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# Production and Characterization of Chitosan from Shrimp (*Penaeus semisulcatus* ) Shell Waste of UAE

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**Abstract.** Chitosan was prepared from shrimp (*Penaeus semisulcatus)* shell waste by a chemical process involving demineralization, deproteinization and deacetylation; conversion of chitin to chitosan (deacetylation) was achieved by treatment with concentrated sodium hydroxide solution (55%) at room temperature (25 ºC). The present study was undertaken to evaluate the influence of deacetylation process during chitosan production on the physicochemical and functional properties of shrimp shell chitosan. Four experimental chitosan samples were prepared with deacetylation for 40 h, for 50 h, with and without stirring as well as for 60 h and were subjected to physicochemical and functional characteristic analysis. Change in duration of deacetylation process yielded some differences in each characteristic; deacetylation for 40 h led to lower viscosity, solubility, water/fat binding capacity and degree of deacetylation and for 60 h resulted in increase in solubility but decrease in viscosity. Stirring during deacetylation process led to lower viscosity, higher degree of deacetylation and higher fat binding capacity of the product. In contrast non-stirred sample produced product with lower degree of deacetylation and higher viscosity. It was concluded that duration of deacetylaylation process should be monitored constantly for optimal chitosan production depending on its intended usages in food, pharmaceutical and biomedical industries.

**Keywords:** shrimp shell waste, deacetylation, chitosan, chitin

## Introduction

Chitosan is a fiber-like substance derived from chitin, a homopolymer of ß-(1→4)-linked N-acetyl-D-glucosamine. Chitin is widely distributed in marine invertebrates, insects, fungi, and yeast (Subasingle, 1995; Austin *et al.*, 1981); however, it is not present in higher plants and higher animals. Generally, the shells of selected crustaceans consist of 30-40% protein, 30-50% calcium carbonate and calciumc phosphate and 20-30% chitin (Acosta *et al.*, 1993; Knorr, 1984). Chitin is widely available from a variety of sources among which, the principal source is shellfish waste such as that of shrimps, crabs and crawfish (Rinaudo, 2006; Allan and Hadwiger, 1979). It also exists naturally in a few species of fungi (Franco *et al.*, 2004; Andrade *et al.*, 2000; Chung *et al.*, 1994). Chitin and chitosan have similar chemical structures (Fig. 1). Chitin is made up of a linear chain of acetylglucosamine groups while chitosan is obtained by removing enough acetyl groups ($CH_3$-CO) from the molecule so that it becomes soluble in most diluted acids. This process is called deacetylation. The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful derivative of chitin (No and Meyers, 1992).

Chitosan is a non toxic, biodegradable polymer of high molecular weight (Zhang and Neau, 2001; Tomihata and Ikada,

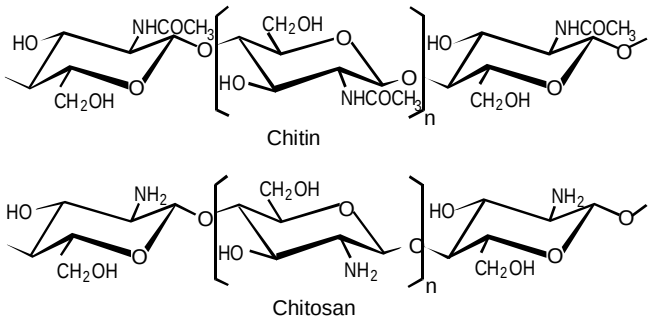


**Fig. 1.** Structure of chitin and chitosan.

1997). Over the last several years, chitinous polymers, especially chitosan, have received increased attention as one of the promising renewable polymeric materials for their extensive applications in the pharmaceutical and biomedical industries for enzyme immobilization and purification, in chemical plants for wastewater treatment and in food industries for use in food formulations as binding, gelling, thickening and stabilizing agent (Prashanth and Tharanathan, 2007; Franco *et al.*, 2004; Knorr, 1984).

Traditional isolation of chitosan from crustacean shell waste consists of four basic steps: demineralization, deproteinization, decolourization and deacetylation (Galed *et al.*, 2008; No and Meyers, 1995). Several procedures have been developed and proposed by many researchers over the years for preparation of chitosan from different crustacean shell wastes (Galed

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*et al.*, 2008; No and Meyers, 1995; No *et al.*, 1989). Some of these formed the basis of chemical processes for industrial production of chitosan. But most of the reported processes were carried out with 45% concentrated sodium hydroxide solution at 100 °C or higher temperature with autoclaving (Galed *et al.*, 2008; Prashanth and Tharanathan, 2007; Domard and Rinaudo, 1983; Horton and Lineback, 1965). Therefore, the specific objectives of this work were to develop an optimum shrimp shell chitosan production process at room temperature (25 °C) with increased alkali strength without decolourization step and to study the influence of deacetylation process on the physicochemical and functional properties of shrimp shell chitosan.

## Materials and Methods

**Shrimp shell chitosan production.** *Penaeus semisulcatus*, *Metapenaeus mastersii* and *Penaeus latisulcatus* are the shrimp species found in UAE waters of which *Penaeus semisulcatus* is the most common and commercially important species. Undersized shrimp shell waste of *Penaeus semisulcatus* was obtained from a commercial shrimp shell processor of Dubai, UAE. Upon receipt, shells (head, body and tail) were washed under running warm tap water to remove soluble organics, adherent proteins and other impurities. The shells were then dried in the oven (Mammert, Germany) at 70 °C for a period of 24 h or longer until completely dried shells were obtained. The moisture content of dried shell was 0.48%. To obtain a uniform size product, the dried shell was ground through a centrifugal grinding mill and sifted with 20-mesh (0.841 mm) and 40-mesh (0.425 mm) sieves. Dried ground shell powder was placed in opaque plastic bottles and stored at room temperature until used. The production of chitosan from shrimp shell waste was carried out with a modified method of No *et al.* (1989). The dried shrimp shell powder (5 kg) was demineralized with 8-10% hydrochloric acid at ambient temperature with a solid to solvent ratio of 1:15 (w/v), in an acid resistant vessel with stirrer for 20-22 h until demineral-i-zation was completed. The demineralized shells were deproteinized with 8-10% sodium hydroxide solution for 20-22 h at 65 °C with constant stirring or without stirring at a solid to solvent ratio of 1:10 (w/v). Samples were then washed with tap water and dried under vacuum for 2-3 h until the powder was crispy. Removal of acetyl groups from chitin was achieved by using concentrated sodium hydroxide solution (55%) with a solid to solvent ratio of 1:10 (w/v). Samples of four experimental shrimp shell chitosans were prepared. The chemical reactions were carried out at room temperature (25 °C). Duration of deacetylation process was 40 h for sample $C_{40}$, 50 h for $C_{50S}$ (with magnetic stirring), 50 h for $C_{50WS}$ (without stirring) and 60 h for $C_{60}$. The resulting chitosans were washed to neutrality in running tap water, rinsed with distilled water, filtered and dried at 60 °C for 24 h in the oven. The obtained shrimp shell chitosan was white to off white in colour and it was not necessary to decolourize or bleach it.

**Physicochemical and functional properties.** ***Measurement of nitrogen.*** Nitrogen of the crawfish chitosan was determined using a microprocessor-based, software-controlled instrument Model-TruSpec CN (Model # FP-428 Leco Corporation, USA). There were three phases during an analysis cycle, i.e., purging, burning and analysis. The encapsulated sample was purged of any atmospheric gases that had entered during sample loading. During the burning phase, the sample was dropped into a hot furnace (850 °C) and flushed with pure oxygen for a very rapid combustion. Finally, in the analysis phase, the remaining combustion product (nitrogen) was measured by the thermal conductivity cell. The final result was displayed as percent nitrogen.

***Ash.*** Ash of the crawfish chitosan was calculated according to the standard method # 923.03 (AOAC, 1990). 2.0 g of chitosan (triplicate) were placed into previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 600 °C for 6 h. The crucibles were allowed to cool in the furnace to less than 200 ºC and then placed in desiccator with a vented top. Crucibles were cooled, weighed and ash content was recorded.

***Degree of deacetylation.*** Chitosan samples prepared in the form of KBr discs were studied for the degree of deacetylation (DD) (Kassai, 2008; Khan *et al.*, 2002). The prepared chitosan KBr discs were kept in desiccators for 12 h and then placed in sealed plates before scanning. The DD of chitosan was established using a FTIR (Fourier Transform Infrared Spectroscopy) instrument (Model # M2000, Midac Corp. USA) with frequency of 4000-4/cm. The degree of deacetylation (DD) of the chitosan was calculated using the baseline reported by Khan *et al.* (2002). The computation equation for the baseline is given below:

$$DD = 100 - [(A_{1655} / A_{3450}) \times 100 / 1.33]$$

where $A_{1655}$ and $A_{3450}$ are the absorbance at 1655 $cm^{-1}$ of the amide-I band as a measure of the N-acetyl group content and at 3450 $cm^{-1}$ of the hydroxyl band as an internal standard to correct for disc thickness. The factor '1.33' denotes the value of the ratio of $A_{1655}/A_{3450}$ for fully N-acetylated chitosan.

***Viscosity.*** Viscosity of chitosan was determined with a Brookfield viscometer (Model DV-II + Brookfield Engineering

Laboratories Inc., Stonghton, MA.). Chitosan solution was prepared in 1% acetic acid at 1% concentration on dry basis. Measurement was made in duplicate using a No. 27 spindle at 50 rpm on solutions at 25 °C with values reported in centipoise (cP) unit.

***Solubility.*** Crawfish chitosan sample (0.1 g in triplicate) was placed in a centrifuge tube (known weight) then dissolved with 10 ml of 1% acetic acid for 30 min. The solution was then centrifuged at 10,000 rpm for 10 min. The supernatant was decanted. The undissolved particles were washed in distilled water (25 ml) then centrifuged at 10,000 rpm. The supernatant was removed and undissolved pellets were dried at 60 °C for 24 h. Finally, the particles were weighed and the percentage solubility was determined.

***Water binding capacity (WBC).*** WBC of chitosan was measured using a modified method of Knorr (1982). Initially a centrifuge tube containing 0.5 g of sample was weighed, 10 ml of water was added and mixing was carried out on a vortex mixer for one min to disperse the sample. The contents were left at ambient temperature for 30 min with intermittent shaking for 5 s every 10 min and then centrifuged (Model # Z383K, HERMLE-National Labnet Company, USA) at 3,500 rpm (6,000 × *g*) for 25 min. After the supernatant was decanted, the tube was weighed again. WBC was calculated as follows:

WBC (%) = [water bound (g)/ initial sample weight (g)]× 100. All experiments were carried out in triplicate.

***Fat binding capacity (FBC).*** FBC of chitosan was measured using a modified method of Knorr (1982). Initially a centrifuge tube containing 0.5 g of sample was weighed, 10 ml of oil (three types of oil were used namely soybean, corn and sunflower oils) were added and mixing was carried out on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s every10 min and then centrifuged at 3,500 rpm (6,000 × *g*) for 25 min. After the supernatant was decanted, the tube was weighed again. FBC was calculated as follows:

FBC (%) = [fat bound (g)/ initial sample weight (g)] × 100. Experiments were performed in triplicate.

**Statistical analysis.** All experiments were carried out in triplicate, Average values (means) and standard deviations were reported. Mean separations were analyzed using the ANOVA and Tukey's student range tests at á = 0.05.

## Results and Discussion

**Yield.** Yield was calculated as the dry weight of chitin obtained from 5 kg of dried shrimp shell powder. The yield of chitin was 20% and that of chitosan ranged from 16-19%. The highest yields were obtained from sample $C_{40}$ (19%), followed by $C_{50WS}$ (18%), $C_{60}$ (17%), and $C_{50S}$ (16%). Results are shown in Table 1. Brzeski (1982) reported about 14% yield of chitosan from krill and 18.6% from prawn waste (Alimuniar and Zainuddin, 1992). The yield of chitosan obtained (15-18%) is lower than that (approximately 23%) of chitin reported in the literature (No and Meyers, 1989). This may be due to loss of sample mass/weight during deacetylation process as we used here 55% concentrated sodium hydroxide solution, whereas in other methods 45% sodium hydroxide solution was used. The moisture content of the shrimp shell chitosan, determined by the gravimetric method (Black, 1965), was in the range of 0.3% to 0.4% (Table 1).

**Table 1.** Proximate analysis of shrimp shell and commercial chitosans (dry weight basis)

| Sample | Yield (%) | Moisture (%) | Nitrogen (%) | Ash (%) |
|---|---|---|---|---|
| $C_{40}$ | 19 | 0.4 (0.25)[a]* | 8.33 (0.02)[a]* | 0.29 (0.07)[a]* |
| $C_{50S}$ | 16 | 0.3 (0.20)[a] | 8.19 (0.01)[a] | 0.3 (0.99)[a] |
| $C_{50WS}$ | 18 | 0.4 (0.25)[a] | 8.11 (0.05)[a] | 0.3 (0.23)[a] |
| $C_{60}$ | 17 | 0.4 (0.22)[a] | 7.91 (0.05)[a] | 0.3 (0.98)[a] |
| Sigma 91** | | 2.5 (0.11)[b] | 8.23 (0.09)[a] | 1.5 (0.25)[a] |

* = numbers in parentheses are standard deviations; means with different letters in each column are significantly different ($P < 0.05$); ** Sigma 91 is a commercial crab chitosan.

**Nitrogen content.** Nitrogen content of the shrimp shell chitosan samples varied between 7.91% and 8.33% on a dry basis, showing no significant differences ($P > 0.05$) in nitrogen content, but the values were slightly higher than that (7.06% to 7.97%) reported by No and Meyers (1995), for chitosan from crab and shrimp shell on a dry basis. This is probably due to the presence of protein residues as mentioned by Rutherford and Austin (1978). Protein is bound by covalent bonds forming stable complex with chitin and chitosan. Thus, it is very difficult to achieve 100% deproteinization. Even with complete deprotinization, nitrogen was still present since chitosan has the amino ($-NH_2$) group.

**Ash.** Table 1 shows the ash content of shrimp shell chitosan in the range of 0.29-0.3%. Ash measurement is an indicator of the effectiveness of the demineralization step for removal of calcium carbonate. Elimination of demineralization step results in products having 31-36% ash (Bough *et al.*, 1978). Some residual ash of chitosans may affect their solubility, consequently contributing to lower viscosity, or can affect other more important characteristics of the final product. A high

quality grade of chitosan should have less than 1% of ash content (No and Meyers, 1995). An ash content of less than 1% from crab chitosans has been reported by No and Meyers (1995). The results presented in Table 1 indicate that the resultant chitosan sample was completely demineralized and contained less than 1% ash.

**Degree of deacetylation.** The degree of deacetylation (DD) of the studied shrimp shell chitosan samples ranged from 55% to 76% (Table 2). According to No and Meyers (1995), DD of chitosan ranges from 56% to 99% with an average of 80%. Sample $C_{50S}$ (76%) had the highest DD, followed by $C_{50WS}$, $C_{60}$ and $C_{40}$ (75%, 74%, and 55%, respectively).

As in the Table 2, $C_{40}$ had a very low solubility and viscosity which may be due to the lower DD value. Therefore, comparison among samples $C_{50S}$, $C_{50WS}$ and $C_{60}$, sample $C_{50S}$ gave lower viscosity (136.6 cP) and higher DD (76%) value which are very important characteristics of chitosan. The medical and pharmaceutical applications of chitosan as antitumor, hemostatic, hypocholesterolemic, antimicrobial and antioxidant depends mostly upon DD and solubility (Jian *et al.*, 2008; Muzzarelli and Muzzarelli, 2005). However, we expected that samples $C_{50S}$, $C_{50WS}$ and $C_{60}$ would have higher DD with higher solubility but the values obtained were lower than the expected ones. According to Kassai (2008) and Khan *et al*. (2002) , the IR spectroscopic method is commonly used for the estimation of chitosan DD values for its advantages: it is relatively fast and does not require dissolution of the chitosan sample in an aqueous solvent. DD values are not only highly dependent on the source and method of purification (No *et al.*, 1989) but also on the type of analytical methods employed, sample preparation and type of instrument used; other conditions may also influence the analysis of DD (Kassai 2008; Khan *et al*., 2002).

**Viscosity.** The viscosity of chitosan solutions, reported in the literature, generally ranges from 60 to 780 cP (Alimuniar and Zainuddin, 1992). This range of viscosity was also observed by Cho *et al.* (1998) for five commercially available chitosans. The results of viscosity, solubility and degree of deacetylation of our shrimp shell chitosans are shown in Table 2.

Bough *et al*. (1978) stated that viscosity of chitosans varied considerably from 60 to 5,110 cP depending on the species. Our shrimp shell samples had viscosity ranging from 90.7 to 170.2 cP. $C_{40}$ had the lowest viscosity (90.7 cP) comparable to that of other samples as of lower solubility may be due to incomplete deacetylation of the sample. Whereas $C_{50WS}$ had a very high viscosity (170.2 cP) (Table 2). Some factors affect viscosity during the production of chitosan such as the degree of deacetylation, molecular weight, concentration, ionic strength, pH and temperature, etc. Moorjani *et al*. (1975) reported that viscosity of chitosan decreased with increasing time of demineralization. The viscosity of chitosan in acetic acid tends to increase with decreasing pH but decrease with decreasing pH in HCl. Intrinsic viscosity of chitosan is a function of the degree of ionization as well as ion strength (Bough *et al.*, 1978). Deproteinization with 3% NaOH and elimination of the demineralization step in chitin preparation, decreased the viscosities of the final chitosan samples (Bough *et al*., 1978). Moorjani *et al.* (1975) stated that it is not desirable to bleach the material at any stage since bleaching considerably reduces the viscosity of the final chitosan product. Our product, prepared without bleaching step, gave lower viscosity which was desirable for preservation of foods against microbial deterioration, formation of biodegradable films and medical applications (Liu *et al.*, 2008; Zeng *et al.*, 2008).

**Solubility.** Three shrimp shell chitosan samples demonstrated excellent solubility ranging from 98.01 to 99% with no significant difference (Table 2), except sample $C_{40}$, which showed comparatively lower solubility (60.3%); it may be due to lower degree of deacetylation. Brine and Austin (1981) noted that lower solubility values suggested incomplete removal of protein and acetyl group. Since solubility of chitosan depends on the removal of acetyl group from chitin therefore lower DD value and the presence of protein contaminants remaining in the sample during the analysis process could adversely interfere with the results.

**Water binding capacity (WBC).** Water binding capacity of shrimp shell and commercial chitosans are shown in Table 3. WBC differed among crawfish chitosan samples, ranging from 299.6 % to 745.4%. There were no significant differences in

**Table 2.** Viscosity, solubility and degree of deacetylation of shrimp shell and commercial chitosans

| Sample | Viscosity (cP) | Solubility (%) | Degree of deacetylation (%) |
|---|---|---|---|
| $C_{40}$ | 90.7 (5.07)*[a] | 60.3 (0.61)[a] | 55 |
| $C_{50S}$ | 136.6 (2.09)[b] | 98.2 (0.66)[b] | 76 |
| $C_{50WS}$ | 170.2 (3.66)[c] | 98.01 (0.45)[b] | 75 |
| $C_{60}$ | 154.29 (2.69)[d] | 99.00 (0.56)[b] | 74 |
| Sigma 91** | 380.15 (3.44)[e] | 89.88 (0.42)[c] | 74 |

* = numbers in parentheses indicate standard deviation; means with different letters in each column are significantly different ($P < 0.05$);
** Sigma 91 is a commercial crab chitosan.

**Table 3.** Water binding capacity and fat binding capacity of shrimp shell and commercial chitosans

| Sample | WBC (%) | Fat binding capacity (%) | | |
|---|---|---|---|---|
| | | Soybean oil | Corn oil | Sunflower oil |
| $C_{40}$ | 299.6 (9.97)*[a] | 258.7(8.9)[a] | 245.5 (4.8)[a] | 255.7 (5.3)[a] |
| $C_{50S}$ | 738.8 (5.6)[b] | 587.3 (5.3)[b] | 599.2 (8.5)[b] | 586.8 (9.9)[b] |
| $C_{50WS}$ | 745.4 (4.9)[b] | 571.5 (7.9)[b] | 583.6 (7.3)[b] | 579.4 (5.6)[b] |
| $C_{60}$ | 732.2 (4.04)[b] | 575.8 (6.5)[b] | 577.5 (6.7)[b] | 566.9 (7.6)[b] |
| Sigma 91** | 538.5 (4.99)[c] | 379.7 (5.9)[c] | 444.3 (5.3)[c] | 398.6 (6.6)[c] |

* = numbers in parentheses indicate standard deviation; means with different letters in each column are significantly different ($P < 0.05$); ** = Sigma 91 is a commercial crab chitosan.

WBC between $C_{50S}$, $C_{50WS}$ and $C_{60}$. These values were in agreement, except $C_{40}$, with those reported by Cho *et al*. (1998) where WBC for chitosans ranged from 458% to 805% for five commercial chitosans from shrimp and crab shell. Sample $C_{40}$ had a lower WBC (299.6 %) than that of other samples; it may be due to lower DA value.

**Fat binding capacity (FBC).** Fat binding capacity (FBC) of four shrimp shell chitosans was measured using three types of oils including soybean, corn, and sunflower oil. The results are shown in Table 3. FBC differed among chitosan products, ranging from 245.5% to 599.2%. Among our crawfish chitosan samples, $C_{50S}$ showed the highest FBC values: 587.3% with soybean oil, 599.2% with corn oil and 586.8% with sunflower oil, although $C_{50S}$ had low viscosity (136.6 cP); $C_{50S}$, $C_{50WS}$ and $C_{60}$ showed no significant difference in FBC.

The sample $C_{40}$ showed the lowest FBC (245.5%-258.7%) as it was not properly deacetylated; it seems higher deacetylation facilitates oil binding capacity of chitosan. Several workers suggested that the DD of chitosan is an important factor which influences fat binding capacity of chitosan (Shahidi *et al.*, 2002). They suggested that increased DD causes increased electrostatic force between chitosan and fatty and bile acid and increased FBC. Moorjani *et al.* (1975) advocated that changing the sequence of steps, when demineralization is conducted prior to deproteinization and finally deacetylation, results in an increase in FBC than when deproteinization is conducted prior to demineralization and finally deacetylation. Amongst the three types of oil used, soyabean oil generally demonstrated more FBC with shrimp shell chitosan samples, whereas sunflower oil showed the least FBC. Regardless of the type of vegetable oils, the four prepared shrimp shell chitosan samples showed desirable FBC ranging from 566.9% (with sunflower) to 599.2% (with corn) which is in agreement with those (314 to 535% with an average of 417%) reported by No *et al.* (2000). Sample $C_{40}$ showed lower value than the reported value. It seemed degree of deacetylation influenced the fat binding capacity of chitosan.

## Conclusion

Throughout the literature on chitosan, the main emphasis is on its quality and physicochemical properties which vary widely with the crustacean species and the preparation methods. Most of the reported preparation methods used high temperature with 45% concentrated alkali and sometimes used autoclave. Based on the reported practice this research study attempted to present a process for the production of shrimp shell chitosan at room temperature (25°C) with increased alkali strength (55%); it could help to develop small industry without wasting energy. This study also demonstrated that the duration of deacetylation process affects the quality of the products. In view of the foregoing, it is our recommendation that for the purpose of achieving uniformity and proper product quality control for particular usage of chitosan, the relationship between the process protocols/conditions and the resulting specific characteristics of chitosan products must be monitored constantly and properly.

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