develop a new management infrastructure for shrimp waste through improvements in the downstream processing of shrimp waste biomolecules technologies to convert this waste into high demand of valuable added products that are compatible with up-scaling industries.

The biomass of shrimp waste sourced from Nigeria has appreciable amount of chitin[12]. Chitin and its most prized derivative chitosan are valued biomaterials that are extracted from shrimp shells. Chitin is a natural polysaccharide, first identified in 1884. This biopolymer is the most abundant polymer after cellulose in nature[13]. Chitin occurs as ordered crystalline micro-fibrils and serves as structural polysaccharides in the exoskeleton of arthropods, especially where reinforcement and strength are required[14,15]. Structurally, chitin is similar to cellulose, but it has acetamide group at the C-2 position of its glucose moiety[16]. It is a linear polymer of 2-acetamido-2-deoxy-\(\beta\)-D-glucose monomeric unit.

Industrially, chemical methods are employed for extraction of chitin and chitosan from marine waste. A wide array of processes were reported by many researchers with detailed review captured by various authors[9,17-19]. Traditionally, chemical extraction of chitin and subsequent conversion into chitosan from waste biomass of shrimp shell involve three major steps: removal of protein using mild alkaline medium (deproteinization), inorganic materials using dilute acidic medium (demineralization) to give chitin and N-acetyl groups (deacetylation) in highly concentrated alkaline medium to give chitosan. Additional steps could be employed to decolorize or purify the extracted crude chitin or chitosan.

Extraction of process parameters such as time, temperature, concentration of acid or alkaline medium and solvent to sample ratio highly influenced specific characteristics of shrimp chitosan such as binding capacity, solubility, molecular weight and degree of deacetylation. Applications of chitosan depend on the quality dictated by its physicochemical and functional properties. Based on specific pharmaceutical applications, molecular weight and degree of deacetylation are key characteristics that need to be optimized during the production of chitosan especially during deacetylation.

For commercial purposes and to reduce the cost of production on a large scale, the heterogeneous method is employed and chitin is treated with a hot concentrated solution of the preferred deacetylation medium for 30 min to few hours and depending on production parameters, chitosan is produced as an insoluble residue with degree of deacetylation up to 99\%[20]. Unlike conventional solvent boiling or autoclaved methods, microwave irradiation of deacetylation methods is receiving much acceptance because of its fast reaction rates resulting in reduced time and resources to complete the process[14].

In this study, the effect of extraction parameters with particular interest during the microwave deacetylation process on the characteristics of chitosan produced from P monodon sourced within the coastal region of Lagos, Nigeria was investigated for applications of controlled release systems for pharmaceutical industries.

2. Materials and methods

2.1. Shrimp waste

Shrimp wastes comprising the head and carapace from processing of P. monodon (Wild Tiger, Fabricus) captured within the Nigeria waterways and sold commercially by Atlantic Shrimpers Limited were collected from a shrimp processing centre at the Inland Port, Ijora-Apapa, Lagos, Nigeria.

2.2. Chemicals

Curcumin, chitosan, sodium tripolyphosphate (Santa Cruz Biotechnology, Germany), bovine serum albumin, methanol and chitosan (Sigma Chemical Company, USA), sodium alginate, ninhydrin, acetic acid (British Drug Houses Laboratories, UK) and other chemicals of analytical grade were obtained. The reagents were prepared in all glass apparatus using deionized or distilled water as required and stored appropriately.

2.3. Preparation of shrimp shell

Shrimp shell and meat portions were separated and the shells collected were dried at a temperature lower than or equal to 40 °C in an aerated oven for 24 h. The dried shell was macerated in the laboratory. The shrimp shell powder obtained was sieved and kept under refrigeration (\(\leq 4\) °C) until it was required for further analysis.

2.4. Processing of chitosan from shrimp shell

Chitosan was processed from shrimp in a three-step procedure[21]. First step was the deproteinization: powdered shrimp shell (50 g) was digested in 4% NaOH (1:10 w/v) at 90 °C for 2 h. The alkali insoluble fractions were separated by centrifugation at 2500 \(\times\) min for 15 min and washed thrice with deionized water. Second step was to demineralize. The deproteinized shrimp (30 g) powder was digested in 2.5% HCl (1:10 w/v) at 60 °C for 6 h. This was followed by separation of crude chitin by centrifugation at 2500 \(\times\) min for 15 min. The residue was then washed 3 times with deionized water. The third was the deacetylation step. For the conventional boiling process, the crude chitin (10 g) was deacetylated with 40% NaOH (1:30 w/v) at 100 °C for 2 h and the step was repeated twice. Whereas for microwave assisted process, crude chitin was deacetylated once in 40% NaOH for 15 min. The deacetylated chitin or crude chitosan fractions were washed with deionized water, air-
dried at 40 °C and stored in a desiccator until required for further analysis.

2.5. Determination of optimized parameters for extraction of chitosan

The effects of selected parameters for deproteinization, demineralization and deacetylation of powdered shrimp shell samples on the physicochemical and functional properties of the extracted chitosan were studied.

2.5.1. Deproteinization parameters

The effects of concentrations of NaOH (3.0%, 3.5%, 4.0%, 4.5% and 5.0% w/v), ratios of weight of shrimp samples and solvent volumes (1:5, 1:7.5, 1:10; 1:12.5 and 1:15), temperatures (27, 50, 60, 70, 80, 90 °C), incubation speeds (100, 150, 200, 250, and 300 r/min) and time (1, 2, 3, 4, 5, 6, 12, 24 h) of digestion on the residual and released protein were evaluated to establish the optimized conditions for deproteinization of the shrimp shells.

2.5.2. Demineralization parameters

The effects of concentrations of HCl (0.4, 0.6, 0.8 and 1.0 mol/L), ratios of weight of deproteinized samples and solvent volumes (1:5, 1:10, 1:15 and 1:20), temperatures (27, 40, 50, 60 °C), incubation speeds (100, 200, 300 and 400 r/min) and time (1, 2, 3, 6 and 12 h) of digestion on residual ash and released calcium contents were evaluated to establish the optimized conditions for demineralization of the deproteinized shrimp shells.

2.5.3. Deacetylation parameters

The effects of concentrations of NaOH (20%, 30%, 40% and 50%), microwave temperatures (80, 110 and 140 °C), ratios of weights of crude chitin and solvent volumes (1:10, 1:20 and 1:30) and time (5, 10 and 15 min) of digestion on solubility, viscosity, degree of deacetylation and molecular weight were evaluated to establish the optimized conditions for deacetylation of the extracted crude chitin.

2.6. Purification of chitosan

The chitosan was extracted using an optimum set of process parameters and purified as described by Qian and Glanville[22] and employed by Puvvada et al.[21].

2.7. Physicochemical and functional properties of chitosan

The moisture, ash and nitrogen contents were determined according to methods of Association of Official Analytical Chemists[23]. Protein was evaluated by biuret’s reaction[24]. Calcium was estimated using titrimetric methods[25]. The solubility was performed in 1% v/v acetic acid[26]. Water binding and fat binding capacities were assessed using soybean, groundnut and olive oils[27]. Viscosities of chitosan solutions were determined at room temperature[28]. The average molecular weight of chitosan samples were determined from calculated intrinsic viscosity. The average molecular weight was obtained from Mark-Houwink equation:

\[ \eta = KM^a \]

where \( \eta \) is the intrinsic viscosity, \( M \) is average molecular weight of the solution, respectively, and \( K \) and \( a \) are the Mark-Houwink constants specific for a given polymer[29].

Concentration of glucosamine was determined using spectrophotometer[30], and the degree of deacetylation was calculated by the following formula:

\[ \text{DDA} = \frac{C_g}{C_s} \times 100 \]

where DDA is the degree of deacetylation, \( C_g \) is the glucosamine concentration in the sample and \( C_s \) is the concentration of the standard curve of curcumin to extrapolate the concentration.

2.8. Chitosan beads loaded with curcumin

Controlled release system for curcumin was prepared using chitosan extracted from P. monodon shell via ionic gelation with anionic tripolyphosphate (TPP) as described by Anchisi et al.[32] with slight modifications. Chitosan (1.5 g) was dissolved in 100 mL of 1% v/v acetic acid and 2.5% w/v TPP was dissolved in distilled water. Blank chitosan beads were prepared by the drop wise addition of 1 mL of the chitosan solution to 25 mL of TPP solution.

For encapsulation of curcumin, 0.1 mL of 2.5 mg/mL of the curcumin dissolved in methanol was added to 0.9 mL of the chitosan solution and mixed vigorously before addition to TPP. Chitosan colloidal beads were formed spontaneously under mild agitation at room temperature. Twenty minutes later, chitosan colloids were filtered and the filtrate was kept for further analysis. The beads were washed thoroughly with deionized water and dried.

2.8.1. Encapsulation characteristics

Following encapsulation of curcumin in chitosan beads, the filtrate (5 mL) containing the residual (non-encapsulated) curcumin was transferred into test tube. The absorbance was read spectrophotometrically at the characteristic wavelength (425 nm) for curcumin following extraction with methanol and regressed onto a standard curve of curcumin to extrapolate the concentration. Encapsulated curcumin was determined by calculating the difference between the total amount of curcumin and the amount of residual curcumin presenting in the filtrate. Encapsulation efficiency, loading capacity and percentage yield as well as cumulative release were determined as follows[32-34].

Encapsulation efficiency (%) = \( \frac{\text{Encapsulated curcumin}}{\text{Total amount of curcumin}} \times 100 \)

Loading capacity (%) = \( \frac{\text{Encapsulated curcumin}}{\text{Total mass of beads}} \times 100 \)

Yield (%) = \( \frac{\text{Total mass of beads}}{\text{Total mass of content and curcumin}} \times 100 \)
2.8.2. Controlled release studies

In vitro release profiles of curcumin from the chitosan beads were carried out by suspending 10 mg of curcumin-loaded beads in 20 mL of simulated gastric and intestinal fluids and incubated with constant agitation at 50 r/min for 2 and 8 h, respectively[33]. The release efficiency (%) was calculated from the amount of curcumin that released into the fluids at a particular time (t) as a percentage of the total amount of curcumin in the carrier[35].

Release efficiency (%) = \( \frac{\text{Released curcumin at a time (t)}}{\text{Total phytoceuticals}} \times 100 \)

Curcumin that released at different time intervals was determined as described above for determination of residual curcumin. Mean release time which was a measure of the rate of the release process was calculated from the amount of the phytoceutical released to the total cumulative release over a given period of time. The time course-release data were subjected to a regression analysis to derive the mean release time using a statistical package (GraphPad Prism version 5.02).

2.8.3. Comparative studies

Physicochemical, functional, encapsulation and release characteristics of crude and purified chitosan from P. monodon shell were compared to other commercially available medium molecular weight or shrimp chitosan samples.

2.9. Statistical analysis

Statistical evaluation of data was performed by GraphPad Prism version 5.02. All data were expressed as mean of three replicates ± SD. Student’s t-test was used for comparisons between the control and test experiments, while One-way ANOVA followed by Bonferroni’s post hoc test was used for multiple comparison.

3. Results

3.1. Effects of processing parameters on deproteinization of P. monodon shell

The deproteinizing effects of varying concentrations of NaOH and volumes of NaOH used per gram of sample on the removal of protein from shrimp shell were shown in Figures 1 and 2, respectively. Increase in NaOH concentration from 3.0% or 3.5% to 4.0% w/v significantly improved the removal of protein bound to the shell \((P < 0.05)\), and further increase to 4.5% w/v did not show significant difference in the amount of protein release. Also, reduction in residual protein content in the deproteinized samples was more pronounced when 4.0% w/v NaOH concentration was used to digest the shrimp shell (Figure 2).

The amount of protein released into the medium significantly increased with the increase in the volume of the NaOH solution per gram of shrimp sample \((P < 0.05)\). However, residual protein levels in deproteinized shrimp samples were not significantly different when above 7.5 mL of the deproteinizing medium was added to every 1 g of shell sample (Figure 2).

The deproteinizing effects of temperature, incubation speed and time on the removal of protein from shrimp shell were shown in Figures 3 and 4. Increase in deproteinization time or temperature significantly increased the removal of protein bound to the shell and reduced the amount of residual protein content in deproteinized shell \((P < 0.05)\). Also, optimum removal of protein was achieved in samples incubated at the speed of 200 r/min.

![Figure 1](image1.png)

**Figure 1.** Removal of protein from shrimp shell following deproteinization in varying concentrations of NaOH (A) and different ratios of weight of sample to volume of NaOH (B) used for digestion.

Bars are expressed as mean ± SD. Bars with different superscript letters within a group are significantly different from each other \((P < 0.05)\).

![Figure 2](image2.png)

**Figure 2.** Amount of residual protein in shrimp shell deproteinised with varying concentrations of NaOH (A) and different volumes of NaOH per gram of sample (B).

Bars are expressed as mean ± SD. Bars with different superscript letters within a group are significantly different from each other \((P < 0.05)\).

![Figure 3](image3.png)

**Figure 3.** Removal of protein from shrimp shell following deproteinization at different temperature (A), incubator speed (B) and time (C) of digestion.

Bars are expressed as mean ± SD. Bars with different superscript letters within a group are significantly different from each other \((P < 0.05)\).
3.2. Effects of demineralization parameters on extraction of *P. monodon* chitin

Figure 5 shows the effects of concentration and volume of hydrochloric acid used as demineralization medium per gram of sample on the amount of calcium released from deproteinized shrimp sample. Increase in the concentration of HCl from 0.4 to 1.0 mol/L and the ratio of demineralizing solvent to solid sample from 5 to 20 mL/g significantly increased the amount of calcium that released from the deproteinized samples (*P* < 0.05). Similarly, increasing the incubator speed significantly increased the release of calcium (*P* < 0.05). In contrast, extending demineralization time from 2 to 6 h had no significant difference on the calcium release from the shrimp shell (Figure 6).

The effects of the selected demineralizing parameters on residual ash content of extracted shrimp chitin were shown in Figures 7 and 8. Increase in concentration or volume of HCl per gram of sample significantly reduced the amount of ash content in the extracted crude chitin sample (*P* < 0.05), though there was no significant difference in ash content of samples digested with 15 or 20 mL of HCl per gram of sample (Figure 7). Also, the amount of ash in HCl-treated samples significantly decreased as demineralization time increased (*P* < 0.05) (Figure 8).
The release of calcium from the deproteinized sample was not temperature-dependent. Optimal demineralization was achieved at 40 °C and very low amount of ash content was determined in chitin extracted at 40 °C.

3.3. Effects of deacetylation parameters on physicochemical properties of P. monodon chitosan

Effects of deacetylation parameters on selected physicochemical properties of chitosan processed from crude chitin were shown on Table 1. Increasing the concentration of the deacetylation medium from 20% to 30% to 40% NaOH significantly increased the solubility, degree of deacetylation, viscosity and molecular weight of chitosan (P < 0.05). Further increase in concentration to 50% NaOH did not show significant difference in all parameters analyzed as compared to chitosan produced with 40% NaOH.

Increasing deacetylation time above 10 min significantly reduced the viscosity and molecular weight of chitosan (P < 0.05). Also, high deacetylation temperature of 140 °C generated by microwave irradiation produced chitosan of lower molecular weight with no net effect on the solubility or degree of deacetylation when compared to values determined in chitosan producing using lower temperature of 110 °C. However, solubility, viscosity and molecular weight of the chitosan were reduced when deacetylating medium was increased from 20 to 30 mL/g of chitin sample and values were significantly lower compared to when 10 or 20 mL of NaOH per g was used for deacetylation.

Table 1: Physicochemical characteristics of chitosan extracted from shrimp shell chitin influenced by selected deacetylation parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Crude samples</th>
<th>Purified samples</th>
<th>Reference (SA)</th>
<th>Reference (SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility (%)</td>
<td>94.23 ± 0.21</td>
<td>94.35 ± 0.19</td>
<td>93.86 ± 0.34</td>
<td>99.60 ± 0.48</td>
</tr>
<tr>
<td>DDA (%)</td>
<td>79.68 ± 5.38</td>
<td>79.17 ± 5.76</td>
<td>83.74 ± 0.45</td>
<td>81.05 ± 0.57</td>
</tr>
<tr>
<td>Viscosity (m²/s)</td>
<td>279.10 ± 29.98</td>
<td>634.80 ± 8.62</td>
<td>143.58 ± 1.85</td>
<td>135.79 ± 3.13</td>
</tr>
<tr>
<td>Molecular weight (kDa)</td>
<td>404 ± 30.6</td>
<td>1109 ± 30.6</td>
<td>541.20 ± 57.70</td>
<td>451.20 ± 57.70</td>
</tr>
</tbody>
</table>

Table 2: Physicochemical and functional properties of crude, purified and commercially available chitosan samples.

- Powdered shrimp shell
- Alkali insoluble fraction
- Crude chitin
- Crude chitosan

Figure 9. Schematic representation of the extraction of chitosan processing from shrimp shell using optimized set of conditions.

3.4. Effects of purification process on physicochemical properties of P. monodon chitosan

Based on the parameters that yielded best responses, crude chitosan was extracted from shrimp shell using the set of conditions depicted in Figure 9. The extracted chitosan was purified and comparative studies on selected physicochemical and functional properties were carried out using commercially available chitosan as reference samples for comparison (Table 2).

Table 2: Physicochemical and functional properties of crude, purified and commercially available chitosan samples.

- Powdered shrimp shell
- Alkali insoluble fraction
- Crude chitin
- Crude chitosan

Figure 9. Schematic representation of the extraction of chitosan processing from shrimp shell using optimized set of conditions.

3.5. Encapsulation characteristics of curcumin loaded into P. monodon chitosan

Encapsulation and release characteristics of curcumin loaded in chitosan processed from P. monodon shell were shown in Table 3. In comparison to encapsulation of curcumin in commercially available chitosan used as a reference, the loading capacity and encapsulation efficiency of the extracted chitosan were significantly lower, whereas the percentage yield was significantly higher (P < 0.05).
simulated gastric fluid (SGF) or simulated intestinal fluid (SIF) were shown in Figure 10. More than 50% of curcumin was released from the beads prepared from *P. monodon* chitosan within 2 h in SGF and 24 h in SIF.

Table 3
Encapsulation and release characteristics of curcumin loaded into formulation developed from chitosan processed from *P. monodon* shrimp shell.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Extracted samples</th>
<th>Reference (SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encapsulation efficiency (%)</td>
<td>68.56 ± 0.85</td>
<td>71.23 ± 0.44*</td>
</tr>
<tr>
<td>Loading capacity (%)</td>
<td>1.04 ± 0.05</td>
<td>1.39 ± 0.05*</td>
</tr>
<tr>
<td>Percentage yield (%)</td>
<td>42.36 ± 2.62</td>
<td>32.99 ± 1.44*</td>
</tr>
<tr>
<td>2-h release efficiency in SGF (%)</td>
<td>60.36 ± 2.13</td>
<td>51.89 ± 1.93*</td>
</tr>
<tr>
<td>Mean release time in SGF (min)</td>
<td>97.31 ± 1.01</td>
<td>120.10 ± 1.02*</td>
</tr>
<tr>
<td>8-h release efficiency in SIF (%)</td>
<td>18.21 ± 0.61</td>
<td>18.77 ± 0.37</td>
</tr>
<tr>
<td>Mean release time in SIF (h)</td>
<td>25.50 ± 1.17</td>
<td>20.33 ± 1.08*</td>
</tr>
</tbody>
</table>

*Values were expressed as mean ± SD and those with asterisk along a row are significantly different from each other (P < 0.05). SC: Santa Cruz.

Figure 10. Cumulative release profiles in SGF at pH 1.2 (A) and SIF at pH 7.4 (B) of curcumin encapsulated in formulations developed from chitosan extracted from *P. monodon* shell and commercially available (reference) chitosan. Values were expressed as mean ± SD.

In comparison to the commercial sample, the release of curcumin from beads prepared with *P. monodon* chitosan in SGF was significantly faster, resulting in lower mean release time (P < 0.05). However, the calculated mean release time in SIF was significantly (P < 0.05) (Table 2) extended in beads prepared with *P. monodon* chitosan, although at a time > 6 h, the release rate in SIF were not significantly different from those prepared with the commercially available chitosan.

4. Discussion

Process parameters such as time, temperature, concentration of acid or alkaline medium and solvent to solid ratio during chemical extraction of chitin and subsequent conversion to chitosan from shrimp shell highly influenced specific characteristics of the biopolymer such as binding capacity, solubility, molecular weight and degree of deacetylation. Applications of chitosan depend on its functional characteristics dictated by its physicochemical properties.

During deproteinization of the shrimp shell using 1 mol/L (4%) NaOH, extending digestion time from 1 h to either 6 or 24 h improved the removal of protein bound to the shell matrix by 27% or 37%, respectively. Also, the deproteinization process was temperature-dependent, and the residual protein in the alkaline insoluble fraction was reduced by 25% when temperature was increased from ambient value of 27 °C to 90 °C and optimum removal was achieved at 90 °C. Results from this study showed that increasing the solvent to sample ratio above 7.5 mL/g of shell sample did increased the removal of protein from shrimp samples but did not alter the residual amount of protein in the deproteinized samples after digestion. Benhabiles *et al.*[36] reported that solvent to sample ratio was a vital parameter in the deproteinization step and absolute removal of protein was achieved when shrimp shell was digested in the 20 mL of 2 mol/L NaOH per gram of samples at 45 °C for 2 h. In this study, optimum removal of protein was achieved using lower concentration (1 mol/L NaOH) and lesser volume (7.5 mL/g) of NaOH at 90 °C with mild agitation (200 r/min) for 2 h. Higher temperature and increased surface area of contact during incubation in the shaker may be responsible for the substantial difference in the amount of NaOH consumed.

In contrast to the alkaline medium used for deproteinization, hydrochloric acid was used as the demineralizing solvent to remove inorganic materials which are mainly CaCO3.[37] For efficient demineralization or decalcification, higher concentration of HCl is required. However, the use of HCl (> 1 mol/L) results in detrimental effects on the viscosity and molecular weight of chitosan[38], which negates or delimits its biomedical use as pharmaceutical excipient.

In this study, increasing the temperature of the medium using lower concentration of 0.6 mol/L HCl had no influence on the amount of calcium titrated in the medium as inorganic marker. Toan[39] showed that extending deproteinization time to 6 h using 0.5 mol/L HCl reduced ash content to less than 1% in partially autolyzed shrimp samples. Extending the time further from 6 to 12 h in this study showed 20% improvement in the demineralization process using diluted HCl. Previous study reported that increasing the sample mass to solvent ratio did not affect the demineralization process when 1.5 mol/L HCl was used[36]. Higher HCl concentration (> 1 mol/L) has been shown to cause absolute demineralization of shrimp shell to crude chitin, thus, increasing the solid to solvent ratio at such concentration may not result in significant changes. However, in this study, when 0.6 mol/L HCl was used to avoid the negative effect of highly acidified medium, increasing the solvent ratio significantly influenced the removal of calcium and reduced ash content of the resultant crude chitin with optimum process achieved at 15 mL per gram of deproteinized shrimp shell sample.

The crude chitin was deacetylated using concentrated NaOH. Production of medium to low molecular weight pharmaceutically graded chitosan from crude chitin was functionally guided by evaluating the solubility, viscosity, molecular weight and degree of
deacetylation in a single step procedure using microwave-assisted process. Literature review and preliminary (unpublished) studies revealed that the microwave process produced chitosan with better characteristics at a shorter time when compared to solvent boiling or autoclaved methods\[13,14,37\]. Deacetylation step is the rate limiting step in the production of chitosan from chitin. Deacetylation process parameters such as time, temperature and concentration of the alkaline medium greatly influenced the physicochemical and functional characteristics of chitosan. Results from this study revealed that microwave-assisted deacetylation process produced absolutely soluble chitosan of at least 80% degree of deacetylation from crude chitin digested with 10 mol/L (50%) NaOH within 10 min. To achieve similar results in studies that use conventional or autoclaving methods, chitin was deacetylated for longer time. Lertsutthiwong et al.\[40\] boiled at a low temperature for about 8 days with renewal of deacetylating medium, and repeated sequence at higher temperatures was employed by Trung et al.\[41\] and autoclaving at reduced pressure for 4 h\[41\] or for about 1 h after steeping in alkaline medium for 24 h\[42\] was adopted by others.

Elevated temperature induced by microwave irradiation has been shown to accelerate degradation of chitosan polymeric chains into lower molecular weight chitosan as the time of deacetylation or concentration of deacetylating medium was increased\[14\]. Al-Sagheer et al.\[37\] processed chitosan of 90% DDA from shrimp chitin using microwave-assisted process with 45% NaOH at 110 °C within 15 min. However, within 10 and 12 min, the production of chitosan was not above 85% degree of deacetylation. In this study, extending the deacetylation time above 10 min or increasing deacetylating temperature above 110 °C generated from the microwave irradiation resulted in degradation of the polymeric structure to produce chitosan of lower viscosity and molecular weight without concomitant effect on degree of deacetylation and solubility. Compared to other variables, solvent to solid sample ratio showed that minimal and no appreciable effect was observed when it was increased above 20 mL/ g, and similar observation was reported by Chang et al.\[20\].

For comparative studies, chitosan was processed from shrimp chitin using optimized set of parameters as described in Figure 9. The chitosan was purified using the method described by Qian and Gnanville\[22\]. The purification process which involved the removal of insoluble fractions, followed by re-precipitation with 1 mol/L NaOH and demetalization with sodium dodecyl sulphate sodium dodecyl sulphate and ethylenediaminetetraacetic acid increased the degree of deacetylation and reduced moisture and ash content as well as viscosity and molecular weight. Steeping in 1 mol/L NaOH during re-precipitation process may be responsible for the degrading of the crude chitosan into lower molecular weight polymer. This process also reduced binding capacities of the purified chitosan to either fat or water. However, the binding capacities and degree of deacetylation of non-purified crude chitosan were compared favourably with the values determined commercially available shrimp chitosan.

The controlled release system for curcumin was developed using the chitosan process from P. monodon. More than two-thirds of the curcumin were successfully loaded into the chitosan microcarriers. The percentage yield was higher compared to those prepared with the commercially available chitosan that served as reference samples. Differences in molecular weight of the biomaterial might be responsible for the large difference in yield. The higher the molecular weight of chitosan was used for encapsulation, the lower the encapsulation efficiencies and release rate would be\[43,44\]. Chitosan carriers in particulate forms or sub-micron sizes are easily eroded in acidic conditions due to the polycationic nature of the polymer. More than half of the curcumin (60%) was eroded from the carrier formulated using the P. monodon chitosan during a 2-hour degradation release study in SGF. However, in SIF, the sustained released and extended mean release time was conferred on the phyto-pharmaceutical.

Valorization of shrimp waste biomass into pharmaceutically graded medium molecular weight chitosan was achieved. The derived high value product was used to develop controlled release formulation. The extracted chitosan confers sustained release characteristics on the hydrophobic curcumin.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**References**
