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Extraction of chitin from prawn shells and conversion to low molecular mass chitosan

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17 **Abstract**

18 Extraction and depolymerisation of chitin and chitosan from prawn shells was carried out using
19 various chemical procedures. Sodium hydroxide and hydrochloric acid solutions were used for
20 deproteination and demineralization, respectively, while acetone was used for decolourisation.
21 The amount of chitin and subsequently chitosan obtained was ~35% and 25% respectively of the
22 dry weight of the shells. The chitin was deacetylated using sodium hydroxide at 100°C and the
23 influence of the concentration of the reagent and duration of the reaction was investigated. The
24 degree of deacetylation (DD) of the chitosan was evaluated by FTIR and NMR spectroscopy and
25 the molecular mass distribution was determined by Gel Permeation Chromatography. It was
26 found that the final DD was significantly higher using 50% sodium hydroxide solution (73% +/-
27 9%) compared to 25% sodium hydroxide solution (40% +/- 5%). It was noted also that the
28 deacetylation reaction was more than 80% completed after 2h but the chitosan produced had
29 higher molecular mass while chitosan produced after 10h had lower molecular mass and higher
30 degree of deacetylation. The molecular mass distribution was bimodal for all the samples and
31 consisted of a broad high molecular mass peak (peak 1) and a sharp low molecular mass peak
32 (peak 2). The Mw of peak 1 decreased from ~ 1.3×10^6 after 2h reaction with sodium hydroxide
33 to 3.1×10^5 after 10h reaction indicating that depolymerisation and deacetylation occurred
34 simultaneously. Peak 2 had a Mw of ~ $2.4 - 9.9 \times 10^3$.

35

36 **1. Introduction**

37 Chitin is the second most abundant of all of the polysaccharides and is found in the shells of
38 crustacea and in the cell walls of certain fungi and algae. It consists of linear chains of (1, 4)–

39 linked 2-acetylamido-2-deoxy-β-D-glucose (Kean and Thanou, 2011; Muzzarelli, 2009; Rinaudo,
40 2006, Rinaudo, 2008; Varum and Smidsrod, 2006). Although chitin itself is insoluble in water,
41 on deacetylation it yields chitosan, which is soluble under acidic conditions. Chitosan has been
42 the subject of many studies in recent years because it is biodegradable, biocompatible and has
43 antifungal and antibacterial activity and has been used to prepare hydrogels, films and fibres for
44 use in, for example, packaging and medical applications (Rinaudo, 2006, Rinaudo, 2008).
45 Considerable attention has been paid in recent years to the method of extraction of chitin from
46 the shells of crustacea (Varum and Smidsrod, 2006; Benhabiles *et al.,* (2012)). The shells consist
47 mainly of chitin, calcium carbonate, protein, lipids and pigments and separation is achieved in
48 three steps: 1) deproteination, 2) demineralisation and 3) removal of lipids and pigments. The
49 chitin extraction steps and its subsequent conversion to chitosan can be carried out chemically or
50 using biological methods, such as microbial fermentation and enzymatic reactions (Acharya et al
51 (2005); Zhang *et al.*, 1999; Gildberg and Stenberg, 2001; Rinaudo, 2006). However, biological
52 methods developed so far do not produce good yields and, therefore, are not economical.

53 The mechanism responsible for the antibacterial activity of chitin and chitosan has been the
54 subject of a number of studies in recent years (Lui et al (2001); Raafat et al (2008); Benhabiles *et*
55 *al.,* (2012)). Raafat et al (2008) studied the influence of chitosan (Mw 50-190 kDa) on the
56 growth of gram positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus simulans*).
57 They concluded that initial contact between the polymer and the anionic bacterial cell wall
58 polymers was due to electrostatic interaction and that teichoic acids played a major role, leading
59 to the disruption of the equilibrium of cell wall dynamics and ultimately to bacterial death. In
60 studies using low molecular mass chitosan (Mw < 5000), Lui et al (2001) have argued that the
61 chitosan molecules can penetrate the bacterial cell wall and bind to DNA molecules thus

62 blocking transcription of RNA. The importance of Mw is highlighted by recent work of
63 Benhabiles *et al.,* (2012) who investigated the antibacterial activity of chitin, chitosan and
64 chitin/chitosan oligomers against a range of gram positive and gram negative bacteria. The
65 viscosity average molecular mass, Mv, of the chitin and chitosan were reported to be 338 kDa
66 and 12 kDa respectively. The chitin/chitosan oligomers were prepared by acid hydrolysis of the
67 chitin and chitosan but precise values of Mv were not reported. They found that while all the
68 chitin / chitosan samples exhibited some degree of antibacterial activity, the chitin / chitosan
69 oligomers were more effective for a greater range of bacteria. They concluded that chitin and
70 chitosan had bacteriostatic activity and caused the bacteria to flocculate and die through lack of
71 nutrients and oxygen while the chitin / chitosan oligomers acted as bactericidal agents and were
72 able to kill the bacteria by penetrating the cell wall.

73 In view of the fact that low molecular mass chitosan has the most effective antimicrobial activity
74 there is a need to develop an efficient extraction process which includes the depolymerisation of
75 the chitin / chitosan. At present depolymerisation is achieved after the extraction process by acid
76 hydrolysis using HCl or by other chemical (Rogozhin *et al* (1988), Allan *et al* (1995, 1997),
77 Sugano *et al* (1992), enzymatic (Aiba *et al* (1992), Pantaleone *et al* (1992), Yalpani *et al* (1994))
78 methods or physical methods such as ultrasonication (Chen *et al* (1997)). Despite the importance
79 of molecular mass on the antimicrobial activity there are very few papers which describe detailed
80 characterisation of the molecular mass distribution of the chitosan. This paper sets out to develop
81 a protocol to produce low molecular mass chitosan as part of the extraction process and to
82 provide a detailed characterisation of the material produced.

83

84 **2. Materials and Methods**

2.1 Materials

Frozen prawn shells [species *Litopenaeus vannamei*] were provided by Findus Group Ltd and varied in weight from 130g to 500g. They were stored in a freezer before use. Hydrochloric acid, acetic acid and acetone were purchased from Fisher while sodium hydroxide, deuterium oxide (D_2O) and deuterium chloride (DCl) were obtained from Sigma-Aldrich.

2.2 Methods

2.2.1. Step 1: Pre-wash with water

Frozen prawn shells were initially hand washed with hot tap water (~60°C) or boiling water (~95°C) while stirring with a mechanical stirrer to remove free prawn flesh residues, lipids and other materials. Finally they were washed with hot distilled water and then dried in an oven at 60°C to constant weight. Washed and dried shells were either crushed to small pieces or powdered and passed through 60-120μm mesh sieves.

2.2.2. Step 2: Deproteination

The washed and dried powdered prawn shells were treated with 5% sodium hydroxide (NaOH) solution (w/v 1:8) and refluxed at 60°C for 2 hours to remove the remaining proteins and other organic materials. After the reaction, the solution was coloured and frothy, therefore, the sample was washed repeatedly with water until most of the colour and frothing disappeared and the resulting solution was near neutral. The sample was finally washed with distilled water and then dried in a vacuum oven at 60°C to constant weight.

Step 3: Decolouration

106 Deproteinated shells were treated with acetone at room temperature for 24 hours to remove
107 pigments. The washed shells were filtered and dried in a vacuum oven at 60°C until constant
108 weight.

109 **Step 4: Demineralisation**

110 The deproteinated and decolourised material was treated with a 0.5 or 1% HCl solution (w/v 1:4,
111 1:10) for 24 hours at 25°C to dissolve the calcium carbonate. The prawn shells were then washed
112 several times with water to remove $CaCl_2$ and other water soluble impurities.

113 The resultant chitin was obtained in the form of a very light brown powder. The content of chitin
114 in prawn shells was determined from the weight differences between the dry weight of the raw
115 materials and the resulting weight of chitin obtained.

116 Alternative procedures were followed in which some of the steps (deproteination and
117 decolouration) were omitted. The experimental details are given in Table 1.

118

119 **2.2.3. Deacetylation of Chitin**

120 Chemical deacetylation was achieved by treatment of extracted chitin with sodium hydroxide
121 (NaOH) solution at elevated temperature using a solid to solvent ratio of 1:5. Effects of various
122 parameters such as NaOH concentration (25%, 50%), temperature (80°C and 100°C) and reaction
123 times (2, 5 and 10 hours) on the deacetylation process were investigated. After the reaction the
124 material produced was washed several times with distilled water until near to neutral pH and
125 dried at 60°C in a vacuum oven until constant weight.

126

127 **3. Characterisation**

3.1. Fourier Transform Infrared Spectroscopy (FTIR)

Chitin and chitosan samples were ground to a very fine powder with KBr and dried thoroughly. The dried mixture was pressed under vacuum in a mould to form a KBr disc containing the sample. FTIR spectroscopic measurements were also performed on films of soluble chitosan. Chitosan samples with a low degree of deacetylation (DD) were dissolved in 20% HCl while samples with a DD of 50% and above were dissolved in a 1% solution of acetic acid. Subsequently, the films were cast on plastic trays and left to dry at room temperature. Neutralisation of protonated $-NH_2$ before obtaining the FTIR spectra was done by leaving the dried films in 1% NaOH solution for 24 hours. This was followed by repeated washing steps using distilled water and a drying step under vacuum at 40°C for 12 hours or until constant weight.

FTIR spectra were recorded using a Perkin Elmer FTIR Spectrometer over the frequency range of 4000–625 cm^{-1}. 16 scans were accumulated at a resolution of 4 cm^{-1}.

FTIR spectroscopy was also used to estimate the degree of deacetylation (DD) of chitosan. The DD of the chitosan samples was calculated from the absorbances at 1658 and 3450 cm^{-1} according to the following equation [Baxter *et al.*, 1992; Muzzarelli 2009]:

$$DD\ (\%) = 100 - [(A_{1658} / A_{3450}) \times 115] \qquad (1)$$

where A_{1658} and A_{3450} are the absorbance at 1658 cm^{-1} of the amide-I band as a measure of the N-acetyl group content and the absorbance at 3450 cm^{-1} of the hydroxyl band as an internal standard to correct for film thickness.

149 The band ratio method of selected bands from their FTIR spectra was also used to determine the
150 DD of the chitosan samples. The bands at 1318 and 1382cm^{-1} were chosen as measuring and
151 reference bands, respectively [Berth *et al.*, 1998]. To measure the peak intensities for these two
152 bands, baselines were drawn between the 1350–1280 cm^{-1} and 1490–1350cm^{-1} wavenumbers,
153 respectively. The DD was determined by using the following equation:

154 $$A_{1318}/A_{1382} = 0.3822 + 0.03133\ DA \qquad (2)$$

155

156 **3.2. NMR spectroscopy**

157 Chitosan samples were analysed by proton Nuclear Magnetic Resonance (^{1}H –NMR)
158 Spectroscopy on a Bruker Avance DRX-500 (500 MHz) spectrometer. The samples for NMR
159 were prepared by dissolving 7-10 mg of chitosan in 1-10% DCl in D_2O, depending on the
160 solubility of the sample. The degree of deacetylation was calculated by using integrals of the
161 peak of the proton of the CH group connected to nitrogen moiety at 3.11 ppm of the deacetylated
162 monomer unit (H1-D) and of the peak of the three protons of the acetyl group (H-Ac) at 1.99
163 ppm of the acetylated monomer unit as shown in equation 3.

164 DD (%) = [(area of 3xH1-D) / (area of 3xH1-D + area of H-Ac)] x100 (3)

165

166 **3.3 Gel Permeation Chromatography**

167 The molecular mass distributions of the chitosan samples were determined by Gel Permeation
168 Chromatography (GPC) coupled with a multi-angle laser light scattering (MALLS) and
169 refractive index detectors. The chromatography system consisted of a HPLC pump and a
170 Rheodyne injection valve fitted with a100µl loop. The column systems used were TSK G5000-

171 PWxl and TSK G6000-PWxl analytical columns protected by a guard column, connected in
172 series. Acetate buffer (0.2 M acetic acid/0.1 M sodium acetate, pH 4.8), used as the eluent
173 (filtered through a 0.22μl filter to remove any insoluble material or dust particles), was pumped
174 at a flow rate of 0.5 ml/min through the column systems. Solutions of each chitosan sample, at a
175 concentration of 2 mg/ml, were prepared in the same acetate buffer used as eluent. Dissolved
176 samples were filtered through a 0.22μl filter to remove any insoluble material or dust particles
177 prior to injection. Filtered samples were injected onto the columns at 40°C. The eluting fractions
178 were monitored by using an Optilab DSP interferometric refractometer coupled with a Dawn
179 EOS Enhanced multi-angle laser light scattering photometer (both instruments from Wyatt
180 Technology Corporation). Depending on the degree of acetylation, the refractive index increment
181 (dn/dc) ranged from 0.151 to 0.219 mg/ml and was experimentally determined using the same
182 refractometer and solvent conditions as for the GPC/MALLS, with the exception that a 500μl
183 loop was used for injection. Signals from the light scattering photometer and the refractometer
184 were recorded and analysed on a PC using the software ASTRA supplied by the manufacturer.
185 Eluent was pumped at a flow rate of 0.5 ml/min. Sample concentrations of 5 mg/ml, 2 mg/ml and
186 1 mg/ml were injected at 40°C.

187

188 **4. Results and discussion**

189 **4.1. Extraction of chitin from prawn shells**

190 The proportions of the various components obtained at the various stages of the extraction
191 process using different procedures are given in Table 2.

192 ***Step 1: Washing with water***

193 The frozen shells were washed with water and it was found that the amount of dried prawn shells
194 left after washing and then drying was ~42 wt% when tap water at ~60°C was used, and ~35%
195 when boiling water (~95°C) was used. This weight loss is attributed to removal of water and
196 residual prawn flesh.

197

198 ***Step 2: Deproteination***

199 The dried shells obtained after washing in Step 1 for Experiments 1-3 and 6, 7 were treated with
200 5% NaOH to remove proteinaceous components together with lipids and pigments. This
201 removed ~ 45 wt % of the material from the prawn shells that had been washed with boiling
202 water at ~95°C and ~54 wt% of the material that had been washed with hot tap water at ~60°C.

203

204 ***Step 3: Decolourisation***

205 The dried shells obtained after Step 1 and Step 2 in Experiments 5 and 6 respectively were
206 washed with acetone to remove organic material. This removed just a small amount of material
207 (0.7-1.4 wt%).

208

209 ***Step 4: Demineralisation***

210 The dried shells obtained after Step 1 for experiment 4, Step 2 for experiments 1-3 and 7 and
211 Step 3 for experiments 5 and 6 were treated with HCl to remove inorganic minerals, mainly
212 $CaCO_3$. The concentration of HCl used was 0.5% in experiments 1-6 and 1% in experiment 7.
213 The weight loss at this stage depended very much on the processing history. For experiments 1-3
214 and 6 the loss was ~ 9%. The loss was greater in experiment 7 using the higher HCl

215 concentration (13.8%). For experiments 4 and 5, in which the deproteination step had been
216 omitted, the loss was ~18%. This higher value is probably due to loss of organic material in
217 addition to $CaCO_3$. Interestingly, Mahlous, *et al.*, (2007) found the mineral content to be 14% for
218 chitin extraction from prawn shells using gamma irradiation.

219

220 The amount of chitin obtained from the prawn shells for experiments 1-3 and 6-7 was ~ 25%
221 based on the dry weight. The very much higher values reported for Experiments 4 and 5 are due
222 to the fact that the deproteination step had been omitted. Chitin yield from shrimp shells has
223 been reported to be 10-30% depending on the shrimp species and the method used [Acosta 1993,
224 Tolaimate *et al.*, 2000, 2003; Benhabiles *et al.*, (2012)]. The yields of chitin from crab shell,
225 squid and crayfish have been reported to be 10%, 40% and 32%, respectively [Tolaimate *et al.*,
226 2000, 2003].

227

228 ***Chitin deacetylation***

229 The chitin obtained after Step 4 was deacetylated using either 25% w/v NaOH (Experiments 2
230 and 3) or 50%w/v (Experiments 1, 4-6) NaOH. The amount of chitosan obtained was ~25wt% of
231 the original weight of the dried prawn shells irrespective of the NaOH concentration.

232

233 **4.2 Characterisation of chitin and chitosan**

234 The FTIR spectra of powdered prawn shells, chitin and chitosan are presented Figures 1 (a-c).
235 The chitin and chitosan were obtained from the conditions outlined in Experiment 7 and these
236 were chosen since these samples were completely free of calcium carbonate. The FTIR spectrum

237 of chitin, presented in Figure 1 (b), includes absorbance bands around 3450, 3262, 3114, 2960,
238 2930, 2888, 1658, 1628, 1560, 1418, 1382, 1318, 1260, 1204, 1158, 1118, 1074, 1026, 952 and
239 896 cm^{-1}. This is consistent with the structure of α-chitin [Acosta *et al.*, 1993]. In addition there
240 is splitting of the amide I band in the chitin spectrum to give two peaks at 1658 cm^{-1} which is
241 attributed to the occurrence of intermolecular hydrogen bonds CO...HN and at 1628 cm^{-1} due to
242 the intramolecular hydrogen bond CO...$HOCH_2$ [Focher *et al.*, 1992] and is characteristic of α-
243 chitin. The bands due to NH stretching at 3262 cm^{-1} and 3114 cm^{-1} are also characteristic of the
244 α-chitin spectrum [Focher *et al.*, 1992]. The bands around 1798, 1420-1430 and 876 cm^{-1} in
245 Figure 1(a) for the powdered shells are due to mineral ($CaCO_3$) are not present in chitin after
246 demineralisation with 1% HCl while these absorbance are present if demineralisation is carried
247 out using 0.5% HCl. The absence of a peak around 1540 cm^{-1} indicates that protein has been
248 removed. The absorption peaks due to mineral and proteins were present in chitin extracted in
249 Experiments 4 and 5 in which Step 2, the deproteination step was omitted.

250 The FTIR spectrum of chitosan (Figure 1 (c)) shows extra bands in the region 1606-1566 cm^{-1}
251 due to primary amine groups while the absorptions at 3450, 3262, 3114 and 1658 cm^{-1} due to
252 amide groups are missing from the deacetylated chitin while absorption at 1632 cm^{-1} due to $-NH_2$
253 deformation of primary amines appears. Absorption at 3398 cm^{-1} appears due to $-NH_2$ stretching
254 absorption in amines in chitosan. The degree of deacetylation of the chitin samples determined
255 from the FTIR spectra was lower for samples treated with 25% NaOH (Experiments 2 and 3)
256 compared to the other samples treated with 50% NaOH as illustrated in Table 3. For some of the
257 experiments the degree of deacetylation was followed as a function of time and was found to
258 increase slightly between 2h and 10h (Table 3). The value obtained after 2h was more than 80%
259 of the final value.

260 The ^{1}H NMR spectra for two of the samples are given in Figures 2 (a) and (b). The degree of
261 deacetylation was determined using equation 3 above and the values obtained are significantly
262 higher than those from FTIR.

263 The GPC RI elution profiles of the chitosan samples obtained from Experiment 6 at different
264 reaction times are shown in Figure 3. The profiles show a bimodal distribution with a broad low
265 intensity elution peak (peak 1) at elution times of ~ 11-22mins and a high intensity elution peak
266 (peak 2) at ~23mins. The polymer molecules eluting at peak 1 for the 2h reaction time have a
267 Mw of 1.3×10^{6} while those corresponding to peak 2 have a Mw of 3.5×10^{3} (Table 4). At the
268 longer reaction times of 5h and 10h, peak 1 elutes at higher elution times, corresponding to Mw
269 values of 9.6×10^{5} and 3.1×10^{5} respectively, while peak 2 elutes at the same elution time but
270 increases in intensity. It is evident, therefore, that depolymerisation is occurring as well as
271 deacetylation as the reaction proceeds. The molecular mass profiles of the other samples at a
272 reaction time of 10h are presented in Figure 4 and show similar profiles and behaviour.
273 Brugnerotto et al., (2001) determined the molecular mass distribution for a number of
274 commercial chitosan samples and also found a broad Mw distribution with average Mw values
275 ranging from $0.6 - 2.1 \times 10^{5}$. Nguyen *et al.*, (2009) provide a review of papers in the literature
276 that report the Mw distribution of chitosan using GPC. Very few, however, actually show elution
277 profiles. Nguyen *et al.*, (2009) themselves, determined the molecular mass distribution of a
278 number of commercial samples of chitosan and reported Mw values of $\sim 2.0 - 3.5 \times 10^{5}$. Whereas
279 for their system most of the chitosan eluted at 14-22 mins they also had a low Mw fraction
280 eluting at ~29-30 mins before the salt peak but made no reference to this latter component.

281

282 **Conclusions**

283 The work described in this paper has demonstrated that chitin can be effectively extracted from
284 prawn shells following deproteination using 5% NaOH and demineralisation using 1% HCl. Low
285 molecular mass chitosan samples with DD >64% and Mw of the major component < 10^4 can be
286 obtained by treating the chitin with 50% NaOH at 100°C for up to 10h.

287

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291

292 **References**

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363

364 **Table 1. Experimental procedure for extraction of chitin from prawn shells and its**

365 **conversion to chitosan.**

Experiments	Experimental details
Experiment 1	Frozen prawn shells crushed and washed with hot tap water (~60°C), deproteinated with 5% NaOH, demineralised with 0.5% HCl (w/v 1:4), deacetylated with 50% NaOH at 100°C for 2, 5 and 10 hours.
Experiment 2	Frozen prawn shells crushed and washed with hot tap water (~60°C), dried and then powdered, deproteinated with 5% NaOH, demineralised with 0.5% HCl (w/v 1:4), deacetylated with 25% NaOH for 2, 5 and 10 h at 80°C.
Experiment 3	Frozen prawn shells crushed and washed with hot tap water (~60°C), dried and then powdered, demineralised with 0.5% HCl (w/v 1:4), deacetylated with 25% NaOH for 2, 5 and 10 h at 100°C.
Experiment 4	Frozen prawn shells washed with boiling water (~95°C), dried and then powdered, demineralised with 0.5% HCl (w/v 1:4), deacetylated with 25% NaOH for 2, 5 and 10 h at 100°C.
Experiment 5	Frozen prawn shells washed with boiling water (~95°C), dried and then powdered, decoloured with acetone, demineralised with 0.5% HCl (w/v 1:4), deacetylated with 50% NaOH for 2, 5 and 10 hours at 100°C.
Experiment 6	Frozen prawn shells were washed with hot tap water (~60°C), dried and then powdered, deproteinated with 5% NaOH, decoloured with acetone, demineralised with 0.5% HCl (w/v 1:4), deacetylated with 50% NaOH for 2, 5 and 10 hours at 100°C.
Experiment 7	Frozen prawn shells were washed with boiling water (~95°C), dried and then powdered, deproteinated with 5% NaOH, demineralised with 1% HCl (w/v 1:10), deacetylated with 50% NaOH for 2, 5 and 10 hours at 100°C.

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368 **Table 2. Experimental results for extraction of chitin from prawn shells and its conversion**
369 **to chitosan**

Experiments:	1	2	3	4	5	6	7
Step 1:Pre-wash with water % wt solids remaining after washing frozen shells with water and drying * = water at ~60°C + = water at ~95°C	42.8*	42.2*	42.2*	35.1^{+}	35.3^{+}	35.6^{+}	35.0^{+}
Step 2:Deproteination % wt of dried shells remaining after treatment with 5% NaOH to remove proteinaceous material	45.44	44.8	44.8	Step omitted	Step omitted	54.5	54.3
Step 3:Decolouration % wt loss of dried deproteinated shells after treatment with acetone to decolourise	Step omitted	Step omitted	Step omitted	Step omitted	1.4	0.7	Step omitted
Step 4: Demineralisation % wt loss of shells after treatment with HCl to demineralise	8.8	9.8	9.8	18	17.6	9.0	13.8
% chitin recovered based on dried shells	36.1	35.03	35.03	82	81	38.15	31.8
% Chitosan recovered after deacetylation of chitin after 10 hours	67.57	73.13	72.79	32.25	31.80	60.7	-
% Chitosan based on dried shells after 10 hours	24.39	25.15	25.04	26.44	25.76	24.29	-

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372 **Table 3. Degree of deacetylation of chitosan as a function of reaction time**

Expt.	Time (h)	Average DD (%) by FTIR	DD (%) by ^{1}H NMR	comments
1	2	73	94	
2	2	34		N/S
	5	38		N/S
	10	41		N/S
3	2	36		N/S
	5	40		N/S
	10	45		N/S
4	2	64		
	5	69		
	10	73		
5	2	64		
	5	69		
	10	73		
6	5	77		
	10	80	98	
7	2	75		
	5	79		
	10	82		

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374 N/S = samples not fully soluble in 0.2 M acetic acid/0.1 M sodium acetate, pH 4.8

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376 **Table 4. Mwt. of chitosan samples as a function of reaction time**

Expt.	Time (h)	Average Mw of Peak 1	Average Mw Of Peak 2	% area Peak 1	%area Peak 2
1	2	$1.3x10^6$	$8.7x10^3$	55	45
4	10	$5.0x10^5$	$9.9x10^3$	50	50
5	10	$4.8x10^5$	$8.4x10^3$	50	50
6	2	$1.3x10^6$	$3.5x10^3$	18	82
6	5	$9.6x10^5$	$5.4x10^3$	16	84
6	10	$3.1x10^5$	$5.9x10^3$	15	85
7	10	$1.9x10^5$	$2.4x10^3$	21	79

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390 **List of Figures**

391

392 **Figure 1.** IR spectra of (a) powdered prawn shells (b) extracted chitin and (c) chitosan from
393 prawn shells

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395 **Figure 2.** ^{1}H NMR spectra of chitosan from prawn shells produced at 100°C for (a) 2 hours and
396 (b) 10 hours

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398 **Figure 3.** GPC RI elution profiles of chitosan from experiment 6 on treating with 50% NaOH at
399 varying reaction times

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401 **Figure 4.** Molecular mass distribution of the various chitosan samples on treating with
402 50%NaOH for 10h at 100°C

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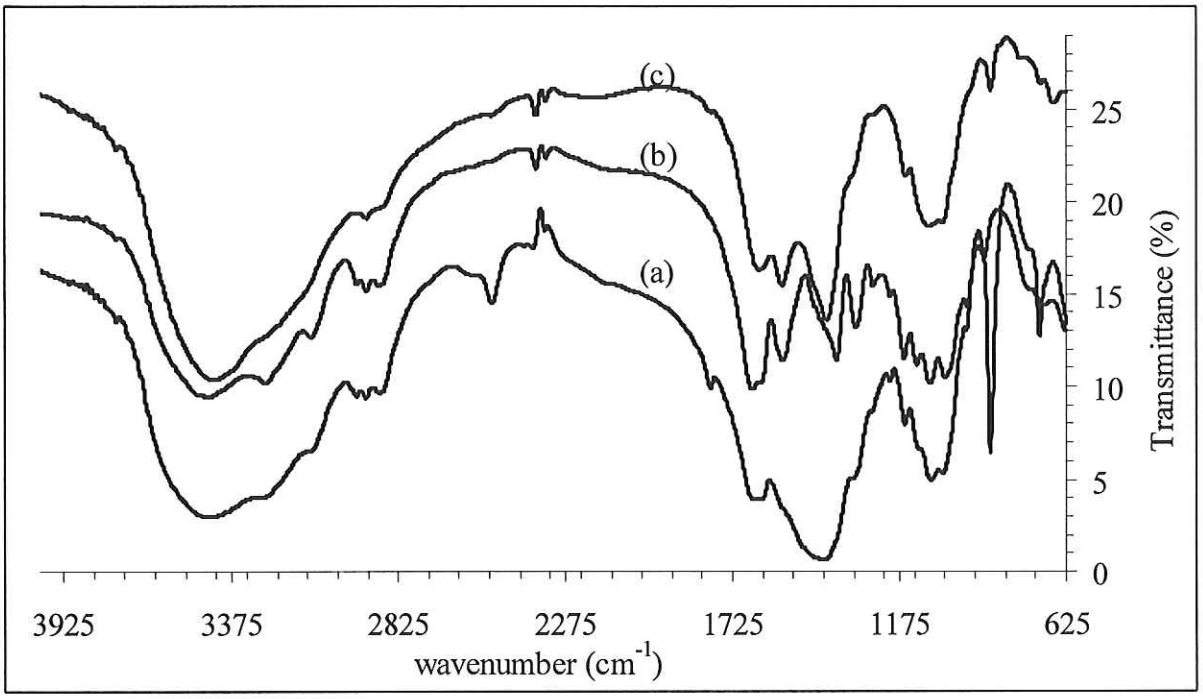
422 **Figure 1.** IR spectra of (a) powdered prawn shells, (b) extracted chitin and (c) chitosan from
423 prawn shells



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427 **Figure 2.** ^{1}H NMR spectra of chitosan produced at 100°C for (a) 2 hours and (b) 10 hours

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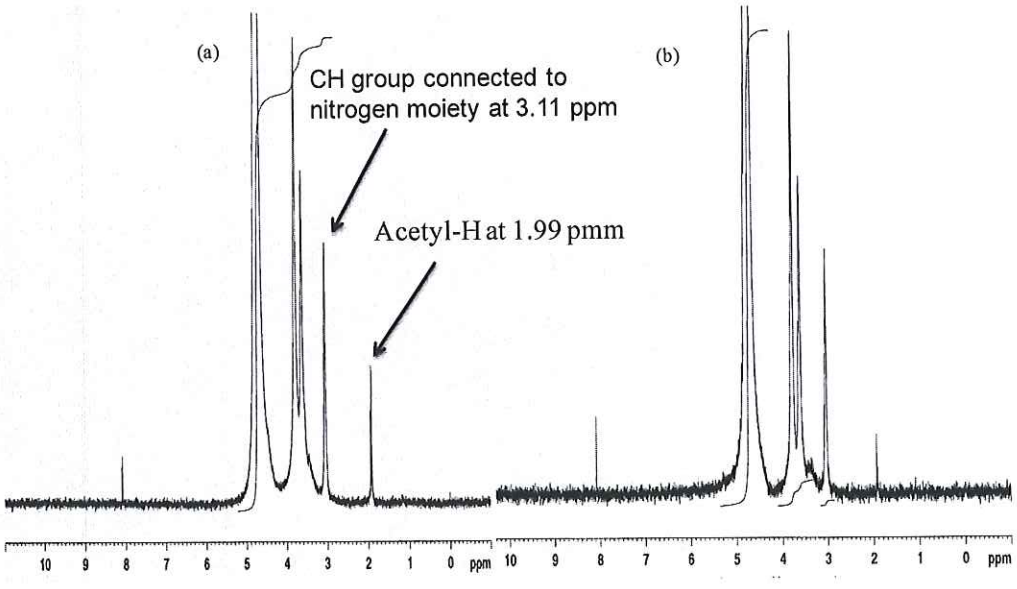

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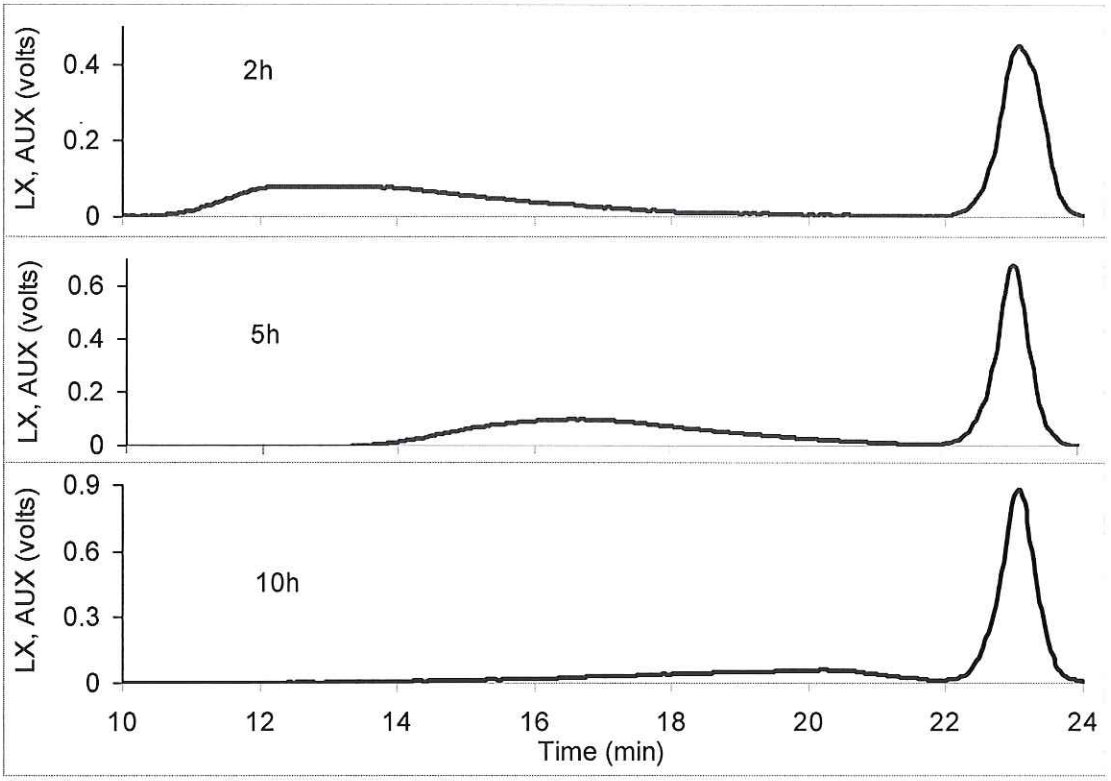
Figure 3. GPC RI elution profiles of chitosan from Experiment 6 at varying reaction times



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446 **Figure 4. Molecular mass distribution of the various chitosan samples on reaction with**
447 **50%NaOH for 10h**

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