



# Physicochemical, Microstructural and Thermal Characterization of Chitosan from Blue Crab Shell Waste and Its Bioactivity Characteristics

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## Abstract

The aim of the present study was to characterize the chitosan synthesized from blue crab (*Callinectes sapidus*, Rathbun, 1986) shell waste and to investigate its physicochemical and microstructural analyses as well as its antioxidant and antibacterial potentials. Microstructural and elemental characterization of the synthesized and commercial chitosan powders were performed using fourier transform infra-red spectroscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy. Thermal analysis were done using thermogravimetric analysis. Antioxidant and antimicrobial analysis were done using by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay and agar well diffusion assay, respectively. The yield of the synthesized chitosan was calculated as 7.47% with moisture and ash contents of  $0.151 \pm 0.0025\%$  and  $0.19 \pm 0.01\%$ , respectively. Synthesized chitosan demonstrated the values of degree of deacetylation (71%), water ( $620.029 \pm 52.59\%$ ) and fat ( $437.93 \pm 15.92\%$ ) binding capacities, solubility ( $94.15 \pm 2.84\%$ ), viscosity ( $463.25 \pm 13.10$  cPs) and whiteness ( $90.23 \pm 0.27\%$ ). Antioxidant activity of synthesized chitosan was found to be moderate ( $55.30 \pm 5.05\%$ ) when compared to ascorbic acid standard ( $87.61 \pm 1.34$ ). Chitosan obtained from blue crab shell also showed moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The findings of the study indicated that blue crab shell waste might be a potentially effective biopolymer source for commercial applications especially in food industry.

**Keywords** Blue crab · Waste · Chitosan · Characterization · Bioactivity

## Introduction

Chitin, is a polysaccharide formed by  $\beta$ -(1-4) *N*-acetyl-D-glucosamine units [1]. It is the second most common biopolymer in the world after cellulose [2] Chitin is the main component of shellfish, such as shrimp, crab and found in the skeleton of insects and also in the structure of cell walls of fungi [3]. Due to its non-toxic, antioxidative, biocompatibility, biodegradability, and renewability properties, it is used in many fields such as food, agriculture, cosmetics, biotechnology and pharmacy. Chitosan is a polysaccharide obtained by deacetylation of chitin which also has many application

areas in different sectors [1]. Commercially, chitin and its derivatives are extracted from shrimp shell, crab shell, crayfish and krill shell [4]. After the 1970s, chitin, chitosan and their derivatives were used in the treatment of water (dye, protein metal ions) and in the food industry (weight control, dietary supplement, antioxidant purpose of coating material). They are also preferred in industrial areas such as paper and textile industry [5]. Crustaceans such as crab, lobster and shrimp consist of 30–40% protein, 30–50% calcium carbonate and 20–30% chitin of their shell. For the production of chitin from the shell wastes, the wastes are treated with alkali and acid to remove protein and mineral substances. Consequently, high quality chitin from shells and also chitosan from this chitin can be obtained with proper treatment methods [6]. The chitin content of blue crab was reported to be 14% [7].

The waste products from blue crabs in large quantities might be an environmental problem which can be solved by proper utilization of the shells. The present study was aimed to produce a value added product from blue crab shell

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waste which is not utilized. For this purpose, chitosan that extracted from blue crab shells were comprehensively characterized and its antioxidant and antibacterial activities were also investigated.

## Experimental

### Materials

Köyceğiz Lagoon, in Muğla/TURKEY, is a geographically important region which is a natural habitat for blue crabs. Blue crabs (100 individuals) obtained from DALKO Cooperative located in Köyceğiz Province were used for chitosan synthesis. Crabs were transferred in ice to the Seafood Quality Control Laboratory approximately in 1 h. All chemicals used for analyses were provided from Merck (Darmstadt, Germany).

### Methods

#### Chitin and Chitosan Extraction

Demineralization, deproteinization, decolorization and deacetylation processes were applied to obtain chitosan. Crabs were gutted and washed under tap water before the extraction process. Shell wastes were dried for a period of 48 h and then were grinded by coffee grinder. Powdered shells were cleaned by washing with distilled water for several times and then dried in the oven at 40 °C for 2 h. For demineralization step of chitin preparation, 40 g of powdered sample was treated with 2 M HCl solution for 24 h at 80 °C to remove minerals. Then, the sample was treated with 2 M NaOH solution at 110 °C for 20 h at a solution ratio of 1:10 (w/v) to remove proteins for deproteinization process. Following the decolorization process with acetone, the precipitate was washed with distilled water for several times, filtered and dried at 40 °C oven then weighed.

Deacetylation process was applied to prepare chitosan from obtained chitin. Chitin was treated with 50% NaOH (w/v) solution at 150 °C for 4 h at a solution ratio of 1:10 (w/v). To obtain chitosan, the precipitate was washed with distilled water for several times until neutral pH and then filtered [8].

#### Yield, Moisture and Ash Contents

Before calculating the chitosan yield, the yield of chitin was found by dividing the weight of the synthesized chitin to the initial shell weight. Then, chitosan yield was calculated by dividing the weight of chitosan to the weight of synthesized chitin before deacetylation [5].

Chitin and chitosan yield were calculated as;

$$\text{Yield of chitin (\%)} = \left[ \frac{\text{Synthesized chitin (g)}}{\text{Crab shells (g)}} \times 100 \right]$$

$$\text{Yield of chitosan (\%)} = \left[ \frac{\text{Synthesized chitosan (g)}}{\text{Chitin (g)}} \times 100 \right]$$

Moisture content was determined by drying 0.1–0.5 g sample at 105 °C with Infrared Moisture Analyzer [9]. Ash content was determined by drying 6 g sample at 550 °C for 6 h [10].

### Color

The color of samples was measured by a lab color meter (Pen Color Art 1L model, Artoksi MSM, Istanbul, Turkey) and was in accordance with the recommendations of the International Commission on Illumination [11]. The measured L\*, a\* and b\* color parameters indicated lightness/brightness, redness/greenness and yellowness/blueness, respectively. The color meter was calibrated with a white standard and the color measurement was repeated three times on different parts of the surface. The whiteness index (WI) value of the synthesized and commercial chitosan samples were calculated based on the following formula [12, 13]:

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

### Solubility

According to the method of Nessa et. al. [14], 0.1 g of chitosan was centrifuged with 10 mL of 1% acetic acid solution at 10,000 rpm for 30 min. Supernatant was discarded and the pellet was washed with 25 mL of distilled water, then was centrifuged at 6000 rpm. Supernatant was discarded, the pellet (undissolved solid) was dried at 60 °C for 24 h in an oven. The amount of the residue was weighted and the percentage of solubility was determined.

### Viscosity

Viscosity of chitosan was determined with a Brookfield viscometer (Model DV-I+Brookfield Engineering Laboratories, Inc., USA.). The 1% solution of chitosan was prepared in 1% acetic acid on a dry basis was used to measure the viscosity. Measurement was made in duplicate using and the viscometer fixed with No. 63 spindle at 60 rpm at 25 °C. The results were reported in centipoises (cPs) units.

### Water Binding Capacity (WBC)

To calculate the water binding capacity; 0.1 g of chitosan and 10 mL of distilled water were vortexed about 5 min. Then, the sample was vortexed again for 30 min for 5 s with 10 min break and centrifuged at 3500 rpm for 30 min with reference [8]. Then calculated as;

$$\text{WBC (\%)} = [\text{Bound water (g)}/\text{Initial chitosan weight (g)} \times 100]$$

### Fat Binding Capacity (FBC)

According to the method of Demir et al. [8], 0.1 g of chitosan and 10 mL of sunflower oil were vortexed about 5 min, then the sample was vortexed again for 30 min for 5 s with 10 min break and centrifuged at 3500 rpm for 30 min. Then calculated as;

$$\text{FBC (\%)} = [\text{Bound oil (g)}/\text{Initial chitosan weight (g)} \times 100]$$

### Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

For examining the SEM images of the synthesized chitosan and commercial chitosan particles, a piece of powder was placed on specimen stub with double-sided adhesive carbon tape. Scanning electron microscopy study was performed on a JSM 7600F Field Emission Scanning Electron Microscope (JEOL, Japan) at an accelerating voltage of 15 kV. Elemental analysis of chitosan samples was also carried out using energy dispersive X-ray spectroscopy (EDS) (Oxford Instruments, UK) combined with SEM.

### FT-IR Spectral Analysis

FT-IR spectrums of synthesized chitosan and commercial chitosan was monitored by FT-IR (Thermo Scientific Nicolet iS10-ATR, USA) at a resolution of  $4 \text{ cm}^{-1}$  in potassium bromide (KBr) pellets and the spectra were recorded in the wavelength interval of 4000 and  $400 \text{ nm}^{-1}$  [14].

Degree of deacetylation (DD) of the prepared chitosan sample was calculated using the formula given below [14, 15]:

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

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

### Thermogravimetric Analysis (TGA)

Thermogravimetric analysis of the synthesized chitosan was performed on a TGA instrument (Perkin Elmer TGA 4000, Perkin Elmer, Waltham, MA). Samples were heated from 30 to  $600 \text{ }^\circ\text{C}$  at a rate of  $10 \text{ }^\circ\text{C min}^{-1}$  under a nitrogen flow rate of  $20 \text{ mL min}^{-1}$ .

### Antioxidant and Antibacterial Activity

The percentage of antioxidant activity (AA%) of synthesized chitosan were assessed by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. [16]. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. The changes in color (from deep violet to light yellow) were read at 517 nm after 100 min of reaction using a UV-Vis spectrophotometer (T80 + Model, PG Instruments, Leicestershire, UK). The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined according to Mensor et al. [17]:

$$\text{AA\%} = 100 - [((\text{Abs sample} - \text{Abs blank})/\text{Abs control}) \times 100]$$

Antibacterial activity of synthesized chitosan obtained from crab shells was compared with commercially available chitosan using agar well diffusion method [18]. For this purpose; a Gram positive strain, *Staphylococcus aureus* ATCC (American Type Culture Collection) 43300 and a Gram negative strain, *Escherichia coli* ATCC 35218 were provided from Ankara Refik Saydam Hifzısıhha Institute, Ankara, Turkey. Microorganisms were cultured in Nutrient Broth (NB) at appropriate temperatures. Inoculums were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland Standard Dilutions. Twenty milliliters of Mueller Hinton Agar (MHA) is sterilized in separated flasks and cooled to  $45\text{--}50 \text{ }^\circ\text{C}$ . After injecting the microorganism cultures to sterile plates ( $1000 \text{ }\mu\text{L}$ ), media was distributed and mixed homogenously. Of the test samples ( $10 \text{ mg/mL}$ ),  $20 \text{ }\mu\text{L}$  were injected to the wells of 6 mm in diameter. After the proper incubation period for each microorganism, antibacterial activity was evaluated by measuring the zone of inhibition against the tested microorganisms. Acetic acid (1.5%) was used as negative control. Measurements were performed in triplicate.

### Results and Discussion

#### Yield, Moisture and Ash Content of Chitin and Chitosan

After in our study, yields of chitin and chitosan were obtained as 10.83% and 7.47%, respectively, which is higher than those reported by Hajji et al. [19] for chitosan extracted

from *Carcinus mediterraneus* shells (5.3%) and by Hamdi et al. [20] for chitosan obtained from the blue crab shells ( $6.94 \pm 0.13\%$ ). There are different chitosan yield amounts in the literature. Baron et al. [21] obtained a yield of 13.7% chitosan using thermoalkaline deacetylation of chitin, extracted from blue crab (*Callinectes sapidus*) waste. Üçgül et al. [6] reported that the percentage of pure chitin by weight of the blue crab was 14% in their study of purification of the chitin from different raw material sources and in textile applications. Özbay et al. [5] in the study of chitin and chitosan efficiency of manta shrimp, cuttlefish and blue crab waste shells, they found chitin and chitosan yields; 14.59% and 12.52%, 2.87% and 1.69%, 10.21% and 7.55%, respectively. Chitosan and chitosan yield of manta shrimp and blue crab waste shells were significantly higher than inner shell of cuttlefish. Nessa et al. [14] produced chitosan using four different acetylation times, 45, 55, 65, 72 h (groups A, B, C, D respectively), from shrimp shell waste. Chitosan yields were obtained 19.6, 16.4, 18.5, 17.7% in groups A, B, C and D, respectively. Küçükgülmez [22] extracted chitin from crayfish (*Astacus leptodactylus*) shell waste, found the deacetylation degree and yield as 25.67% and 26.44%, respectively. Hossain and Iqbal [23] produced chitosan from shrimp shell wastes, found yield of chitosan as 15.4%. Fernandez-Kim [24] reported that during acetylation, the loss of sample mass/weight from excessive removal of acetyl groups from the polymer (i.e. the conversion of chitin to chitosan) affect the chitosan yield.

Moisture contents of chitosan synthesized from blue crab shells and commercial chitosan sample were  $6.97 \pm 0.70\%$  and  $8.24 \pm 0.23\%$ , respectively (Table 1). Low moisture content values of chitosan is reported to enhance the quality and provide better shelf stability [25]. Similar to our study results Parthiban et al. [26] reported that the result of final moisture content of synthesized chitosan from crab shell were 7.62%. Baron et al. [21] calculated the moisture and ash contents of the extracted chitosan from blue crab (*Callinectes sapidus*) waste as  $8.7 \pm 0.4\%$  and  $1.5 \pm 0.1\%$ , respectively. Özbay et al. [5] investigated the chitin and chitosan efficiency of manta shrimp, cuttlefish and blue crab waste shells and found

the moisture content of chitosan as 1.56, 1.51 and 1.49%, respectively. Nessa et al. [14] produced chitosan using four different acetylation times, 45, 55, 65, 72 h (groups A, B, C, D respectively) from shrimp shell waste and found moisture content as; 0.45, 0.34, 0.43, 0.44% respectively. Kumari et al. [4] characterized the chitosan synthesized from fish scales, crab and shrimp shells and obtained moisture content as 0.009, 0.0004, 0.0048% respectively.

Ash content which is an indicator for effective demineralization process affects the solubility of chitosan contributing to low viscosity. As an important parameter of characterization, ash content may also affect the features of the final product [24]. The ash contents of chitosan synthesized from blue crab shells and commercial chitosan were found to be  $0.19 \pm 0.01\%$  and  $0.53 \pm 0.04\%$  (Table 1). On contrary to our results, Baron et al. [21] reported that the ash value of extracted chitosan ( $1.5 \pm 0.1\%$ ) was higher than that of commercial chitosan (0.6%). It was reported that the high amount of ash content might be due to an ineffective demineralization process [21, 27]. No and Meyers [28] suggested that the ash content of high quality chitosan should be  $< 1\%$ . Sarbon et al. [13] found that ash content of extracted chitosan from mud crab shells ( $5.97 \pm 0.90\%$ ) was lower than that of commercial chitosan ( $7.55 \pm 0.05\%$ ). On the other hand, Kucukgulmez et al. [22] indicated that the snow crab possessed lower ash content (0.59–0.61%). According to Kumari et al. [4], crab chitosan had more ash content compared to the fish and shrimp chitosan.

## Color

The whiteness characteristic of chitosan powder is quite important for commercial production and customer satisfaction [29]. The whiteness indexes of synthesized chitosan and commercial chitosan were  $90.23 \pm 0.27$  and  $97.05 \pm 0.39$ , respectively (Table 1). The whiteness value of synthesized chitosan was similar to the commercial chitosan. Similarly, Nouri et al. [29] found that whiteness index (WI) of chitosan samples from extracted shrimp shells were between 93 and 95%, approximately. Povitas and Laokuldilok [30] reported

**Table 1** The moisture and ash content, yield, viscosity, solubility, whiteness index, water binding capacity (WBC) and fat binding capacity (FBC) of synthesized and commercial chitosan from crab shells

Sample	Commercial chitosan	Synthesized chitosan
Moisture	$8.24 \pm 0.23\%$	$6.97 \pm 0.70\%$
Ash	$0.53 \pm 0.04\%$	$0.19 \pm 0.01\%$
Yield	*	7.47%
Viscosity	$440 \pm 13.10$ cPs	$362 \pm 6.27$ cPs
Solubility	$98.37 \pm 1.42\%$	$94.15 \pm 2.84\%$
Whiteness Index (WI)	$97.05 \pm 0.39$	$90.23 \pm 0.27$
Water binding capacity (WBC)	$803.41 \pm 26.45\%$	$620.29 \pm 52.59\%$
Fat binding capacity (FBC)	$484.42 \pm 13.39\%$	$437.93 \pm 15.92\%$

\*As it is a commercial product, the yield cannot be calculated

that the whiteness of chitosan (77–79) was significantly higher than chitin (72.82). In addition, the color of chitosan was reported to be associated with the carotenoid pigment astaxanthin [12].

### Viscosity

The viscosity of the synthesized chitosan obtained from blue crab shells and commercial chitosan were  $362 \pm 6.27$  cPs and  $440 \pm 13.10$  cPs, respectively (Table 1). Deproteinization process that is applied to remove the protein of the chitin cause higher viscosity values for chitosan. This process is directly related to the reagent and the shell amount. Other parameters such as pH, temperature, ionic strength, molecular weight, deacetylation degree and the extraction process also effect the viscosity of chitosan [13].

### Solubility

At least 85% deacetylation degree of chitosan shows high solubility in acetic acid [23]. Chitosan with high solubility is an important feature especially for medicine and food applications. In our study solubility of the synthesized chitosan from blue crab shells and commercial chitosan was found as 94.15% and 98.37%, respectively (Table 1). Demir et al. [8] characterized the chitin and chitosan from blue crab and reported the chitosan solubility as  $99.29\% \pm 0.001$ . Kumari et al. [4] reported that moisture contents of chitosan synthesized from fish scales, crab and shrimp shells was as 78, 70, 60% respectively. Nessa et al. [14] synthesized chitosan using four different acetylation times; 45, 55, 65, 72 h (groups A, B, C, D respectively) from shrimp shell wastes and found the solubilities of A, B, C, D groups of chitosan as; 44.3, 96.01, 97.2 and 97.06%, respectively. Hossain and Iqbal [23] produced chitosan from shrimp shell wastes and obtained the deacetylation degree and the solubility as 81.24% and 97.65%, respectively. Solubility also depends on the operating temperature [4] and the removal of acetyl group [28] in the deacetylation process. As a result, the solubility values appear to be correlated with other studies.

### Water (WBC) and Fat Binding Capacity (FBC)

In our study, water binding capacities of the synthesized chitosan from blue crab shells and commercial chitosan were obtained as 620.29% and 803.41%, respectively (Table 1). In a study of Kumari et al. [4] who synthesized chitosan from fish scales, crab and shrimp shells, reported the water binding capacities of extracted chitosan as 492, 358, 138%, respectively. Demir et al. [8] extracted chitin and chitosan from blue crab and reported that the water binding capacity of chitosan as  $582.59\% \pm 58.67$ . Nessa et al. [14] synthesized chitosan using four different acetylation times, 45, 55, 65,

72 h (groups A, B, C, D respectively) from shrimp shell wastes and reported the water binding capacities as 345.6, 741.2, 748.4 and 738.3%, respectively. Hossain and Iqbal [23] produced chitosan from shrimp shell wastes and found the water binding capacity as 537.29%.

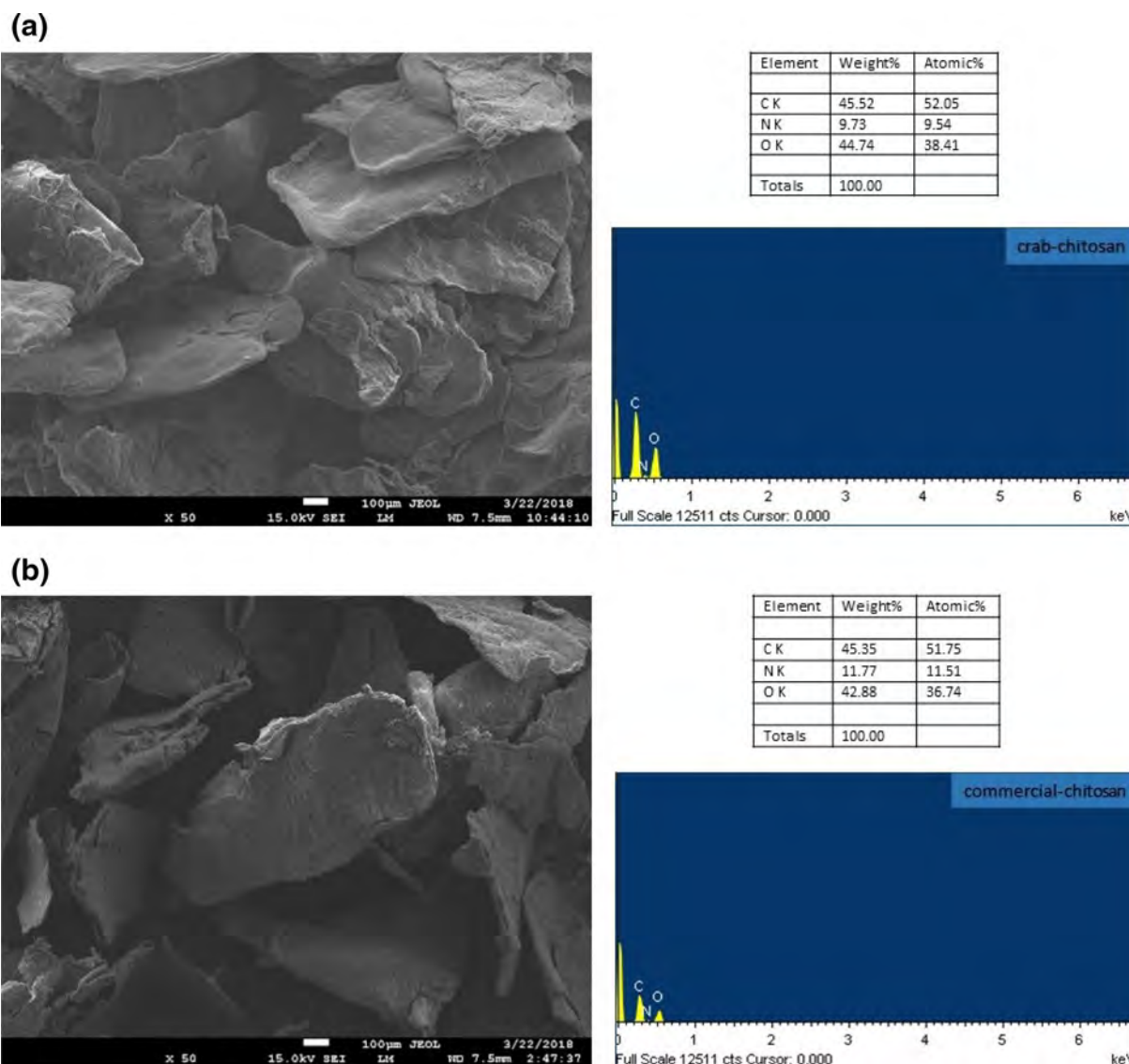
In our present study, fat binding capacity of the synthesized chitosan from blue crab shells and commercial chitosan was obtained as 437.93% and 484.42%, respectively (Table 1). Demir et al. [8] studied the properties of chitin and chitosan from blue crab and found the fat binding capacity as;  $372.21\% \pm 9.29$ . Nessa et al. [14] synthesized chitosan using four different acetylation times, 45, 55, 65, 72 h (groups A, B, C, D respectively) from shrimp shell wastes and reported the fat binding capacities (with sunflower oil) as 588.8, 579.9, 568.9%, respectively. Hossain and Iqbal [23] produced chitosan from shrimp shell wastes and found the fat binding capacity of chitosan as 427.98%. Kumari et al. [4] characterized the chitosan synthesized from fish scales, crab and shrimp shells and obtained fat binding capacities as 226, 246, 104%, respectively.

Depending on the source and preparation procedure, commercial chitosan usually has a deacetylation degree varying from 70 to 95%, WBC, 458 to 805% and FBC, 314 to 535% [24]. Similar to Nouri et al. [29], the results of our study were in the range of those values.

### Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

SEM images and EDS results of the synthesized and commercial chitosan samples are given in Fig. 1. SEM images displayed that chitosan particles are well-dispersed and have flake-shaped morphology (Fig. 1a). Similar to our results, Yen et al. [31] reported that commercial chitosan samples showed layers of crumbling flakes. In this study, prepared chitosan powder has almost same regular microstructural conformation with the commercial chitosan powder. In a study of Kucukgulmez et al. [22], SEM images of chitosan extracted from *Metapenaeus stebbingi* shells exhibited porous morphology on some areas and layers of flakes had been observed which is similar to the present study.

Chitosan is a linear amino polysaccharide with too much nitrogen content [32]. EDS spectrums of the both chitosan samples show that elemental analysis results show great similarity (Fig. 1a, b). Carbon, nitrogen and oxygen peaks were found to be almost same with the commercial chitosan sample which figures out the purity of the prepared chitosan powder. C/N ratios were calculated according to the carbon and nitrogen values of chitosan derived from blue crab shells were found to be 5.45% and 4.5% respectively. Similar to our study, Kumari et al. [4] found that C/N ratio of crab chitosan as 6.2%.



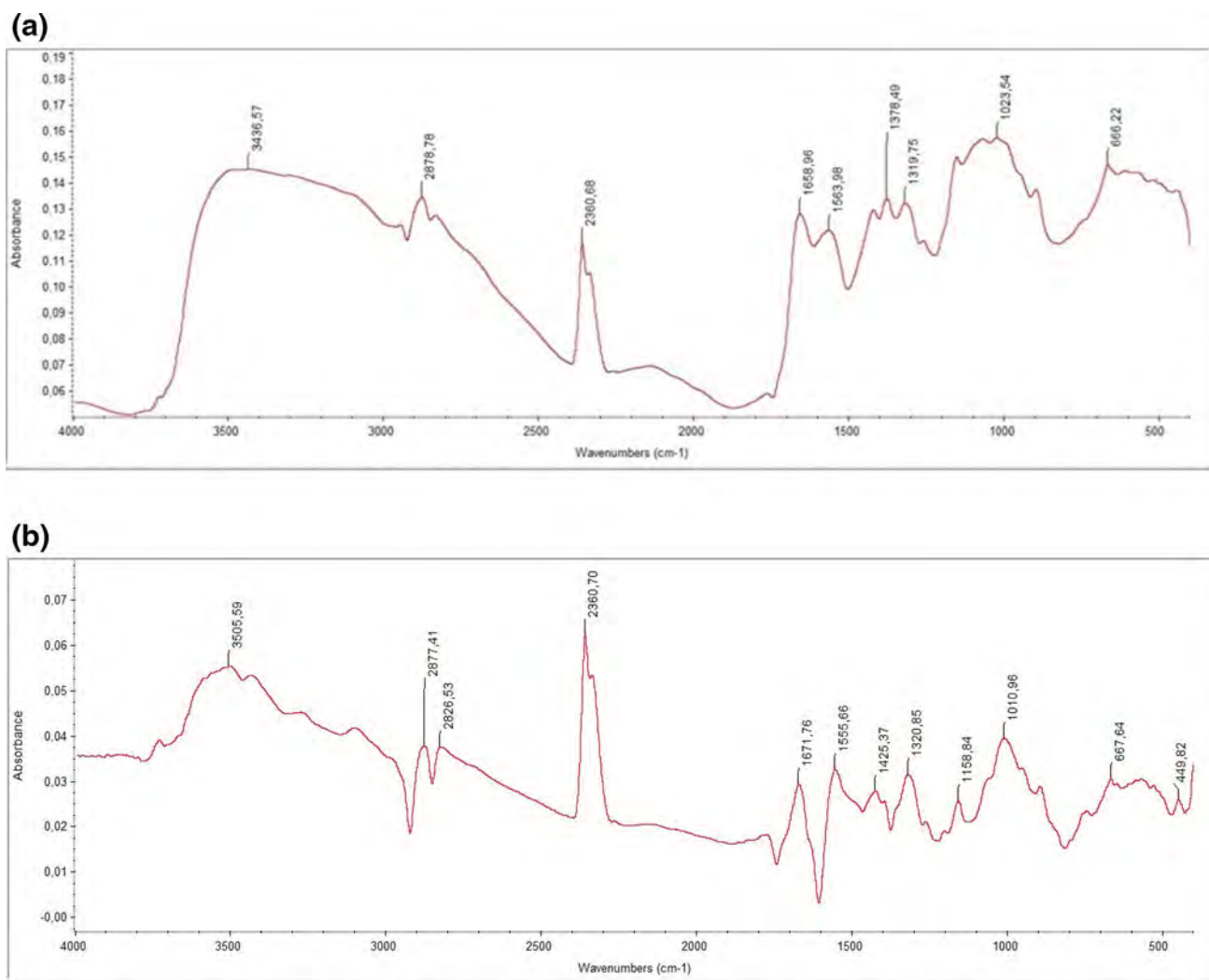
**Fig. 1** SEM image and EDS spectrum of; crab chitosan **(a)** commercial chitosan **(b)**

## FTIR Analysis

Synthesized chitosan sample was analyzed by using FTIR and compared with that of commercial chitosan. It was found that chitosan synthesized from crab spectrum is very similar to that of commercial chitosan. The matching value of the commercial chitosan and crab chitosan was found to be 73.54% (Fig. 2). FTIR spectra of commercial chitosan sample exhibited different bands at  $3443\text{ cm}^{-1}$ ,  $2878\text{ cm}^{-1}$ ,  $2360\text{ cm}^{-1}$ ,  $1658\text{ cm}^{-1}$ ,  $1563\text{ cm}^{-1}$ ,  $1319\text{ cm}^{-1}$  and  $1023\text{ cm}^{-1}$ . The spectra of prepared crab chitosan showed FTIR bands at  $3505\text{ cm}^{-1}$ ,  $2877\text{ cm}^{-1}$ ,  $2360\text{ cm}^{-1}$ ,  $1671\text{ cm}^{-1}$ ,  $1555\text{ cm}^{-1}$ ,  $1425\text{ cm}^{-1}$ ,  $1320\text{ cm}^{-1}$ ,  $1158\text{ cm}^{-1}$  and  $1010\text{ cm}^{-1}$ . FTIR results of the extracted

crab chitosan revealed out that the functional groups are similar to that of commercial sample.

Kumari et al. [4] who synthesized crab chitosan expressed that the peak  $1660\text{ cm}^{-1}$  was reduced due to deacetylation and two peaks at  $1624\text{ cm}^{-1}$  and  $1403\text{ cm}^{-1}$  were seen due to the formation of  $\alpha$ -chitosan. In the same study, it was reported that FTIR peaks for shrimp-chitosan observed  $3441\text{ cm}^{-1}$ ,  $2918\text{ cm}^{-1}$ ,  $1603\text{ cm}^{-1}$ ,  $1624\text{ cm}^{-1}$ ,  $1593\text{ cm}^{-1}$ ,  $1420\text{ cm}^{-1}$ ,  $1362\text{ cm}^{-1}$  and  $1010\text{ cm}^{-1}$  were representing for the angular deformation of OH present in structure of chitosan,  $-\text{CH}$  stretching, vibration modes of amide I,  $-\text{NH}_2$  bending vibration in amino group, vibration of OH, CH in the ring,  $-\text{NH}$  primary, secondary and tertiary bonds, and C–O stretching in acetamide, respectively. Sarbon et al.



**Fig. 2** FTIR spectrum results of commercial chitosan (a) and chitosan synthesized from crab (b)

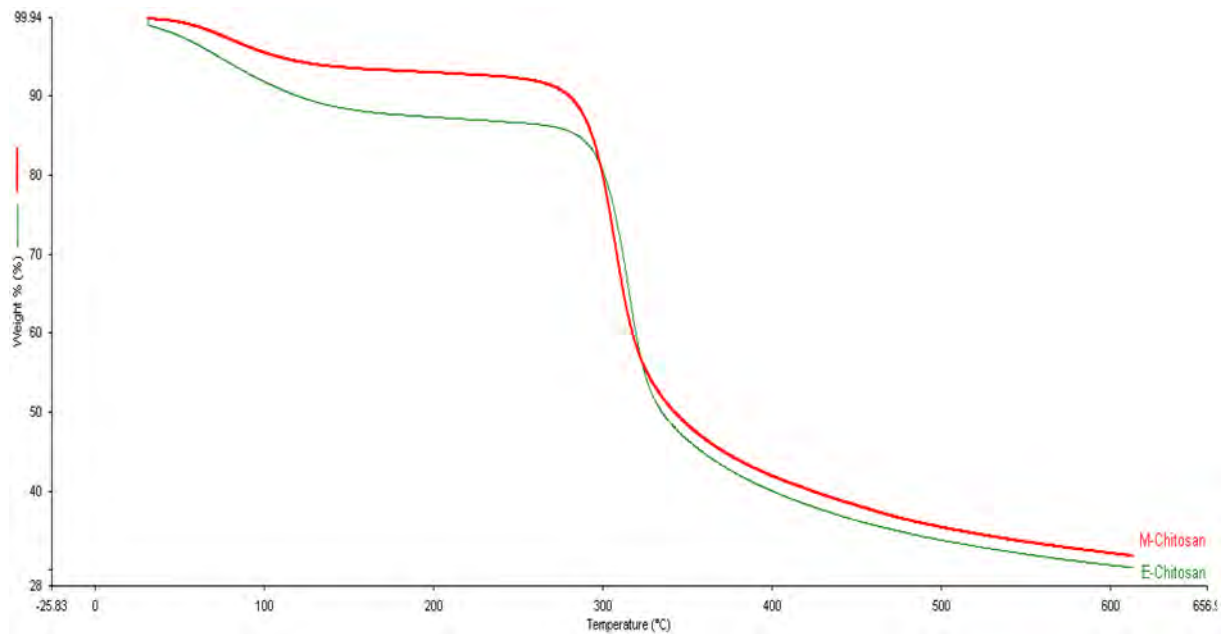
[13] reported similar IR absorption frequencies for commercial chitosan and extracted chitosan from mud crab. They indicated that depending on the deacetylation process of chitosan, different deacetylation degrees showed different peaks in the FTIR spectra due to the absorption band of the N–H group and O–H group at different IR. The FTIR bands observed in the present study are in close match with the literature [4, 33, 34]

Degree of deacetylation of chitosan is vital because, it influences the physical, chemical and biological properties of chitosan. The degree of deacetylation determines mainly the content of free amino groups in the polysaccharide [35]. The degree of deacetylation of chitosan depends on the crustacean species and the preparation methods and ranges from 56 to 99% with an average of 80% [36]. In our study, the degree of deacetylation calculated by FTIR method of chitosan from blue crab shells was 71%. Similar to our study results, the degree of deacetylation of chitosan derived from

crab are calculated by FTIR method and the values found to be 70% [4]. Knaul et al. [37] stated that chitin with degree of deacetylation of 75% or above is known as chitosan. The pharmaceutical potential of chitosan such as antimicrobial, antioxidant, antitumor, hemostatic and hypocholesterolemic activities is reported to be directly related to its solubility and deacetylation degree [38, 39].

### TGA

TGA analysis of commercial chitosan and chitosan synthesized from blue crab shell is shown in Fig. 3. Two stages of decomposition were observed for both chitosan samples. Similar to the degradation process which starts in the range of 50–100 °C is recognized by evaporation of water molecules. This was followed by the second stage of decomposition between 300 and 500 °C resulting in the degradation of saccharide structure of the molecules [4]. It was observed



**Fig. 3** TGA results of the commercial chitosan (red) and synthesized chitosan (green) (Color figure online)

**Table 2** Antioxidant and antibacterial activity of synthesized chitosan from blue crab shells

Sample	DPPH radical activity (%)	Zone of Inhibition (mm $\pm$ SD)	
		<i>E. coli</i>	<i>S. aureus</i>
Synthesized chitosan	55.30 $\pm$ 5.05	13.5 $\pm$ 0.71	12.5 $\pm$ 0.71
Commercial chitosan	55.56 $\pm$ 2.89	14.0 $\pm$ 0.00	13.0 $\pm$ 0.00

that commercial chitosan shows very similar degradation to crab chitosan. TGA analysis results revealed out that chitosan extracted from blue crab have lower thermal stability. Andrade et al. [40] also reported that the decomposition occurred in the ranges of 50–100 °C and 400–500 °C for shrimp (*Litopenaeus vanammei*) and crab (*Ucides cordatus*) chitosan. Kaya et al. [41] suggested that the greatest decomposition was observed at 389 °C for chitin and 295 °C for chitosan from Bat guano (*Rhinolophus hipposideros*).

### Antioxidant and Antibacterial Activity

The scavenging ability of synthesized chitosan on DPPH radical was calculated as 55.30  $\pm$  5.05% at 10 mg/mL concentration. The results of DPPH radical activity for commercial chitosan were similar to those of synthesized chitosan from blue crab shells (Table 2). At the same concentration, Sarbon et al. [13] reported 30  $\pm$  0.001% DPPH scavenging activity for chitosan extracted from mud crab (*Scylla olivacea*) and indicated that the effective concentration for

scavenging activity on the DPPH increases with a decrease in the concentration. For chitosan synthesized from the pen of *Doryteuthis singhalensis*, Ramasamy et al. [42] found the scavenging activity of DPPH radicals was 49.98% at 10 mg/mL. They concluded that the scavenging ability of chitosan might be reduced after sulfation or might be enhanced after *N*-alkylation of the disaccharide. Our results demonstrated that synthesized chitosan from blue crab shells has moderate free radical scavenging activity.

The antibacterial activity of chitosan synthesized from blue crab shells was evaluated against two common pathogenic bacteria; a Gram negative strain *E. coli* and a Gram positive strain *S. aureus* (Table 2). Similar to commercial chitosan, chitosan synthesized from blue crab shell inhibited effectively the growth of *E. coli* and *S. aureus* as evidenced by the inhibition zone of 13.5 mm and 12.5 mm, respectively. There are different environmental (pH, microorganism species) and structural (molecular weight of chitosan, degree of deacetylation, its derivative form, its concentration and original source.) factors that affect the antimicrobial activity of chitosan [43]. In a report of Kaya et al. [41] who used disc diffusion method for testing the antimicrobial activity of  $\alpha$ -chitosan obtained from blue crab (*Callinectes sapidus*), the inhibition zone diameters varied between 15.28 and 20.21 mm for human bacterial pathogens, between 15.51 and 16.25 mm for fungal pathogens and between 14.22 and 15.75 mm for fish bacterial pathogens. Hajji et al. [44] who investigated the antimicrobial properties of chitosan extracted from cuttlefish (*Sepia officinalis*) bones, crab (*Carcinus mediterraneus*) shells and shrimp



(*Penaeus kerathurus*) waste, reported the inhibition zones of chitosan as  $17 \pm 0.05$ ,  $14 \pm 0.3$  and  $10 \pm 0.8$  against *E. coli*;  $14 \pm 0.5$ ,  $12 \pm 0.0$  and  $9 \pm 0.5$  against *S. aureus*, respectively. It was suggested that the antimicrobial potential of chitosan can be related to its metal binding capacity that inhibits the enzymatic activity of cells which cause microbial death [45]. According to Zheng and Zhu [46], the growth of *S. aureus* was inhibited as the molecular weights of chitosan increased whereas the growth of *E. coli* was suppressed as the molecular weight decreased.

## Conclusion

Blue crab (*C. sapidus*) is a widely distributed species throughout the world and can be commercially cultivated. The shell of this seafood should be evaluated as an alternative biological source of chitosan, as it is a waste product after consumption of the blue crab as a food source. The results of the present study revealed out that synthesized chitosan can be a good alternative to the synthetic antioxidants. The chitosan from blue crab shell waste showed antimicrobial activity against common pathogenic microorganisms tested in the present study. It can be suggested that the characterized chitosan might be served as a natural alternative to commercial chitosan and to the synthetic antioxidants/antimicrobials.

## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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