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Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf

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ABSTRACT

Chitin in the α and the β forms has been extracted from different marine crustacean from the Arabian Gulf. The contents of the various exoskeletons have been analyzed and the percent of the inorganic salt (including the various elements present), protein and the chitin was determined. Deacetylation of the different chitin produced was conducted by the conventional thermal heating and by microwave heating methods. Microwave heating has reduced enormously the time of heating from 6–10 h to 10–15 min, to yield the same degree of deacetylation and higher molecular weight chitosan. This technique can save massive amount of energy when implemented on a semi-industrial or industrial scale. The chitin and the obtained chitosan were characterized by elemental analysis, XRD, NMR, FTIR and thermogravimetric measurements. XRD analysis showed that chitosan has lower crystallinity than its corresponding chitin; meanwhile its thermal stability is also lower than chitin.

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1. Introduction

About 45% of processed seafood consists of shrimp, the waste of which is composed of exoskeleton and cephalothoraxes (Ibrahim, Salama, & El-Banna, 1999; Venugopal & Shahidi, 1995), the latter has become a problem for the environment. This waste represents 50–70% of the weight of the raw material; however it contains valuable components such as protein and chitin (CH) (Roberts, 1992; Shahidi & Synowiecki, 1991). Chitin, next to cellulose, is the second most common polysaccharide on earth, with a yearly production of approximately 10^{10}–10^{12} Tons (Roberts, 1992). This polymer consists of a linear chain of linked 2-acetoamido-2-deoxy-β-D-glucopyranose units.

Chitin is usually isolated from the exoskeletons of crustacean, mollusks, insects and certain fungi. Three different polymorphs of chitin are found in nature; the α-chitin, being the most common structure and corresponding to tightly compacted orthorhombic cells formed by alternated sheets of antiparallel chains (Minke & Blackwell, 1978); the β-chitin, adopts a monoclinic unit cell where the polysaccharide chains are disposed in parallel fashion (Gardner & Blackwell, 1975); and γ-chitin, however it has not been completely identified, an arrangement of two parallel and one antiparallel sheet has been proposed (Rudall, 1963). Roberts (1992) has suggested that γ-chitin can be a combination of α and β structures rather than as a different polymorph. α-Chitin is usually isolated from the exoskeleton of crustaceans and more particularly from shrimps and crabs. β-Chitin can be obtained from squid pens, while γ-chitin exists in fungi and yeast.

Because chitin has a compact structure, it is insoluble in most solvents. Therefore, the chemical modifications of chitin are performed (Peter, 1995). The most common derivative is chitosan, derived by partial deacetylation of chitin (Muzzarelli, 1977; Roberts, 1992). When the degree of deacetylation (DDA) reaches higher than 50%, chitosan becomes soluble in acidic aqueous solutions and it behaves as a cationic polyelectrolyte.

Potential and usual applications of chitin and its derivatives, mainly chitosan, are estimated to be more than 200 (Brzeski, 1987). These polymers have antimicrobial activity, besides being biocompatible and biodegradable (Mathur & Narang, 1990; Muzzarelli, 1977; Ravi Kumar, 2000). They display a wide range of applications in different fields, e.g. in cosmetics, agriculture, food, pharmacy, biomedical, paper industry and also as absorbent materials for wastewater treatment (Bautista-Baños et al., 2006; Rashidova et al., 2004; Sashiwa & Aiba, 2004). Chitosan has been used to modify the surface of nonwoven fabrics and polypropylene films to improve antimicrobial properties (Abdou, Elkholy, Elsabee, & Mohamed, 2008; Elsabee, Abdou, Nagy, & Eweis, 2008).

Several techniques to extract chitin from different sources have been reported. The most common method is referred to as the chemical procedure. The chemical method for isolation of chitin from crustacean shell biomass involves various major steps:

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elimination of inorganic matter (calcium carbonate) in dilute acidic medium (demineralization), and usually demineralization is accomplished by using HCl. Followed by extraction of protein matter in alkaline medium (deproteinization), and it is traditionally done by treating shell waste with aqueous solutions of NaOH or KOH. The effectiveness of alkali deproteinization depends on the process temperature, the alkali concentration, and the ratio of its solution to the shells. As an alternative to the chemical process, a biological process using microorganisms has been evaluated for the demineralization (Hall & Da Silva, 1992; Shirai et al., 1998) and the deproteinization (Jung et al., 2006; Shirai et al., 1998). Recovery of the protein fraction of the shrimp waste by enzymatic hydrolysis has widely been investigated (Gildberg & Stenberg, 2001; Mizani, Aminlari, & Khodabandeh, 2005; Synowiecki & Al-Khateeb, 2003).

Chitin is industrially converted into more applicable chitosan; a structural modification of chitin often performed by alkaline hydrolysis. It is soluble in aqueous acidic medium due to the presence of amino groups.

The degree of deacetylation of (DDA) of chitosan has been found to influence its physical, chemical properties (Illanes et al., 1990) and its biological activities (Hisamatsu & Yamada, 1989). A number of precise and sensitive methods have been derived to achieve the quantitative determination of chitosan and its degree of deacetylation (DDA). Among them, is the dye adsorption method (Maghami & Roberts, 1988), Fourier transform infrared (FTIR) (Baxter, Dillon, Taylor, & Roberts, 1992; Miya, Iwanoto, Yoshikawa, & Mima, 1980; Shigemasa, Matsuura, Sashiwa, & Saimoto, 1996), the first derivative UV method (Muzzarelli & Rocchetti, 1985; Tan, Khor, Tan, & Wong, 1998), NMR methods (Hirai, Odani, & Nakajima, 1991; Raymond, Morin, & Marchessault, 1993; Vårum, Anthonsen, Grasdalen, & Smidsrød, 1991), and potentiometric titration (Raymond et al., 1993).

The objective of the present work is to isolate the useful polymers chitin from the waste byproducts of the seafood industry in the State of Kuwait. The obtained chitin will be characterized and deacetylated to the more useful chitosan. Two methods have been used to convert chitin to chitosan, the conventional thermal heating and by microwave heating methods.

2. Methods

2.1. Extraction of chitin

2.1.1. Raw materials preparation

The different local resources used to extract chitin are described in Table 1. The shells of these species were scraped free of loose tissue, washed, dried, and grounded to pass through a 250 μm sieve, then subjected to demineralization and deproteinization (Scheme 1). The reference chitin-crab shells (CH-Ref) was obtained from Sigma.

Table 1
Crustaceans of the Arabian Gulf (Kuwait).

Chitin source (Latin name)	English name	Max. length (cm)
Penaeus semisulcatus (de Haan)	Grooved Tiger Prawn CH-TP	20
Metapenaeus affinis (Milne-Edwards)	Jinga Shrimp CH-JS	15
Portunus pelagicus (Linne)	Blue Swimming Crab-Male CH-Cr-M	20
Portunus pelagicus (Linne)	Blue Swimming Crab-Female CH-Cr-F	20
Thenus orientalis (Lund)	Scyllarid Lobster CH-Lob	25
Sepia spp.	Cuttlefish CH-Cut	35

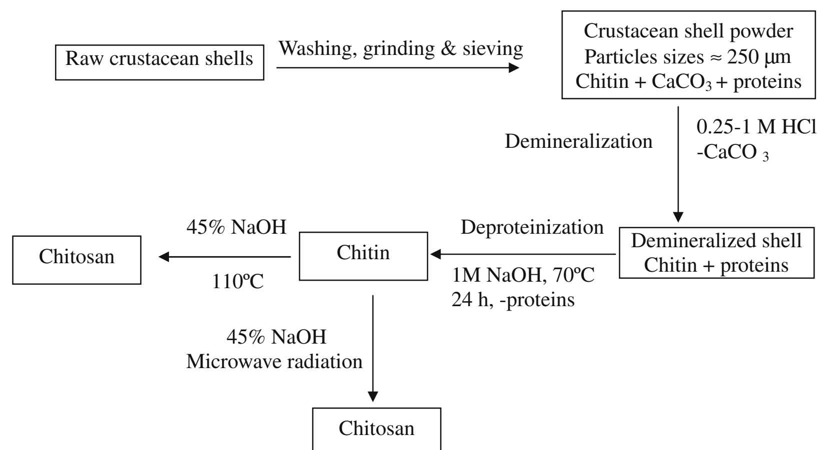
2.1.2. Demineralization

Demineralization was carried out in dilute HCl solution. The mineral content in the exoskeleton of crustacean is not the same for each species, hence studied chitin resources do not need the same treatments. All species except for cuttlefish were treated with 0.25 M HCl solution at ambient temperature with a solution-to-solid ratio of 40 mL/g, whereas 1.0 M HCl was used to demineralize the cuttlefish pens.

The resulting solid was washed with distilled water until neutral. Then, the demineralized samples were dried and weighed. The number of baths and their duration (15–180 min) were dependent on the species. It was observed that the emission of CO_2 gas was more or less important according to the studied species. It also depends upon the mineral content of different species and penetration of the shells by hydrochloric acid. It was found that the larger the mineral content the greater the gas emission. The CO_2 emission was stronger in case of cuttlefish than other species. The percent of mineral contents of different species is given in Table 2.

2.1.3. Deproteinization

Deproteinization of chitin was carried out using 1.0 M NaOH (20 mL/g) at 70 °C. The treatment was repeated several times. The absence of proteins was indicated by the absence of color of the medium at the last treatment, which was left overnight. The resulting solution then washed to neutrality. Finally, it was washed with hot ethanol (10 mL/g) and later boiled in acetone to remove any impurities. The purified chitin was then dried. The chitin con-



Scheme 1. Isolation of chitin and preparation of chitosan.

Table 2
Chemical composition of raw shells from local crustaceans (Kuwait).

Chitin source	Ca (ppm)	K (ppm)	Na (ppm)	Mg (ppm)	Fe (ppm)	Total ash in raw shell/g
CH-TP	754.6	15.04	35.29	48.88	1.900	0.29
CH-JS	781.1	13.73	38.88	47.30	2.040	0.37
CH-Cr-M	846.8	12.21	39.26	63.28	1.580	0.66
CH-Cr-F	655.6	25.72	62.08	49.92	1.720	0.37
CH-Lob	762.8	14.27	40.32	71.81	2.440	0.45
CH-Cut	840.6	8.730	28.59	1.070	1.410	0.89

Table 3
Mineral content of raw shells from local crustaceans (Kuwait).

Chitin source	% $CaCO_3$	% Protein	% Chitin
CH-JS	52.03	28.84	19.13
CH-TP	45.66	37.59	16.75
CH-Cr-M	68.87	10.33	20.80
CH-Cr-F	65.50	14.36	20.14
CH-Lob	61.81	16.93	21.26
CH-Cut	91.25	1.35	7.40

tent was determined from the weight differences of the raw materials and that of the chitin obtained after acid and alkaline treatments. Ash content of dried chitin was determined by burning the samples at 600 °C in a muffle furnace.

2.2. Deacetylation

Two methods have been used to prepare chitosan from chitin. First, chitin that was extracted from different species was treated with 45% NaOH (15 mL/g) at 110 °C. Kurita (2001) has indicated that deacetylation of chitin can be highly facilitated by steeping in strong sodium hydroxide at room temperature before heating. We adapted this method of steeping for our samples for one day before conversion by heat. All chitosan samples were purified by dissolving in 2% acetic acid and reprecipitating them out in 20% NaOH solution. Samples were then washed with distilled water until neutral and freeze-dried prior treatment with freezing under methanol and later lyophilized under −70 °C and stored for further use, (Scheme 1). To decrease the long processing times typically required to achieve *N*-deacetylation, an alternative microwave method was used. A mixture of chitin and 45% NaOH was placed in a conical flask, covered tightly with cotton, and then subjected to microwave radiation. The mixture then cooled with cold water and after filtration chitosan was washed to neutral pH and freeze dried using VIRTIS Freezemobile 5EL with sentry microprocessor control freeze dryer.

The deacetylation kinetics were followed in both methods by monitoring the DDA% as a function of time. In the first method deacetylation was performed at different heating times of 2, 4, 6, 8 and 10 h, while with the microwave heating method the duration of subjecting microwave radiation to chitin/NaOH mixture was 6, 8, 10, 12 and 15 min at 600 W.

2.3. Characterization

2.3.1. Determination of the ash content in chitin

The ash content was determined by heating a sample of raw material (≈1 g) at 600 °C and weighing the remaining product after cooling in a desiccator. The mineral contents of the ash were analyzed using inductively coupled plasma optical emission spectroscopy analysis (ICP-GBC INTEGRA XM). Prior to the analysis, the solid samples were digested in concentrated nitric acid in microwave reactor (QWAVE 2000) until complete dissolution had occurred.

2.3.2. Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were measured by KBr-supported sample of chitin and chitosan over the frequency range 4000–400 cm^{-1} at resolution of 4 cm^{-1} using a model 2000 Perkins–Elmer spectrometer. The sample was thoroughly mixed with KBr, the dried mixture was then pressed to result in a homogeneous sample/KBr disc.

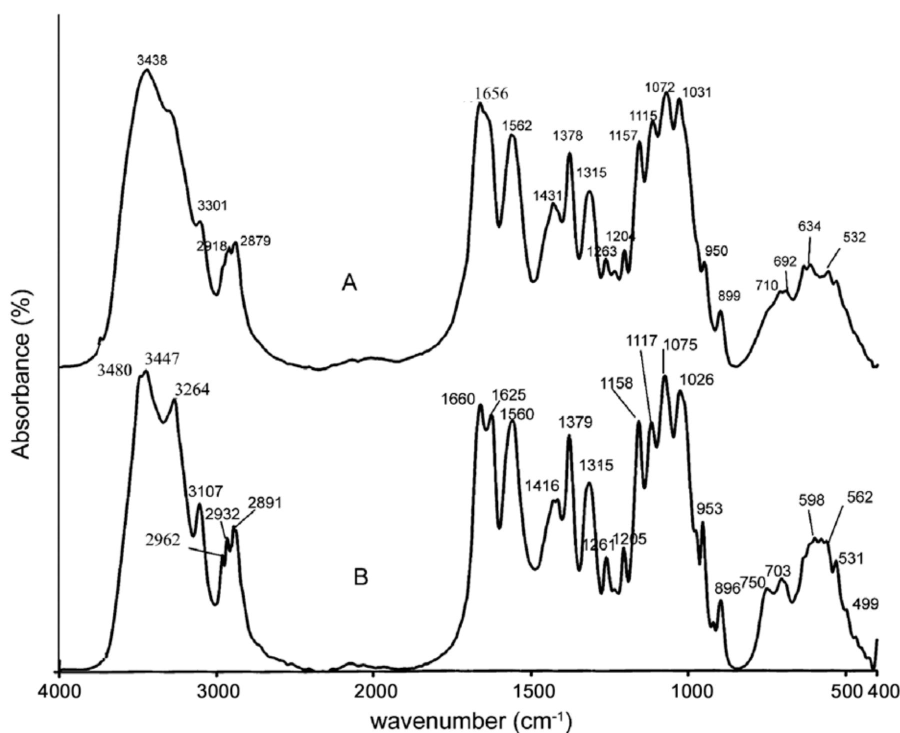


Fig. 1. FTIR spectra of β-chitin from CH-Cut (A) and α-chitin from CH-TP(B).

2.3.3. X-ray powder diffractometry (XRD)

The XRD measurements on powder samples were carried out (at $2\theta = 5–40°$ and RT) using a model D500 Siemens diffractometer (Germany) equipped with Ni-filtered Cu Kα radiation ($\lambda = 1.5406$ Å). The diffractometer was operated with 1° diverging and receiving slits at 50 kV and 40 mA and a continuous scan was carried out with a step size of 0.015° and a step time of 0.2 s. The crystalline index (I_{CR}) was calculated from the normalized diffractograms and the apparent size crystallites $D_{ap}[1\,1\,0]$ was determined according to the method currently applied to polysaccharide diffraction studies (Focher, Beltrame, Naggi, & Torri, 1990) after mathematical treatment of the peaks corresponding to its deconvolution and application of the Lorentzian function. The intensities of the peaks at 1 1 0 lattices (I_{110}, at $2\theta \cong 20°$ corresponding to maximum intensity) and at $2\theta \cong 16°$ (amorphous diffraction) were used to calculate ICR using Eq. (1) while the values of $D_{ap}[1\,1\,0]$ were calculated according to Klug and Alexander (1974) Eq. (2).

$$I_{CR} = \frac{I_{110} - I_{am}}{I_{110}} \times 100 \quad (1)$$

$$D_{op[110]} = \frac{K\lambda}{\beta_o \cos\theta} \quad (2)$$

where K is a constant (indicative of crystallite perfection and was assumed to be 1; λ (Å) is the wave length of incident radiation; β_o (rad) is the width of the crystalline peak at half height and θ (rad) is half the Bragg angle corresponding to the crystalline peak.

2.3.4. Elemental analysis

The average degree of acetylation (DA) of chitin samples was determined from data of elemental analysis, which was carried out by using LECO CHNS-932 equipment. Following equation (Xu, McCarthy, Gross, & Kaplan, 1996) used to calculate the DA values:

$$DA = \frac{(C/N) - 5.14}{1.72} \times 100 \quad (3)$$

where C/N is the ratio carbon/nitrogen as determined by elemental analysis.

2.3.5. Thermogravimetry analysis (TGA)

TGA was performed using a 10 mg sample from ambient to 600 °C at a heating rate of 10 °C/min in a dynamic (50 mL/min) synthetic air atmosphere using TGA-50 Shimadzu automatic analyzer.

2.3.6. Scanning electron microscopy (SEM)

The surface morphology of chitin and chitosan was observed using SEM. The dried sample of chitin and chitosan was ground and then coated with gold under vacuum using a sputter coater. The scanning electron microscopy (SEM) was conducted using a JEOL JSM-630 J scanning electron microscope operated at 20 kV.

2.3.7. Determination of the intrinsic viscosity of chitosan

Viscosity measurements were performed using Herzog Ubbelohde viscometer HVU 481 at 25 ± 0.1 °C. Chitosan samples were dissolved in 2% acetic acid/0.1 M KCl, and the viscosity-average molecular weight of chitosan was calculated from the viscosity-molecular weight equation (Rinaudo, Milas, & Le Dung, 1993):

$$[\eta] = 0.078 * M_v^{0.76} \quad (4)$$

2.3.8. Nuclear magnetic resonance NMR

NMR spectra were recorded using Bruker AVANCE II 600 spectrometer in 2% deuterated acetic acid in D_2O solution. The experiments were run at 70 °C, temperature at which the solvent (HOD) peak does not interfere with any chitosan peaks. After dissolution, approximately 1 mL of the chitosan sample solution was transferred to 5 mm NMR tube. The sample tube was inserted in the magnet and allowed to reach thermal equilibrium for 10 min before performing the experiment.

3. Results and discussion

3.1. Chemical composition of raw material of crustacean shells

Chitin was isolated from six sources, two kinds of marine shrimp shells, crab female and crab male shells, cuttlefish pens and lobster shells, all from the Kuwait region of the Arabian Gulf. The chemical composition of the source materials are shown in

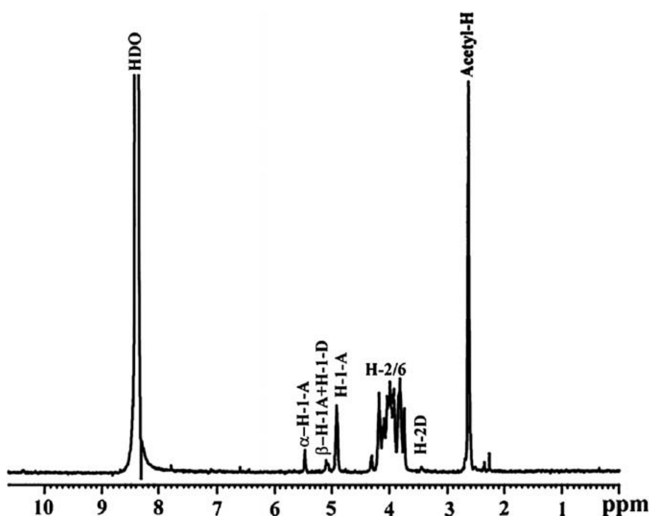


Fig. 2. ^{1}H NMR spectrum (600 MHz) of α-chitin in concentrated DCl at 25 °C.

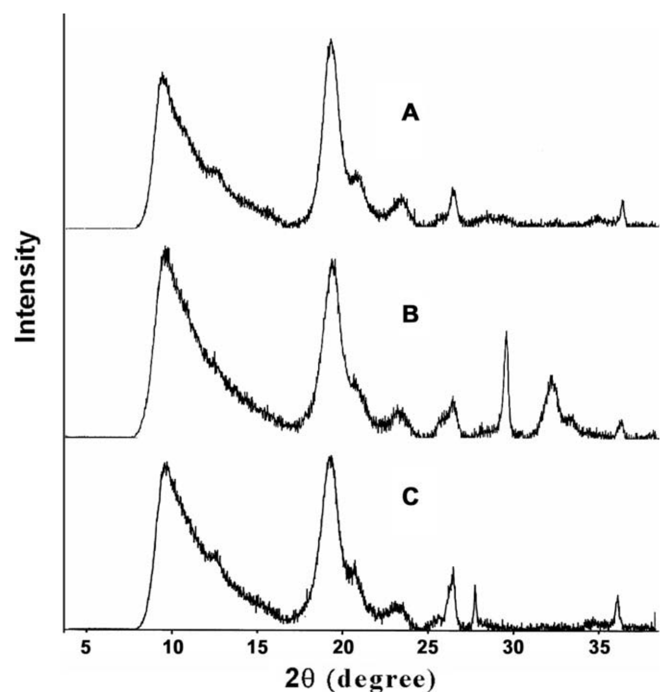


Fig. 3. XRD patterns of CH-JS (A), CH-Cr-M (B), CH-Lob (C).

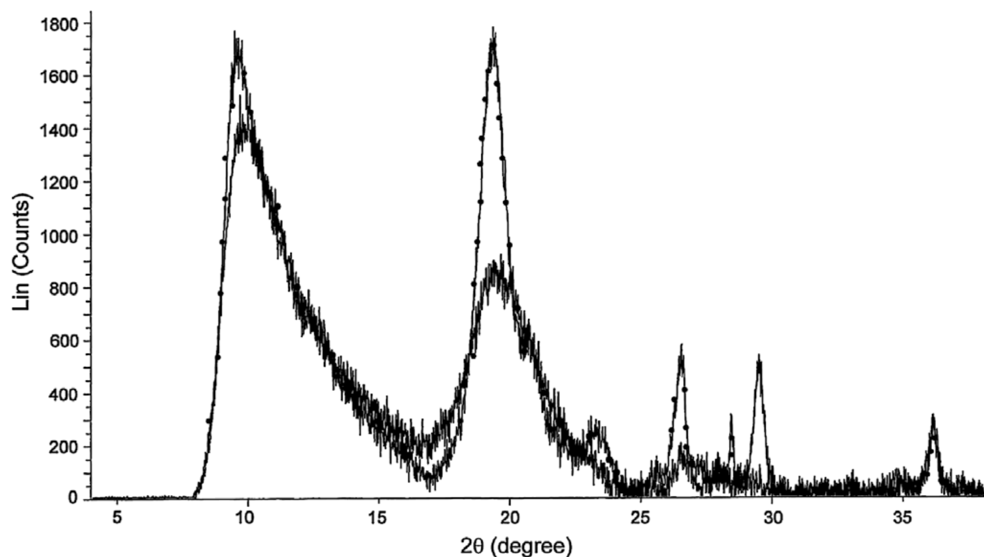


Fig. 4. XRD patterns of β-chitin from CH-Cut (—) and α-chitin from CH-ON (-·-·-·-).

Table 4
Degree of F_A values of Chitin by elemental analysis.

CH-TP	CH-JS	CH-Cr-M	CH-Cr-F	CH-Lob	CH-Cut
0.96	0.97	0.9	0.94	0.9	0.98

Table 2. The percentage of inorganic matter ($CaCO_3$) was found to be lowest in the shrimp 45% in CH-JS and 52% in CH-TP and highest in the cuttlefish (CH-Cut) 91%. Crab male shell contains higher inorganic material (68.87%) than crab female (65.50%). Cuttlefish pen (CH-Cut) was found to have a low level of protein (1.35%). The higher protein contents were found in shrimp CH-JP (37.59%) and CH-TP (28.84%). Female crab CH-Cr-F has slightly higher protein content (14.36%) than male crab CH-Cr-F (10.33%). The raw crustacean shells contain 17–21% chitin whereas a lower percentage of chitin was found in the squid species (7.4%).

In all crustacean shells studied, the most common elements were Ca, Mg, Na, K and Fe (Table 3). Calcium was by far the most abundant and then followed by Mg. From the comparison of the results in Table 3, it shows that the source has an influence on the percent of each element. Cuttlefish pens have the highest percent of Ca metal and the smallest amount of Mg, Na and Fe compared to the other crustacean shells. The mineral contents in female and male crab are quite different. While female crab contained the highest amount of Na and K, Male crab found to have high content of Ca and Mg. Both species of shrimp contained almost the same mineral contents.

3.2. Chitin characterization

3.2.1. FTIR analysis

Spectra of β-chitin from CH-Cut and α-chitin from CH-TP are shown in Fig. 1A and B, respectively. Different patterns occur in the α-chitin and β-chitin. The differences in the IR spectra of chitin can be used to distinguish between α-chitin and β-chitin. (i) Due to the different arrangement between α-chitin and β-chitin, amide I band in α-chitin spectrum splits at 1660 cm^{-1} which is attributed to the occurrence of intermolecular hydrogen bond CO...HN and at 1625 cm^{-1} due to the intramolecular hydrogen bond CO...$HOCH_2$ (Focher et al., 1992; Lavall, Assis, & Campana-Filho, 2007; Pearson, Marchessault, & Liang, 1960; Rinaudo, 2006). However, a single band is observed in case of the β-chitin at 1656 cm^{-1} which is commonly assigned to the stretching of the CO group hydrogen bonded to amide group of the neighboring intra-sheet chain (Lavall et al., 2007; Rinaudo, 2006). (ii) The strong band at 1430 cm^{-1} is seen in the spectrum of β-chitin while a distinct band at 1416 cm^{-1} occurs in the spectrum of α-chitin which is in agreement with Lavall et al. (2007). (iii) The band due NH stretching at 3264 cm^{-1} and 3107 cm^{-1} can be seen clearly in the of α-chitin spectrum but these are weak and not easily observed in β-chitin. Focher et al. (1992) assigned these bands to CO...NH intermolecular bonding and H bonded NH group. (vi) OH-out-of plane bending at 703 cm^{-1} and NH-out-of plane bending at 750 cm^{-1} can be observed in the spectrum of α-chitin while they are less well defined and shifted to 682 cm^{-1} and 710 cm^{-1} in the spectrum of β-chitin. This remarkable difference between the two types of chitin is due to a relatively low crystalline and loosely ordered structure showing weaker inter- and intramolecular hydrogen bonding in β-chitin (Kurita et al., 2005) compared to that of the α-chitin.

3.2.2. NMR analysis

Chemical composition of chitin was obtained by ^1H NMR spectrum using concentrated DCl as solvent. Fig. 2, shows the ^1H NMR spectrum (600 MHz) of α-chitin in concentrated DCl at 25 °C. H-1 of deacetylated units resonate at 5.1 ppm, overlapping with β-ano-

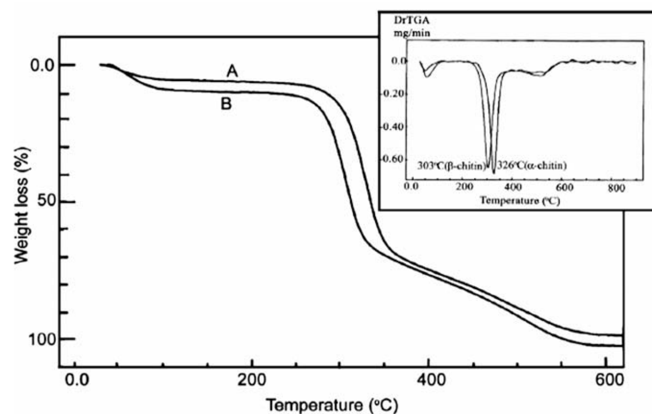


Fig. 5. TGA thermograms for α-chitin from CH-Cr-M (A) and β-chitin from CH-Cut (B). The inset figure shows DSC thermogram for α-chitin and β-chitin.

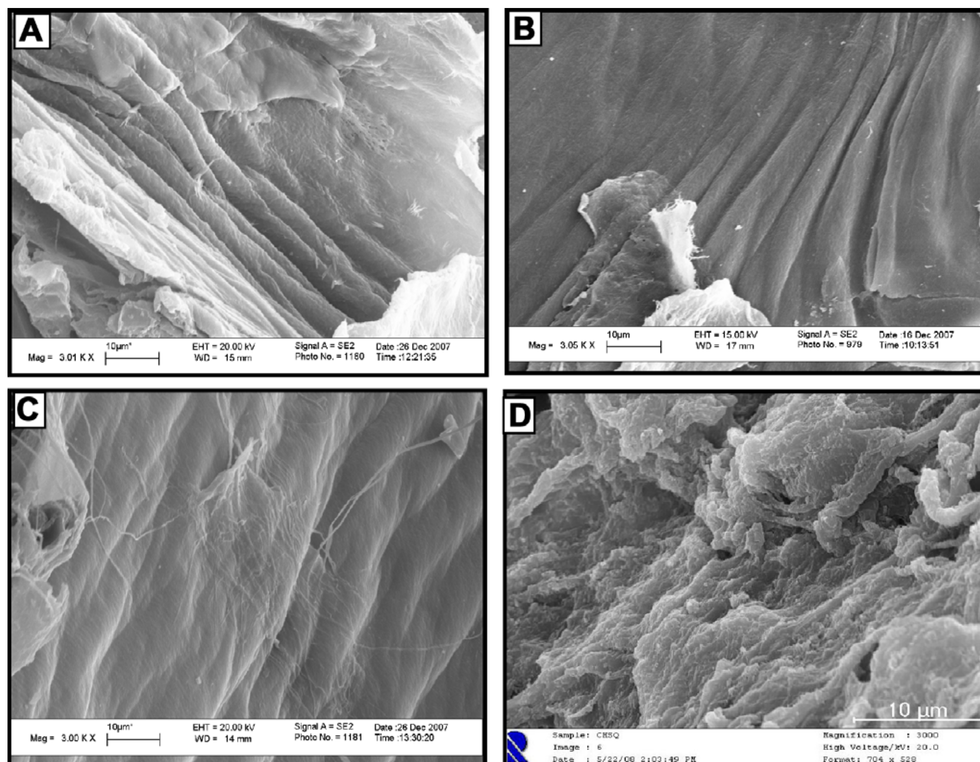


Fig. 6. SEM micrographs for α-chitin from CH-JS (A), CH-Cr-F (B), CH-Lob (C) and β-Chitin from CH-Cut (D).

meric proton. H-1 of internal acetylated units peak at 5 ppm. Acetyl protons are found at 2.6 ppm while H2–6 of the ring appeared between 3.6 and 4.4 ppm. H-2D of internal deacetylation units resonate at 3.4 ppm. The absence of methyl proton resonance from protein between 1.0 and 1.5 in ^{1}H NMR spectra of chitin gives a good indication of the purity of chitin sample (Einbu & Vårum, 2008).

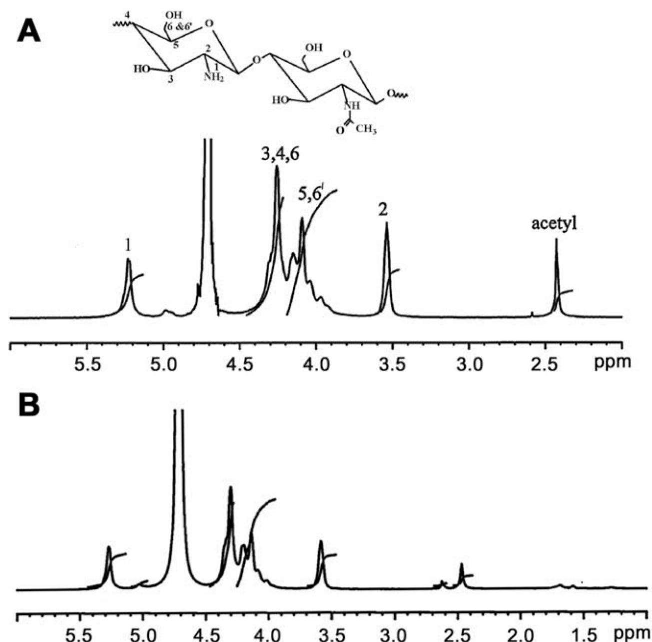


Fig. 7. The 600 MHz ^{1}H NMR spectrum measured at 70 °C for chitosan 83% DDA (A) and 90% DDA (B).

3.2.3. X-ray powder diffractometry of chitin

XRD analysis was applied to detect the crystallinity of the isolated chitin. Depending on the source of raw material, different XRD patterns were observed. The XRD patterns of α-chitin (Fig. 3) for CH-JS (A), CH-Cr-M (B) and CH-Lob (C), show five sharp crystalline reflections at 9.6°, 19.6°, 21.1°, 23.7° and 36°.

Two additional sharp peaks are found in the XRD patterns of crab male (CH-Cr-M) and female (CH-Cr-F) at 29.3° and 32.1° and one additional sharp peak at 27.7° was recognized in the XRD patterns of CH-Lob. X-ray diffraction exposed the differences between α-chitin and β-chitin more clearly due to the different arrangements adopted by these polymorphs. Fig. 4 shows XRD patterns of β-chitin from CH-Cut and α-chitin from CH-TP. The XRD profile of the α-chitin exhibits well-resolved and intense peaks, while a broad diffuse scattering and less intense peaks are found for the β-chitin at 9.6° and 19.6°. This indicates that α-chitin is a more crystalline polymorph because of its antiparallel compact structure.

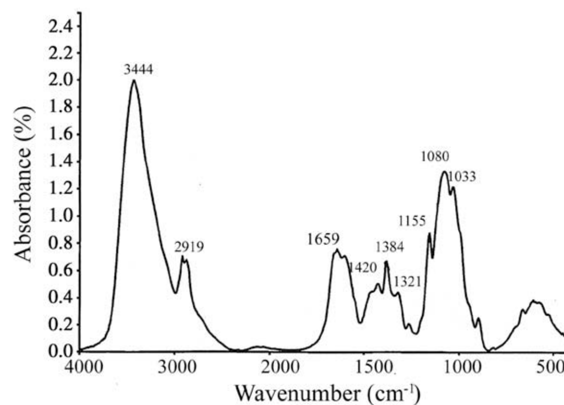


Fig. 8. FTIR spectrum for chitosan.

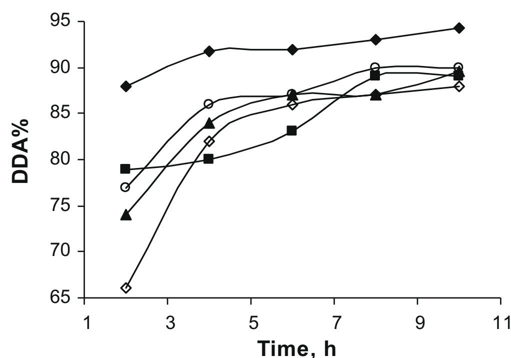


Fig. 9. Effect of time on the DDA% under traditional heating method for (♦) CH-Cut, (○) CH-Cr-M, (▲) CH-Ref, (■) CH-TP, (◇) CH-Cr-F.

The crystalline index and the average diameter of its crystallites were calculated from the X-ray diffraction data and are presented in Table 4. This data shows that both shrimp species have nearly the same crystallinity and that the male crab is more crystalline than the female crab. Also these data confirm that β-chitin is less crystalline than all α-chitin. Average diameter of crystallites for all α-chitin was found to be similar and about twice that of the β-chitin and these results concur highly with results given by Lavall et al. (2007).

3.2.4. Degree of N-acetylation

The basic repeating unit of chitin is *N*-acetyl-D-glucosamine. Although most of the C-2 amino groups within chitin are acetylated, free amino groups are also present to some extent because of deacetylation during deproteinization process in the alkaline medium. Therefore, chitin samples have different degrees of acetylation depending on their sources of origin and mode of isolation. The average degree of acetylation (DA) of chitin samples was determined from data of elemental analysis and is given in Table 4. Chitin from shrimp shell have F_A of 0.96 (CH-TP) and 0.97 (CH-JS), i.e. contains a small but significant fraction of de-*N*-acetylated unit. β-Chitin (CH-Cut) contains the highest degree of *N*-acetylation among the studied species. On the other hand CH-Cr-M and CH-Lob found to have about 10% of de-*N*-acetylated unit.

3.2.5. Thermogravimetry analysis (TGA)

TGA curves of chitins are shown in Fig. 5, (A) for CH-Cr-M (representative of α-chitin) and (B) for CH-Cut (β-chitin). Both curves show that weight loss occurs in two stages. The first stage starts around 60 °C (weight loss WL ≈ 5%) and the second stage starts around 326 °C for α-chitin and 303 °C for β-chitin with weight loss about (65–73%). The first stage is assigned to the loss of water be-

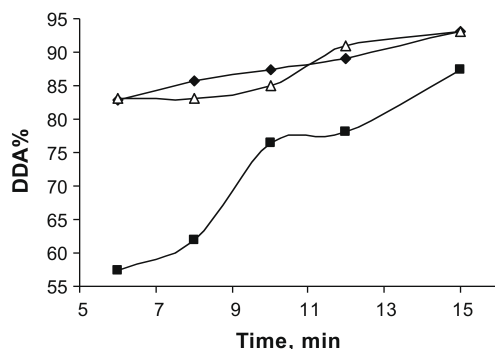


Fig. 10. Effect of time on the DDA% under microwave heating method for (♦) CH-Cut, (△) CH-Ref, (■) CH-TP.

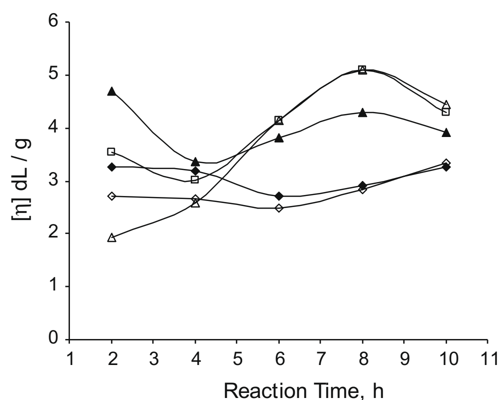


Fig. 11. Effect of time under traditional heating on the intrinsic viscosity [η] of chitosan obtain from (♦) CH-Ref, (□) CH-Cr-M, (▲) CH-Cut, (△) CH-Cr-F (◇) CH-TP.

cause polysaccharides usually have a strong affinity for water and therefore may be easily hydrated.

The second one corresponds to the thermal decomposition of chitin. The decomposition temperature of CH-Cr-M (α-chitin) is higher than that of CH-Cut (β-Chitin). This result indicates that α-chitin exists as a stable structure toward thermal decomposition than β-chitin.

3.2.6. Scanning electron microscopy (SEM)

Fig. 6 shows SEM photographs of powder α-chitin from CH-JS (A), CH-Cr-F (B), and CH-Lob (C) and β-Chitin from CH-Cut (D). A very uniform with a lamellar organization and dense structure was observed clearly for α-chitin, whereas the surface of β-chitin appears less crystalline and different from α-chitin.

3.3. Deacetylation of chitin

3.3.1. Preparation of chitosan

To avoid long heating times, chitosan was prepared by chitin deacetylation in 45% sodium hydroxide solution using microwave radiation technology. Microwave heating, as an alternative to conventional heating techniques, has been proved more rapid and efficient for chemical reactions. The chitosan results from microwave method were compared with that of the traditional method by refluxing chitin in the same alkali concentration. To speed up the process, the chitin was steeped in concentrated sodium hydroxide for 24 h at room temperature before subjecting chitin to microwave radiation or heating in refluxing method (Abdou, Nagy, & Elsabee, 2007). The degree of deacetylation for soluble chitosan

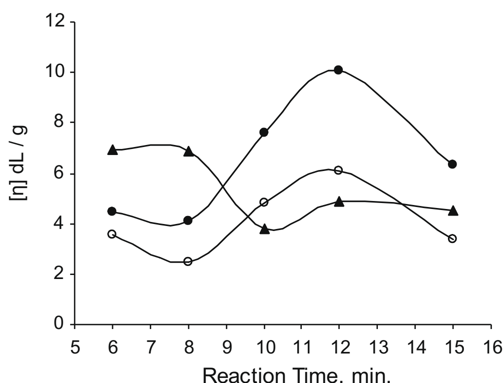


Fig. 12. Effect of time under microwave heating method on the intrinsic viscosity [η] of chitosan obtain from (▲) CH-Cut, (○) CH-Ref, (●) CH-TP.

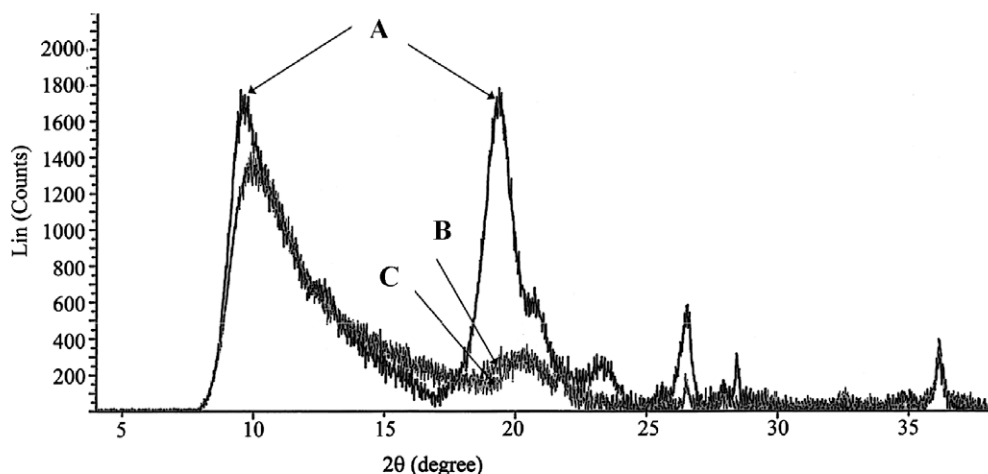


Fig. 13. XRD patterns of α-chitin of CH-TP(A), its corresponding chitosan prepared under microwave heating (B), chitosan prepared under traditional heating (C).

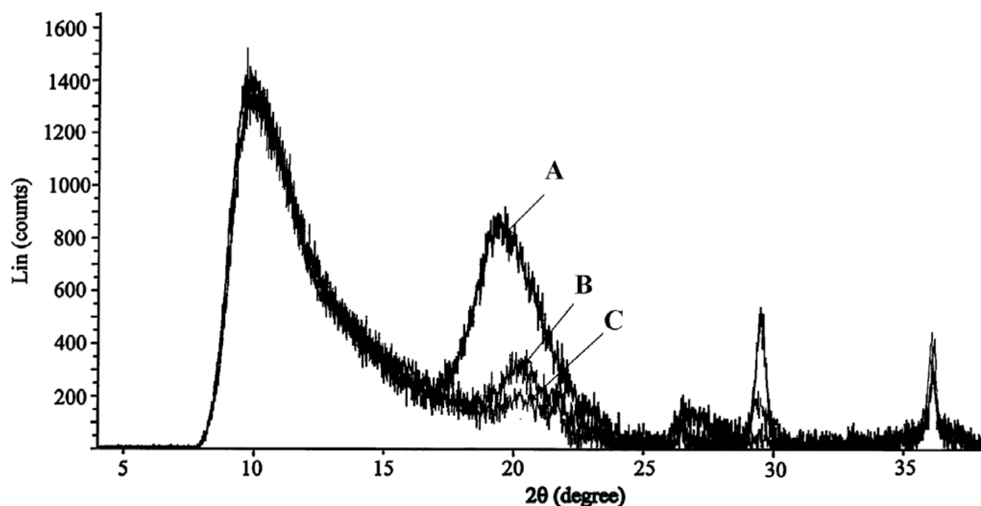


Fig. 14. XRD patterns of β-chitin of CH-Cut (A), its corresponding chitosan prepared under microwave heating (B), chitosan prepared under traditional heating (C).

was determined by ^{1}H NMR. Fig. 7 represents the 600 MHz ^{1}H NMR spectrum measured at 70 °C for chitosan (DDA% = 83% (A) and 90% (B)). The DDA was calculated using integrals of (H1-D, $\delta \approx 5.2$) and the peak of the three protons of acetyl group (H-Ac, $\delta \approx 2.4$) (Lavertu et al., 2003).

$$\mathrm{DDA}(\%) = \left(\frac{\mathrm{H1D}}{\mathrm{H1D} + \mathrm{HAc}/3}\right) \times 100 \quad (5)$$

Fig. 8 represents FTIR spectrum for chitosan. The bands at 1320 cm^{-1} and 1420 cm^{-1} were chosen to measure the DA values according to Brugnerotto et al. (2001). The DDA% values of chitosan were calculated using Kasaai, Arul, and Charlet (2000) formula.

$$\mathrm{DDA}\% = \frac{6.857 - \mathrm{C/N}}{1.7143} \quad (6)$$

where C/N is the carbon/nitrogen ratio measured from the elemental composition of the chitosan samples. The average values of DDA% reported in this article are average of the three methods.

3.3.2. Kinetics of deacetylation

Figs. 9 and 10 show the results of deacetylation of chitosan under both conventional and microwave heating, respectively, at different times. In general DDA% of chitin occurs rapidly in the early stages of both processes, conventional and microwave heating, and then slows down until a plateau is reached. The percentage of DDA increases with increasing time of reaction reaching maximum 88–94.4% after 10 h of refluxing using traditional heating methods depending on the source of chitin. On the other hand, using microwave heating, the highest DDA% values (87.5–93) were obtained after 15 min of microwave radiation. In case of β-chitin (CH-Cut) deacetylation rate was performed faster as compared to α-chitin in both methods. The deacetylation percentage above 90 was obtained after 15 min in microwave heating as compared to that in conventional heating method, which took 8–10 h to reach to approximately the same DDA%. In this way microwave heating method reduces the reaction of deacetylation by a big factor from 8–10 h to 15 min saving thus enormous amount of energy, if implemented on an industrial scale.

3.3.3. Viscosity of chitosan

The variation of intrinsic viscosity values for traditional and microwave-heating methods with time of reaction are given in Figs. 11 and 12, respectively. Both methods show an increase in viscosity with time of reaction and then showing a decrease at longer heating time. Maximum viscosity was found to be at 8 h in traditional heating method (2.9–5.1 dL/g), however in the microwave heating method the viscosity increases to a maximum

after 12 min in the range 4.9–10.1 dL/g depending on the source of chitin. These results proved that chitosan produced using microwave technique has higher molecular weight than using the traditional method.

3.3.4. Crystallinity of chitosan

Figs. 13 and 14 represent XRD for α-chitin (CH-TP) (A), β-chitin (A), and their corresponding chitosan under microwave heating (B) and traditional heating (C), respectively. Both Figures show that the crystallinity of chitin was reduced after deacetylation reaction. Peaks corresponding to the angle 2θ–20° in XRD of chitosan were less resolved and shifted to higher 2θ. Strong reflection at 2θ around 9–10° which is due to incorporation of bound water molecules into crystal lattice slightly shifted in α-chitin. α-Chitin with a crystallinity of 89.4% produced chitosan with crystallinity indices of 37% under microwave heating (12 min) and 30% under traditional heating (8 h). β-Chitin with a crystallinity of 71% produced chitosan with crystallinity indices of 33% under microwave heating (12 min) and 10% under the traditional heating (8 h). This indicates that chitosan obtained under microwave heating exhibits higher crystallinity than that under traditional heating.

4. Conclusions

α-Chitin and β-chitin have been isolated from local marine sources of Kuwait, by treatment with dilute HCl solution for demineralization, and dilute NaOH for deproteiniztion. In FTIR spectra, the amide I band is split for α-chitin, and the amide I for β-chitin is a single peak. The XRD, SEM results indicate that α-chitin is a more crystalline polymorph because of its parallel structure.

α-Chitin and β-chitin were hydrolyzed using traditional and microwave heating method. Chitosan produced from microwave heating reduced the time of deacetylation from ~8 h to few minutes (~15 min) to reach to the same DDA% as the traditional method. Also chitosan from microwave heating proved to have higher molecular weight and crystallinity.

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