Environmentally hazard and toxic exoskeletons of marine crustaceans wastes in synthesizing *N*-deacetylated chitin

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Abstract: High molecular weight chitosan was synthesized from shrimp shells. Chemical structure of chitosan was confirmed using FT-IR, XRD, TGA, and SEM analyses. Physicochemical properties of chitosan such as molecular weight (800 000 g/mol), deacetylation degree (80%), ash content (1%) indicating effectiveness of demineralization step in removing minerals, water binding capacity (4220%), and fat binding capacity (537%), were determined. FT-IR spectra exhibited characteristic joint band at 3365 cm^{-1} due to –OH and $–NH_2$ regarding to the conversion of chitin acetyl group to NH_2 of chitosan.

Keywords: chitosan, shrimp shells, scanning electron microscopy, thermal gravimetric analysis, *X*-ray diffraction.

Zastosowanie niebezpiecznych i toksycznych dla środowiska egzoszkieletów skorupiaków morskich w syntezie *N*-deacetylowanej chityny

Streszczenie: Z muszli krewetek zsyntetyzowano chitozan o wysokiej masie cząsteczkowej. Strukturę chemiczną chitozanu potwierdzono metodami FT-IR, XRD, TGA i SEM. Określono właściwości fizykochemiczne chitozanu takie jak masa cząsteczkowa (800 000 g/mol), stopień deacetylacji (80%), wskazującą na skuteczność procesu demineralizacji zawartość popiołów (1%), zdolność wiązania wody (4220%) i zdolność wiązania tłuszczu (537%). Widma FT-IR wykazały charakterystyczne pasmo przy liczbie falowej 3365 cm^{-1}, pochodzące od grup –OH i $–NH_2$, wynikające z przekształcenia grupy acetylowej chityny w NH_2 chitozanu.

Słowa kluczowe: chitozan, skorupki krewetek, skaningowa mikroskopia elektronowa, analiza termograwimetryczna, dyfrakcja rentgenowska.

One of the greatest problems that the world is facing today is that of environmental pollution, increasing with every passing year and causing serious and irreparable damage to the earth. Environmental pollution consists of five basic types of pollution, namely: air, water, soil, noise, and light. There has been a considerable progress in recent years in transforming the marine crustacean's wastes into economically valuable products. Shrimp exoskeleton (shells) and cephathoraxes consist of about 30–40% of raw shrimp weight and are discarded as waste being a major portion of the processed seafood industry. Shrimp shells are non-biodegradable and take up a big portion of physical space as well as create pollution [1]. However, the potential for shrimp shells to be chemically modified into chitosan has become more and more interesting issue in recent years [2–4]. Biopolymers are easily available from its natural sources by extraction. These natural sources are renewable due to their ability to be replenished through plantations or growth of animals. Typically, they are categorized by their biological compositions into a few major groups including: polysaccharides, proteins, lipids, polyphenols, and others [5]. Among them, polysaccharides, like cellulose, chitin, and chitosan, are commonly used. Chitin and chitosan are the second most available biopolymers after cellulose. They are received mostly from exoskeleton of crustaceans such as shrimps, but are also available in fungi and some insect's wings [1]. In some studies, chitosan was reported to be obtained from squilla (mantis shrimp), crab [6, 7], and silkworm chrysalides [8]. The long-term objective of this study is the preparation of high molecular weight chitosan from exoskeleton of fishery waste materials such as shrimp shells, hazardous and toxic for environment, by extraction of chitin from shrimp shells, which is expected to undergo deacetylation reaction yielded 80% chitosan. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor [9].

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EXPERIMENTAL PART

Materials and methods

Shrimp shells wastes were obtained from fish market. X-ray diffraction (XRD) was employed for the crystallinity tests of the polymer. X-ray diffraction was performed using a Philips model PW 3710 (Philips, USA) diffractometer with CuKα radiation (λ = 0.1542 nm) source closed in sealed tube, operating at 40 kV and 30 mA. The diffraction patterns were obtained for 2θ degree from 10 to 80 and scanning rate of 1 min^{-1}. The basal spacing of the silicate layer, d, was calculated using Bragg's equation $k = 2d \cdot \sin\theta$, where θ is the diffraction position and k is the wavelength. The scan speed 2θ/s of 0.040 was used. Scanning electron microscopy (SEM) was performed using Philips apparatus, model XL 30C (USA). The films were cut and mounted on a brass stubs with double-sided adhesive tape and were coated with 50 Å layer of gold using SCD-040 Balzers sputter. The specimen were finally characterized by SEM at accelerating voltage of 5, 15, and 20 kV, and a different magnification of the original specimen size. The materials used in this research were purchased from Alfa company (El-Marwa Tower, 112 Emtedad Waley El-Ahd, Hadayek El-Kobba).

Extraction of chitin from shrimp shell

Shrimp shells were dried in sun for two days and when became crispy, they were grounded into powder (Fig. 1).

Fig. 1. Grounded shrimp shells

Shrimp shells samples were immersed in 8% HCl for 24 h with the solid to solvent ratio of 1:6, then they were rinsed with water several times to remove $CaCO_3$ and retreated again with 20% HCl, which showed no bubble generation.

The samples were heated under reflux in 7% NaOH for 48 h at 60–75°C at a solid to solvent ratio of 1:10% w/v.

Fig. 2. Decolorization of chitin sample

The residue was washed with distilled water to remove NaOH and dried giving chitin.

A suitable amount of acetone was added to chitin residue under reflux conditions and kept for 2 h. Afterwards, the colored solution was separated from the sample by filtering and washed several times with acetone (Fig. 2).

Chitin (10 g) was put into 70, 80, and 90% NaOH solutions at 60°C for 2, 5, and 10 h, respectively, filtered, and the residue was washed with water (deacetylation from chitin to chitosan).

Determination of chitin to chitosan deacetylation degree

The degree of deacetylation was measured using the modified acid-base titration method. Briefly, chitosan (0.1 g) was dissolved in 30 ml HCl at room temperature with 5–6 drops of methyl orange added. The red solution was titrated with 0.1 mol/l NaOH solution until it turned orange. The deacetylation degree (*DD*) was calculated using the following formula (1):

$$DD = \frac{C_1V_1 - C_2V_2}{M \cdot 0.09914} \cdot 0.016 \qquad (1)$$

where:

C_1 – concentration of standard HCl aqueous solution (mol/l),

C_2 – concentration of standard NaOH solution (mol/l),

V_1 – volume of standard HCl aqueous solution used to dissolve chitosan (ml),

V_2 – volume of standard NaOH solution consumed during titration (ml),

M – weight of chitosan (g),

The number 0.016 (g) is the equivalent weight of NH_2 group in 1 ml of standard 1 mol/l HCl aqueous solution and 0.09914 is the proportion of NH_2 group by weight in chitosan.

$$DD = \frac{0.1 \cdot 1.30 - 0.1 \cdot 20.5}{0.1 \cdot 0.09914} \cdot 0.016 = 80\% \qquad (2)$$

Preparation of chitosan transparent thin film

1 g of chitosan powder was dissolved in 1% acetic acid and stirred for 30 min. Afterwards the unreacted material was removed and the filtered solution was casted on 7 cm petri dish. The solution was allowed to dry for 5 days forming the separated thin film.

Determination of water and fat binding capacity

1 g of sample was put into a centrifuge tube and 20 ml of water or soybean oil was added and the solution was mixed on a vortex mixer for 2 min to disperse the sample. The content was left at ambient temperature for 45 min with shaking for 5 s every 15 min and after that it was centrifuged at 3200 rpm for 25 min. The supernatant was decanted and the tube was weighted again. Water bind-

ing capacity (*WBC*) and fat binding capacity (*FBC*) were calculated as follows [10] (Formula (3) and (4)):

$$WBC = \frac{\text{water bound (g)}}{\text{sample weight (g)}} \cdot 100 \quad (3)$$

$$FBC = \frac{\text{fat bound (g)}}{\text{sample weight (g)}} \cdot 100 \quad (4)$$

Calculation of ash content of chitosan

$$\text{Ash \%} = \frac{\text{weigh of residue (g)}}{\text{sample weigh (g)}} \cdot 100 \quad (5)$$

1 g of chitosan sample was placed into ignited, cooled, and tarred crucible. After that the sample was heated in a muffle furnace preheated to 650°C for 4 h [11, 12].

Determination of moisture content by gravimetric method

The water mass was determined by drying the sample to constant weight and measuring the sample before and after drying.

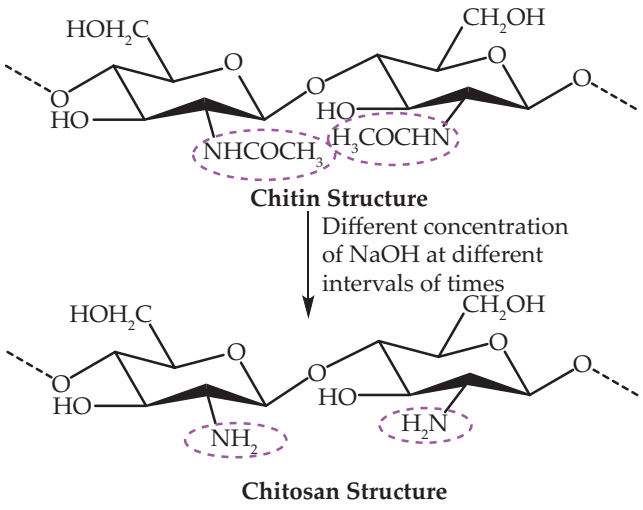
$$\text{Moisture \%} = \frac{\text{wet weight (g)} - \text{dry weight (g)}}{\text{wet weight (g)}} \cdot 100 \quad (6)$$

RESULTS AND DISCUSSION

Chitin is a long chain polymer of *N*-acetylglucosamine, mainly found in cell wall of fungi, exoskeletons of arthropods like crustaceans and insects, radula of mollusks, and beaks and internal shells of cephalopods such as octopuses and squid. Chitin has many derivatives, of which chitosan plays a main role in food packaging and other industrial applications.

Chitosan and chitin, the naturally occurring and renewable polymers, are biocompatible, non-toxic, biodegradable, and adsorptive [13]. Owing their film-forming properties, chitin [14] and chitosan [15] can be successfully used as food wraps [16], however, among them both, chitosan is widely used in the edible film industry. Because of its ability to produce semi-permeable film, chitosan coating can be expected to modify the internal atmosphere as well as decrease the transpiration loss [17–22].

Chitin was extracted from marine crustaceans wastes, such as shrimp shells, dried in sun light for two days until they became crispy and grinded by mortar pestle. The shrimp powder was treated by 8% HCl to remove $CaCO_3$, and the resulted sample was refluxed in 7% NaOH until deproteinization occurred. The dye coating of the sample was removed by refluxing in acetone for 2 h. The conversion of chitin to chitosan (80%) by *N*-deacetylation was carried out using acid-base titration (Scheme 1). It is believed, that by using the FT-IR spectrum strategy, it is possible to differ between chitin and chitosan. The FT-IR (Fig. 3) showed the absence of acetyl group and presence of amino group at ν = 3365 cm^{-1}. Chitosan was purified using 2% acetic acid and re-precipitated with 20% of NaOH solution. Conversion of surface morphology of chitosan was enhanced by preparation of smooth chitosan thin film by dissolving chitosan powder in weak acidic solution of 1% acetic acid, stirring for 30 min, and casting samples in petri dish. The scanning electron microscopy (SEM) was used for analyses at selected point locations on the sample and could be helpful in qualitative analysis of chemical compositions using EDS function. Data were collected over a selected area of the sample surface. Normally ranging from approximately 5 cm to 20 microns in width, the area of observation can be imaged in a scanning mode in magnification ranging from 20× to approximately 30 000× and with spatial resolution of 10 to 200 μm. The SEM of shrimp shells (Fig. 3a) and chitin (Fig. 3b) confirmed the microfibrillar crystalline structure. Crude chitosan reflected a lamellar organization with a dark contrast of straps and shrinkage on surface. Subsequently, purified chitosan revealed two types of alpha and gamma chitosan at different magnification range and accelerating voltage (5 and 20 kV). Also, chitosan film exhibited a clearer and smoother surface morphology and some slight small particles laying on a smooth surface (Fig. 4). For practical applications, further studies on measuring would be essential. Physicochemical properties of chitosan, like solubility, deacetylation degree, ash content, moisture, molecular weight, fat binding capacity *FBC*, and water binding capacity *WBC*, were investigated and listed in Table 1.



Scheme 1. Conversion of chitin to chitosan

The X-ray diffraction (XRD) investigation indicated characteristic crystalline peaks, which correspond with two kinds of chitosan, alpha and gamma, revealing a comparable degree of crystallinity (Fig. 5). A high molecular weight chitosan structure was also assigned by infrared spectroscopy (FT-IR), which showed a characteristic joint

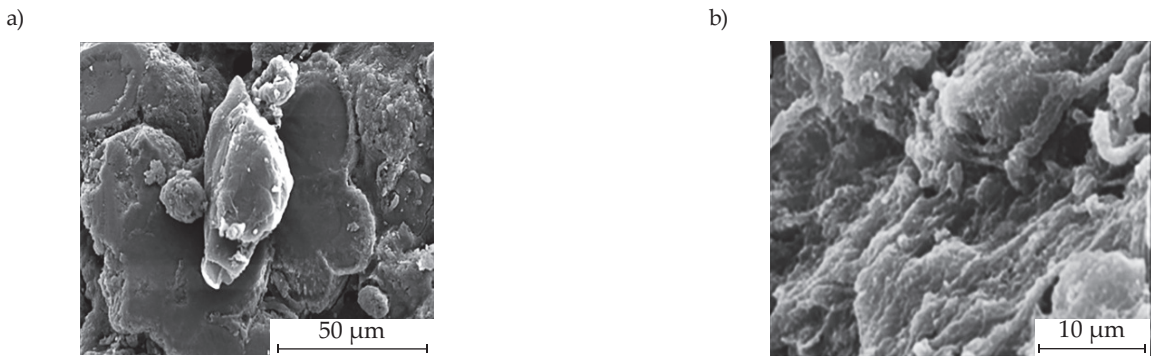


Fig. 3. SEM of: a) shrimp shell powder, b) chitin

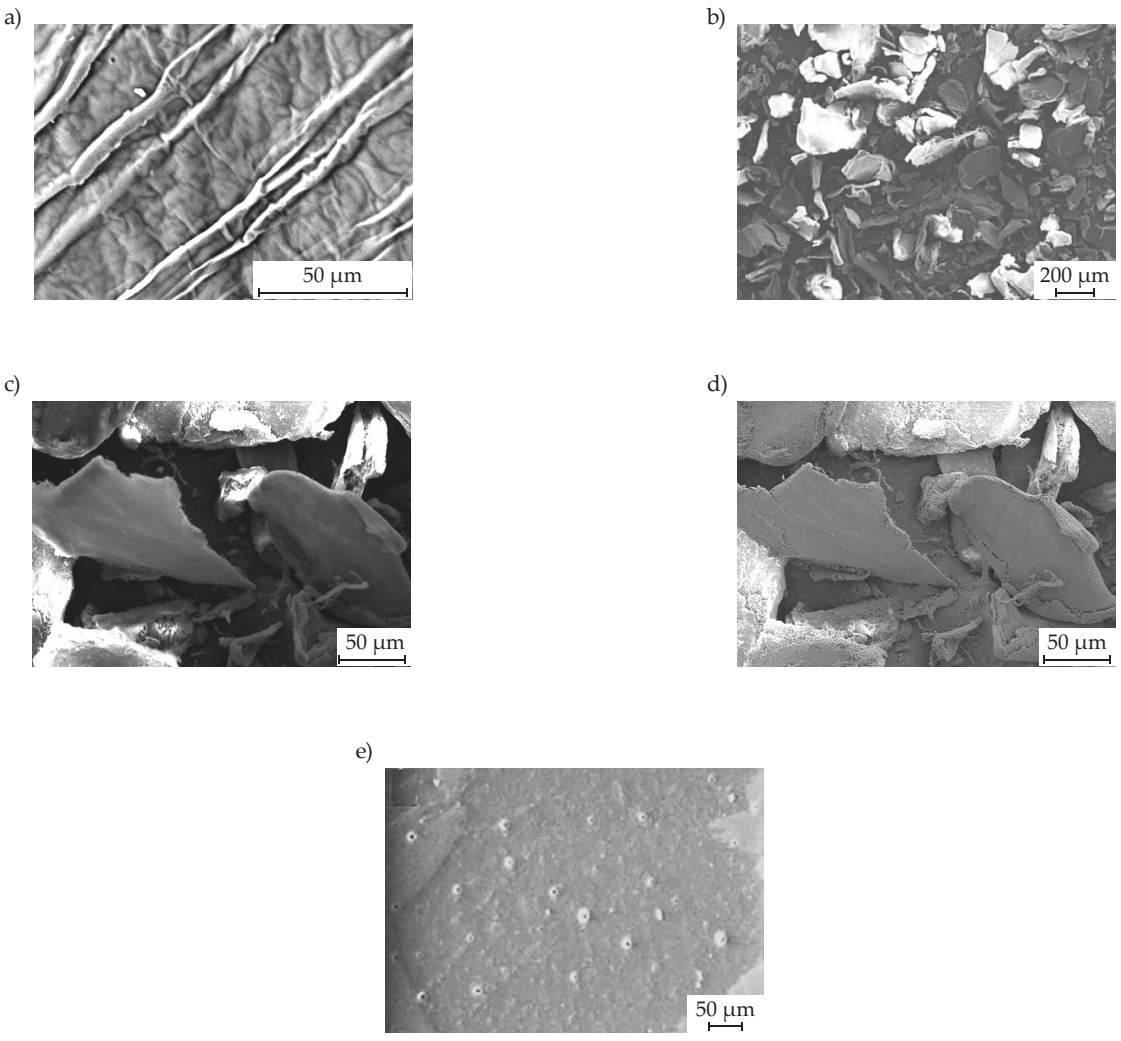


Fig. 4. SEM of: a) chitosan powder before purification, b)–d) chitosan powder after purification at different average velocity and different magnification, e) chitosan film

T a b l e 1. Physicochemical and functional properties chitosan

Solubility	*DD* %	Ash %	Moisture %	Molecular weight, kDa	*FBC* %	*WBC* %	Yield %
1% CH_3COOH	80	1	1	800 000	523	4220	20

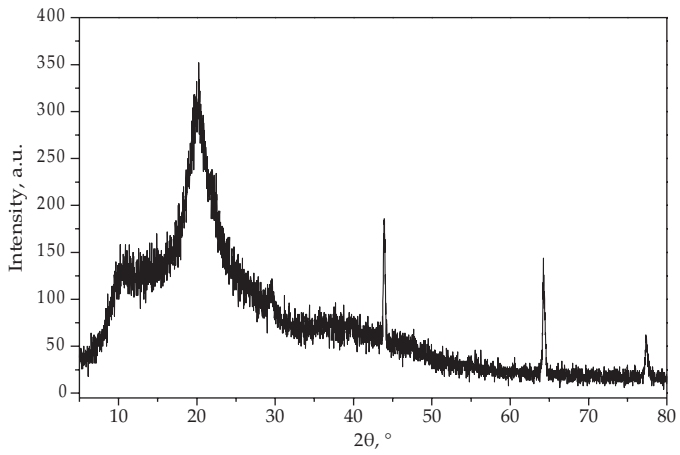


Fig. 5. XRD of chitosan crystals

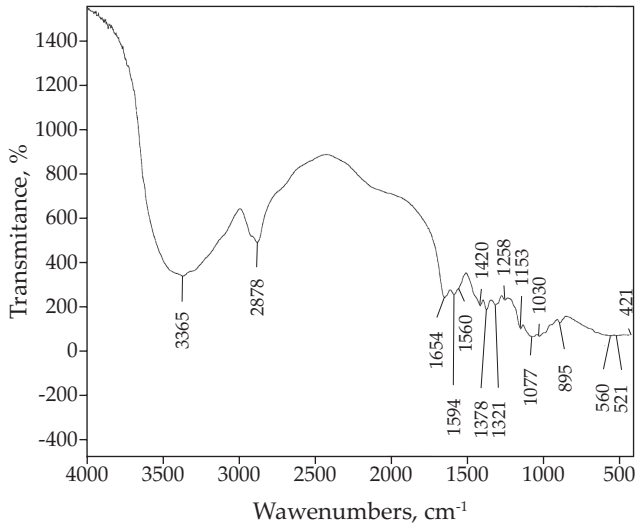


Fig. 6.FT-IR spectrum of chitosan

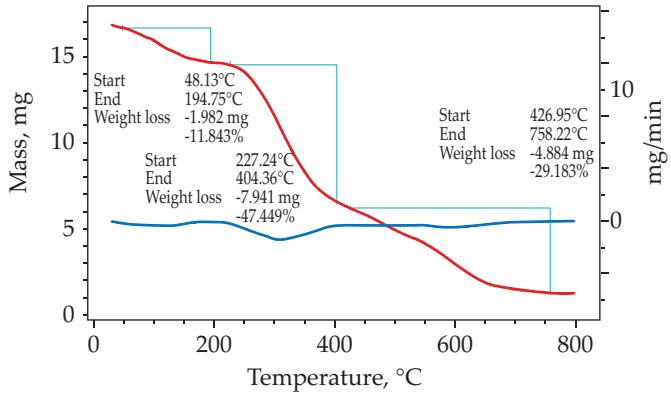


Fig. 7. TGA of chitosan

band at 3365 cm^{-1} corresponding to a hydroxyl group and an amino group stretching, respectively. On the other hand, the FT-IR spectrum had absorption bands at 2878, 1654, and 1321 cm^{-1}. These bands correspond to –CH, traces of carbonyl group of 20% acetyl group in chitosan chain backbone $–CH_3CO–$, and –C-O-C– linkage (Fig. 6).

Thermal gravimetric analysis (TGA) exhibited thermal stability of chitosan with first decomposition peak observed in range of 48–194°C regarding to the moisture vaporization. The other weight loss at 227–404°C owed to the first degradation of chitosan structure (glyosidic linkage). The final weight loss at 426–758°C owed to decomposition of glucosamine rings (Fig. 7).

CONCLUSIONS

The article focuses on the possibilities of minimizing the level of environmental pollutants, hazard and toxic marine crustaceans such as shrimp shells, by extraction of biodegradable and biocompatible chitin. Characteristics of chitin and chitosan are also given. Highly molecular weight chitosan was prepared by deacetylation of chitin from shrimp shells. Increasing the temperature and concentration of NaOH would increase the degree of chitin deacetylation, and increasing the heating time would reduce the degree of deacetylation, as investigated by FT-IR, XRD, SEM and TGA.

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