Physicochemical characterization of chitosan extracted from *Metapenaeus stebbingi* shells

Aygul Kucukgulmez^{a,*}, Mehmet Celik^a, Yasemen Yanar^a, Didem Sen^b, Hurriyet Polat^b, A. Eslem Kadak^a

^a Cukurova University, Faculty of Fisheries, Department of Fishing and Fish Processing Technology, Adana, Turkey

^b Izmir Institute of Technology, Faculty of Science, Department of Chemistry, Izmir, Turkey

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ABSTRACT

In this study, chitosan was extracted from *Metapenaeus stebbingi* shells. In order to determine physicochemical characteristics of the extracted chitosan, the yield, moisture and ash contents, degree of deacetylation, molecular weight, water and fat binding capacities, apparent viscosity and colour properties were measured using a variety of techniques including Fourier transform infrared spectroscopy, scanning electron microscopy and X-ray diffraction. In addition, the physicochemical characteristics of the chitosan extracted from *M. stebbingi* shells were compared to commercial chitosan. The degree of deacetylation was calculated by the titration method and elemental analysis. The molecular weight was determined by viscosimetric methods. The results of the study indicate that shrimp shells are a rich source of chitosan as 17.48% of the shell's dry weight is consisted of this material. Extracted chitosan exhibited a lower molecular weight, higher degree of deacetylation, higher viscosity and higher water and fat binding capacities compared to the commercial chitosan.

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1. Introduction

Chitin (β -1,4-poly-*N*-acetyl-D-glucosamine) is the second most common polymer after cellulose in nature, existing in the shells of crustaceans like crab, shrimp and lobster as well as in the exoskeleton of marine zooplankton, the cuticle of insects and the cell walls of fungicide. Chitosan (poly- β -1,4-2-amino-2-deoksi- β -D-glucopiranoz) is derived by deacetylation of chitin (Knorr, 1984; No & Meyers, 1989; Ruiz-Herrera, 1978). Due to its biodegradability, biocompatibility, nontoxic and wound healing properties and haemostatic activity, chitosan has received increased attention as one of the promising renewable polymeric materials for various applications (Knorr, 1984; Yen, Yang, & Mau, 2009). It is commonly used in different sectors including especially food, chemistry, biotechnology, agriculture, veterinary, cosmetic, medicine, dentistry, environmental protection, textile, paper making and packaging (Coward-Kelly, Agbogbo, & Holtzapple, 2006; Duarte de Holanda & Netto, 2006; Healy, Green, & Healy, 2003; Lamarque et al., 2005; Seo, King, & Prinyawiwatkul, 2007; Shahidi & Synowiecki, 1991).

Chitosan is commercially produced from crustaceans in general, and shells of shrimp are quite important amongst them. *Metapenaeus stebbingi*, caught from the Aegean and Mediterranean Sea, is a highly demanded crustacean both in Turkey and abroad. However,

there is no detailed study in Turkey regarding the use of shells of this shrimp species. The waste amount of the shell not used in the factories presents a big potential. This waste is normally likely to affect human health adversely as it could lead to environmental pollution. For this reason, the utilisation of this source would not only be beneficial to the industry, but to the community health as well.

The aim of the present study was to perform a characterization of the chitosan extracted from *M. stebbingi* shells with chemical methods. In the characterization of the chitosan, the yield, moisture and ash contents, degree of deacetylation, molecular weight, water and fat binding capacities, apparent viscosity, colour properties were measured. In addition, chitosan extracted from *M. stebbingi* was compared to commercial chitosan.

2. Materials and methods

2.1. Materials

Shell materials were obtained from the wastes of *M. stebbingi*. Fresh samples of shrimp processing discards, comprising intact cephalothorax and abdominal exoskeleton, were collected from a local shrimp factory. Representative shrimp wastes were selected, packed in plastic bags and stored at -20°C before and during transportation to the laboratory. Shells were completely separated from the shrimp wastes in laboratory, washed in pure water and dried at 60°C .

* Corresponding author. Tel.: +90 322 338 6646; fax: +90 322 338 6439.

E-mail address: akucukgulmez@cu.edu.tr (A. Kucukgulmez).

2.2. Extraction of chitosan

Chitosan was extracted by the method of Chang, Tsai, Lee, and Fu (1997). Deproteinization and demineralization steps were carried out with 2.5 N NaOH at 65 °C for 6 h and 1.7 N HCl at 25 °C for 6 h, respectively. The chitin residue was treated with nine volumes of hydrogen peroxide and dried at 90 °C for 2 h. Chitosan was prepared by alkali treatment of chitin using 50% (w/v) NaOH in distilled water at 120 °C. The reactants were filtered, washed with deionized water to neutral pH and dried at 90 °C for 2 h.

2.3. Characterization of chitosan

2.3.1. Yield, moisture and ash contents

The chitosan yield was calculated by comparing the weight measurements of the raw material to the chitosan obtained after treatment. The moisture was determined after drying samples for 24 h at 105 °C, and the ash content was determined by heating at 530 °C for 20 h.

2.3.2. Determination of the deacetylation degree

2.3.2.1. Potentiometric titration. The degree of deacetylation (DD) of chitosan was determined by a potentiometric titration method. 250 mg portion of chitosan were dissolved in 10 ml of 0.30 M HCl and after being diluted to 50 ml with ultrapure water, it was titrated with 0.10 M NaOH. The consumed volume of NaOH solution, which corresponds to the amount of amine groups in chitosan, is obtained from the difference between two inflection points of acid–base titration (Tolaimate et al. (2000)).

2.3.2.2. Elemental analysis. A LECO CHNS-932 elemental analysis apparatus was used to determine the amount of C and N in chitosan. In this case, samples were heated to a temperature of 1000 °C and approximately 2 mg of chitosan was placed inside a silver capsule and dropped into the CHNS-932 furnace, where it was completely combusted. This instrument relies upon infrared detection to measure the weight percent of carbon, while nitrogen was measured by thermal conductivity detection. The DD of chitosan was determined by the formula below (Kasaai, Arul, & Charlet, 2000):

$$DD = \frac{C/N - 5.145}{6.861 - 5.145} \times 100$$

2.3.3. Molecular weight determination

For the determination of viscosity–average molecular weight, eight chitosan solutions at different concentrations were prepared in 0.2 M NaCl/0.1 M AcOH. The solvent was chosen according to the deacetylation degree results from the study of Wang et al. (2006). The efflux times of the solutions were measured in triplicate using an Ubbelohde capillary viscometer in a constant-temperature water bath at 25 °C. The running times of the solution and solvent were recorded as seconds (s) and used to calculate intrinsic viscosity.

The Mark–Houwink equation $[\eta] = KM^\alpha$ was used to calculate the molecular weight of chitosan. In this equation $[\eta]$ defines the intrinsic viscosity, K and α are constants the values of which depend on the nature of the polymer and the solvent, as well as on the temperature. M is relative molecular weight. Values of K and α were $1.64 \times 10^{-30} \times DD^{14}$ (ml/g) and $1.02 \times 10^{-2} \times DD + 1.82$, respectively, and DD is the degree of deacetylation of chitosan expressed as the percentage (Wang et al., 2006).

2.3.4. Apparent viscosity

A chitosan solution was prepared in 1% (v/v) acetic acid at 1% (w/v) concentration on a moisture-free basis. The chitosan solution was then filtered using filter paper to remove insoluble materials.

The viscosity of chitosan was determined with an AMVn Automated Micro Viscometer (Anton Paar, Graz, Austria). Measurements were made in triplicate at 20, 25, 30, 35 and 40 °C. Values were reported in centipoises units (cP).

2.3.5. Colour measurement

The chitosan sample was put in a transparent petri dish. The colour of chitosan was measured using a Hunter lab (Hunter Associates Laboratory, Inc., Reston, VA, USA), which was standardised with a calibration white plate ($X = 78.83$, $Y = 83.79$, $Z = 88.60$, $L^* = 3.47$, $a^* = -0.12$, $b^* = -0.32$). The results were recorded as L^* , a^* , b^* . Chroma, hue and whiteness values of the sample were calculated based on the following equations (Seo et al., 2007):

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

$$\text{Hue} = \text{Arctan}(b^*/a^*)$$

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

2.3.6. Water and fat binding capacity

Water binding capacity (WBC) and fat binding capacities (FBC) of chitosan were measured using a modified method of Wang and Kinsella (1976). Water and fat absorption were initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding 10 ml of water or soybean oil and mixing 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 every 10 min and centrifuged at 3500 rpm for 25 min. After the supernatant was decanted, the tube was weighed again. WBC and FBC were determined as follows:

$$\text{WBC (\%)} = \text{Water bound/sample weight} \times 100;$$

$$\text{FBC (\%)} = \text{Fat bound/sample weight} \times 100$$

2.3.7. Fourier transform infrared spectroscopy (FTIR)

IR characterization of chitosan was performed with a Nicolet Magna 550 FT-IR instrument. Chitosan samples were prepared in the forms of thin film (Niamsa & Baimark 2009). For the preparation, 2% w/v chitosan was dissolved in 2% acetic acid solution (AcOH), poured on a petri dish, and finally dried at 60 °C for 16 h under vacuum. In this preparation method, the important thing is the equal film thickness for all samples. The spectra of the chitosan samples (in the forms of thin film) were obtained with a frequency range of 4000–400 cm^{-1} at 4 cm^{-1} resolution and 16 scan.

2.3.8. Scanning electron microscopy (SEM)

SEM characterization was carried out using a Philips XL-30S FEG type instruments in a vacuum environment. Prior to analysis, the chitosan was sprinkled onto adhesive Al or C tapes and was supported on metallic discs and coated with Au. Images of the sample surfaces were recorded at different areas and magnifications.

2.3.9. X-ray diffraction (XRD)

X-ray diffraction data were collected on a Philipps X'Pert Pro diffractometer using Cu $K_{\alpha 1}$ radiation ($\lambda = 0.154056$ nm) in 5–45 θ values. Chitosan was prepared by compressing it in the cassette sample holder without any adhesive substances.

2.4. Statistical analysis

Analysis of variance was performed using the SPSS statistical package program (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

The present study represents the first attempt to investigate various physicochemical properties of chitosan extracted from *M. stebbingi* shells. The results of yield, moisture, ash, deacetylation degree, molecular weight, water and fat binding capacities of chitosan extracted from *M. stebbingi* shells and commercial chitosan are shown in Table 1.

The chitosan yield of *M. stebbingi* shells, being about 17.5%, justifies its use as an economic way of producing chitosan on an industrial scale due to the availability of *M. stebbingi* and the low cost of the sources. Similar to the present study, a chitosan yield of 14.6% was reported from the carapax of *Penaeus monodon* (Hongpattarakere & Riyaphan, 2008). When the commercial chitosan was compared to the extracted chitosan, similarities were found in moisture (1.07–1.33%, respectively) and ash contents (0.59–0.61%, respectively) ($p > 0.05$). The results show similarities with the moisture and ash content of chitosan obtained from different sources (*Artemia urmiana*, snow crab processing) (Kamil, Jeon, & Shahidi, 2002; Tajik, Moradi, Rohani, Efrani, & Jalali, 2008).

The deacetylation degree, molecular weight and also the order of its repetitive units are important parameters for chitosan, as they affect its properties (Lamarque et al., 2005). The deacetylation degree of chitosan is important for its use in the industry. From this regard, certain researchers (Li, Dunn, Grandmaison, & Goosen, 1992; Muzzarelli & Rocchetti, 1985) suggested that the term chitosan should be used when the degree of deacetylation is above <70%. In the present study, the deacetylation degrees of the commercial chitosan and the extracted chitosan were determined as 86.92% and 92.19%, respectively, by the elemental analysis and potentiometric titration.

The molecular weight of chitosan is one of the most important properties as it considerably affects the physicochemical and functional properties (Yen et al., 2009). The molecular weight of extracted chitosan (2.20 kDa) was lower than commercial chitosan (3.52 kDa). The difference in the molecular weight is caused by the difference in the deacetylation degree, and the different sources of the chitosan. In addition, several factors in the production of chitosan, such as the high temperature, concentration of alkali, reaction time, previous treatment of the chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress may also influence the molecular weight of chitosan (Guo, Kikuchi, Matahira, Sakai, & Ogawa, 2002; Li et al., 1992; Nemtsev, Gamzazade, Rogozhin, Bykova, & Bykov, 2002; Oh, Kim, Chang, & Kim, 2001).

The water binding (WBC) and fat binding capacities (FBC) of commercial chitosan are lower than the extracted chitosan. Water and fat binding capacities of different commercial chitosans were reported as 458–805% and 314–535%, respectively, by Cho, No, and Meyers (1998). WBC and FBC of six commercial chitosan products observed by No, Lee, and Meyers (2000) were in the range of

Table 1

Physicochemical characteristics of extracted and commercial chitosan (mean $\pm$ standard deviation of triplicate determinations).

	Extracted chitosan	Commercial chitosan
Yields (%)	17.48 $\pm$ 0.64	–
Moisture (%)	1.33 $\pm$ 0.08	1.07 $\pm$ 0.09
Ash (%)	0.61 $\pm$ 0.03	0.59 $\pm$ 0.07
Deacetylation degree (%)	92.19 $\pm$ 2.56	86.92 $\pm$ 1.62 ^b
Molecular weight (kDa)	2.20 $\pm$ 0.03	3.52 $\pm$ 0.02 ^a
Water binding capacity (%)	712.99 $\pm$ 11.98	492.67 $\pm$ 10.14 ^a
Fat binding capacity (%)	531.15 $\pm$ 12.26	383.04 $\pm$ 10.02 ^a

^a Indicates a significant difference between two groups ($p < 0.01$).

^b Indicates a significant difference between two groups ($p < 0.05$).

Table 2

Apparent viscosities (cP) of extracted and commercial chitosan at various temperatures (mean $\pm$ standard deviation of triplicate determinations).

(°C)	Extracted chitosan	Commercial chitosan
20	56.52 $\pm$ 0.14	38.21 $\pm$ 0.03 ^a
25	46.14 $\pm$ 0.07	31.88 $\pm$ 0.00 ^a
30	38.18 $\pm$ 0.02	27.09 $\pm$ 0.01 ^a
35	31.69 $\pm$ 0.06	23.30 $\pm$ 0.00 ^a
40	26.73 $\pm$ 0.01	20.35 $\pm$ 0.00 ^a

^a Indicates a significant difference between two groups ($p < 0.01$).

355–611% and 217–477%, respectively. The WBC (492.67%) and FBC (383.04%) of commercial chitosan in the present study were compatible to those reported by Cho et al. (1998) and No et al. (2000).

Table 2 shows the apparent viscosities of extracted and commercial chitosan at various temperatures. The viscosities of both produced and commercial chitosans decreased with increasing temperature, and there were significant differences between the two groups at all degrees of temperature ($p < 0.05$). In a similar study, Jeon, Kamil, and Shahidi (2002) obtained chitosans with 86.40–91.30% of deacetylation degree and values of 14, 57 and 369 cP for apparent viscosities through the deacetylation of chitin extracted from snow crab (*Chionoecetes opilio*) processing waste. The researchers reported that the chitosan viscosity was closely related to the deacetylation time and that the chitosan reached its highest viscosity with a short deacetylation time.

The colour values of extracted and commercial chitosan are given in Table 3. In colour measurements, chroma, hue and whiteness values were calculated in addition to L^* (lightness), a^* (redness) and b^* (yellowness) values. No significant difference

Table 3

Colour measurement of extracted and commercial chitosan (mean $\pm$ standard deviation of triplicate determinations).

	Extracted chitosan	Commercial chitosan
L^*	82.74 $\pm$ 0.44	83.14 $\pm$ 0.62
a^*	−0.23 $\pm$ 0.11	−0.12 $\pm$ 0.00
b^*	18.49 $\pm$ 0.95	13.58 $\pm$ 0.02 ^a
Chroma	18.49 $\pm$ 0.94	13.58 $\pm$ 0.02 ^a
Hue	−0.51 $\pm$ 1.79	−1.56 $\pm$ 0.00
Whiteness	74.69 $\pm$ 0.80	78.35 $\pm$ 0.48 ^a

^a Indicates a significant difference between two groups ($p < 0.01$).

Fig. 1. FTIR spectra of chitosan extracted from shrimp shells.

Fig. 2. SEM images of chitosan (a) 1000 $\times$, (b) 5000 $\times$, (c) 10,000 $\times$, (d) 15,000 $\times$.

was observed between the L^* , a^* and hue values ($p > 0.05$), while the b^* and chroma values of the chitosan synthesised in the study were found lower compared to commercial chitosan ($p < 0.05$). Similarly, chitosan extracted from crawfish was subjected to ozone application for 20 min and its L^* , a^* , b^* , chroma and hue values were determined as 80.91, -1.82 , 28.86, 25.60 and 94.17, respectively. In addition, the colour of chitosan was reported to be associated with the carotenoid pigment astaxanthin (Seo et al., 2007).

Fourier transform infrared spectroscopy was used to determine the structure of chitosan. The infrared spectrum for extracted chitosan is given in Fig. 1. According to this spectrum, the peak observed around 1555 cm^{-1} is attributed to N–H bending of the secondary amide II bands of $-\text{CONH}-$. However, the amide I band, which is usually observed around 1655 cm^{-1} , cannot be observed. Other bands that are observed in the region of $1380\text{--}1460\text{ cm}^{-1}$, are attributed to the symmetric and asymmetric bending vibrations of the methyl groups. According to the study of Li, Weng, Wu, and Zhou (1998) the peak at 1415 cm^{-1} indicates the C–H bending vibrations of $-\text{CH}_2-$. When the spectra given in Fig. 2 are observed, the small peak around 2900 is ascribed as $-\text{CH}_2-$, $-\text{CH}_3$ groups. The C–O stretching of structure is observed at 1075 cm^{-1} according to the literature. The broad band around 3300 is usually attributed to intermolecular $-\text{H}$ bands. The out plane bending $-\text{OH}$ vibrations are observed at 670 cm^{-1} . Strong amide II bands could not be observed, which is due to the high deacetylation degree of the produced chitosan.

The studies in the literature about FTIR spectroscopy related with chitosan show some characteristic peaks, which are at 2940 cm^{-1} ($-\text{CH}_3$, $-\text{CH}_2$), 1655 cm^{-1} (C=O stretch vibration of secondary amide I band), 1555 cm^{-1} (N–H bending vibration of amide II band), 1570 cm^{-1} (N–H bending vibration of primary amides) and 1070 cm^{-1} (C–O stretching) (Choi, Kim, Pak, Yoo, & Chung, 2007; Lee, Hong, Kajiuchi, & Yang, 2005).

The morphology of chitosan was studied by SEM and the SEM images with different magnifications and different areas of chitosan are shown in Fig. 2. The extracted chitosan was observed to have layers of flakes, and porous could be seen on some areas. In some parts of chitosan, fibril structures can easily be distinguished.

When the magnification became higher in some parts of chitosan, crumbling flakes were observed with fibril structures as in the study of Yen et al. (2009).

The X-ray diffraction pattern for chitosan was given between 5 and 40 of 2θ in Fig. 3. The prepared chitosan has two peaks at $2\theta = 20.2$ and at $2\theta = 22.4$, the latter peak is weaker than the first one, whereas, in the literature, many XRD patterns of chitosan have two characteristic peaks which are usually around $2\theta = 10$ and $2\theta = 20$ (Yen et al., 2009). The reason of having different characteristic peaks might be caused by the source of chitin, as suggested by Yen et al. (2009). The chitosan chains are shown to crystallize in an orthorhombic unit cell by Okuyama, Noguchi, Miyazawa, Yui, and Ogawa (1997).

The crystal size of samples (t) was also calculated by applying of the following Debye Scherrer equation using the XRD data:

$$t = \frac{0.9\lambda}{\beta \cos \theta}$$

Fig. 3. X-ray diffraction patterns of chitosan extracted from shrimp shells.

where 0.9 is the shape factor, λ is the X-ray wavelength, typically 1.542 Å, β is the line broadening at half the maximum intensity in radians, and θ is the Bragg angle, which is the angle of the incident beam and the lattice plane, which comes from the theoretical basis of the X-ray diffraction. The estimation regarding the crystal size of the sample (t) is calculated as 8.157 nm.

4. Conclusions

This study investigated the physicochemical characteristics of chitosan extracted from the shells of *M. stebbingi* waste, which is discarded without being used and causes environmental pollution. The results of the study were also compared with commercial chitosan extracted from crab. Based on the results it is suggested that there is good potential for the production of chitosan in Turkey, from crustacean shells, which constitute a significant amount of waste in nature, which this way could be prevented.

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