Arab Journal of Nuclear Science and Applications, 46(1), (53-66) 2013

Chitin and Chitosan Extracted from Irradiated and non-Irradiated Shrimp Wastes (Comparative Analysis Study)

E. M. El-Nesr[1], A. I. Raafat[1], Sh. M. Nasef[1], E. A. Soliman[2] and El-Sayed A. Hegazy[1].
[1]*Polymer Chemistry Department, National Center for Radiation Research and Technology, P.O. Box 29 Nasr City, Cairo, Egypt*
[2]*Chemistry Department. Faculty of Science. Ain Shams University, Cairo, Egypt.*
Received: 3/7/2012 **Accepted: 2/9/2012**

ABSTRACT

Keeping the importance of chitin and chitosan in mind, an attempt has been made on the extraction of chitin and chitosan from local shrimp waste using a chemical method. The influence of using ionizing radiation before chemical treatment on the time needed for deproteination and demineralization process was investigated. Deacetylation process was conducted by the conventional thermal heating. The degree of deacetylation (DDA%) was determined by potentiometric titeration method. The intrinsic viscosity and the weight average molecular weight (MW) were determined by viscometric method. Comparison between physicochemical properties of chitin and chitosan extracted from both non irradiated and irradiated shrimp waste was studied using Fourier Transform Infrared spectroscopy (FTIR), X–ray diffraction analysis (XRD) and thermogravimetric analysis (TGA). The results showed that irradiation causes the reduction of time of deproteination, demineralization and deacetylation processes. Also, chitosan prepared from irradiated sample has higher solubility, DDA% and lower molecular weight.

Keywords: Shrimp shell waste / Gamma Irradiation / Chemical modification / Chitin/ chitosan.

INTRODUCTION

Waste is directly linked to human development, both technologically and socially. In living organisms, waste is the unwanted substances or toxins that are expelled from them. More commonly, waste refers to the materials that are disposed of in a system of waste management. Biodegradable waste is a type of waste, typically originating from plant or animal sources, which may be broken down by other living organisms. Arthropods, the most abundant animal group, which includes insects and crustacean. Crustacean shells such as crab, shrimp, prawn, krill and lobster are constituted mainly of a matrix made of chitin and protein, hardened by mineral salts. The amount of each component can vary widely among species and also in an intra-specific way as a function of season, age, gender and other environmental conditions. Depending on the species there are minor components such as lipids and pigments, among others. The chitinous waste could be considered hazardous as a result of uncontrolled dumping. In the sea, it leads to eutrophication and a high load of biological oxygen demand (BOD) while on land it can result in pathogen-borne problems[1,2].

The processing of shellfish waste poses a major technological problem. Shells are largely insoluble and very resistant to natural biodegradation. It is, however, the constituents of such shells which make them worthy of further processing consisting, in general, of (30-40%) protein, calcium carbonate (30-50%) and chitin (20-30%) on a dry basis[3] and these percentage vary with crustacean species and seasons[4,5]. several schemes have been suggested for the utilization of this waste product,

involving the extraction of chitin,[6,7,8] and the recovery of protein[9,10], lipids (pigments)[11,12,13] and other compounds[14,15].

Chitin is widely available from a variety of source among which, the principal source is shellfish waste such as shrimps, crabs, and crawfish[16]. It also exists naturally in a few species of fungi. Chitin is associated with proteins and, therefore, high in protein contents. Chitin fibrils are embedded in a matrix of calcium carbonate and phosphate that also contains protein. The matrix is proteinaceous, where the protein is hardened by a tanning process[17]. Acorrding to the literature[18], chitin represents 14-27% of the dry weight of shrimp processing wastes. Chitin is the second most abundant natural polysaccharide after cellulose and consists of a linear chain of linked 2-acetoamido-2-deoxy-ß-D-glucopyranose units[19]. Because of the compact structure of chitin, it is insoluble in most solvents and to dissolve it, highly toxic solvents such as lithium chloride and dimethylacetamide are used. Therefore, the chemical modifications of chitin are needed to improve its solubility[20,21].

The most common derivative of chitin is chitosan which possesses free amine groups which are an active site in many chemical reactions and can be obtained by partial deacetylation of chitin[17]. When the degree of deacetylation (DDA%) reaches higher than 50%, chitosan becomes soluble in acidic aqueous solutions and it behaves as a cationic polyelectrolyte. Chitosan has been widely used in vastly diverse fields, ranging from waste management to food processing, medicine and biotechnology[22]. It becomes an interesting material in pharmaceutical application[23,24] due to its biodegradability[25,26] biocompatibility[27] and low toxicity[28]. The Degree of N-acetylation (DDA) of chitosan influences not only its physicochemical characteristics[29] but also its biodegradability[26] and immunological activity[30].

The use of ionizing radiations such as ?-radiation can produce electronic excitation as well as ions and may also produce ions and free radicals in excited states. Thus, it is important that possible chemical effects due to electronically excited groups as well as to ionized groups may occur[31,32].

The objective of the present work is the improvement of the extraction method of chitin from shrimp waste by using radiation to obtain more important natural polymer chitosan. The obtained chitin will be characterized and deacetylated to the more useful chitosan. The influence of gamma irradiation on chitin and chitosan production processes such as time needed for deproteination, demineralization and deacetylation were investigated.

MATERIALS AND METHODS

Materials

Local shrimp head and shell waste samples were obtained from Rod El-Farag Fish Market in Cairo. The reference chitin and chitosan were obtained from (Sigma-aldrich Lab. Germany). Sodium hydroxide, hydrochloric acid, ethanol and acetone, were obtained from (El-Nasr for pharmaceuticals and chemicals company, Egypt).

Techniques

Gamma Radiation Source

Irradiation of samples was carried out using gamma rays Co-60 source at the dose rate of 3.32 kGy/h.

Extraction of chitin from shrimp waste

Shrimp wastes were washed several times with tap water to remove meat residues and other contaminants and then desiccated at room temperature to a constant weight (1500 g). The dried waste samples were minced to powder with a food blender and passed through a 250 µm sieves. Then, Shrimp waste powders were divided into three parts; first non irradiated to gamma ray, second and third packaged in PE bags and irradiated at doses of 20 & 25 kGy, respectively. Then, non- irradiated and irradiated samples were subjected to demineralization and deproteination.

1. Demineralization

Demineralization was carried out at room temperature using 1 M hydrochloric acid bathes. The number of bathes depends on the emission of CO_2 gas. The resulting solid was washed with distilled water until neutrality. Then, the demineralized samples were dried and weighed.

2. Deproteination

Deproteination was performed using alkaline treatments with 1 M sodium hydroxide solutions at 100°C. The treatment was repeated several times. The number of bathes depends on clarity of the solution. Absence of proteins was indicated by the absence of color of the medium at the last treatment cycle which was left overnight. Washing with distilled water was then carried out up to neutrality. Finally, it was washed with hot ethanol then boiled in acetone to remove any impurities. The purified chitin was then dried. The chitin content was determined from the weight differences of the raw materials and that of the chitin obtained after acid and alkaline treatments.

3. Deacetylation of Chitin

The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with strong alkaline solution. Preliminary experiments were carried out by steeping different chitin samples for one day in strong sodium hydroxide solution (40 wt %) at room temperature, then refluxing in the same alkali at 135°C. Samples were then washed with distilled water until neutral. Finally, it was washed with hot ethanol and later boiled in acetone to remove any impurities. The purified chitosan was then dried at room temperature and weighed.

Determination of degree of deacetylation

Three methods were used to determine the degree of deacetylation; potentiometric titration, IR base line and Nuclear magnetic resonance (NMR).

1. Potentiometric titration:

Potentiometric titration was carried out according to the method described elsewhere[33], in brief, Chitosan (0.1 g) was dissolved in 20 ml of 0.1 M standard hydrochloric acid solution. The titrant was a solution of 0.05 M sodium hydroxide. pH meter used for pH measurements under continuous stirring, the titrant was added until the pH value reached 2.00 which taken as starting point, the standard sodium hydroxide was then added stepwise and the pH values of the solution were recorded and a curve were drawn between solution pH and volume of alkali added, which produced an integral curve with two inflection points and allows the determination of DDA% of the chitosan.
The degree of acetylation DDA% was calculated using following equation:-

$$DDA\% = \left(\frac{203Q}{1+42Q} \right) \times 100 \qquad (1)$$

Where, $Q = \frac{N\Delta V}{m}$, ?V is the volume difference of NaOH solution between the two inflection points (in liter), N is the concentration of NaOH (in mol/l in this paper 0.05 mol/l) and m is the dry weight of chitosan (in g), and 203 and 42 are the respective molecular weights of N-acetyl glucosamine (Chitin skeleton unit) and a acetyl group, respectively.

2. Fourier transform infrared (FT-IR) spectroscopy

Chitin and chitosan samples prepared quantitatively in the forms of potassium bromide (KBr) disks according to the literature[34]. Briefly, a disk was made from 40 mg of chitin or chitosan powder and 120 mg of KBr. The spectra of chitin and chitosan samples were obtained using an FT-IR spectrophotometer (JASCO FT/ IR-6300, Japan in the range of 400-4000 Cm^{-1}). The degree of deacetylation (DDA%) of the chitosan samples was calculated using baseline method as proposed in literature[35,36]. The computation equation is given below:-

$$DDA\% = 100 - [(A_{1655} / A_{3450}) \text{ X } 100 / 1.33] \quad (2)$$

where A_{1655} and A_{3450} were the absorbance at 1655 cm^{-1} of the amide-I band as a measure of the N-acetyl group content and 3450 cm^{-1} of the hydroxyl band as an internal standard to correct for film thickness or for differences in chitosan concentration powder form. The factor '1.33' denoted the value of the ratio of A_{1655} / A_{3450} for fully Nacetylated chitosan.

3. Nuclear magnetic resonance (NMR)

The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^1H spectra were run at 300 MHz in dimethylsulphoxide (DMSO-d6). Chemical shifts are quoted in d and were related to that of the solvents. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 e.V. The degree of deacetylation (DDA%) of the chitosan samples was calculated using ^1H.NMR which was proposed by literature[37] from the following equation:-

$$DDA\% = 100 - [(1/3.I_{CH3} \text{ X } 100) / I_{H1}] \quad (3)$$

Where I_{CH3} was the integral of the hydrogen atom in $NHCOCH_3$ group and I_{H1} corresponded to the hydrogen atom of C1 in glucosamine units.

Intrinsic Viscosity and Average Molecular Weight:

Chitosan samples were dissolved in a solvent mixture (0.3 M acetic acid and 0.2 M sodium acetate). An Ubbelhode capillary viscometer was used to measure the passage time of the solutions flowing through a capillary at room temperature. The intrinsic viscosity of the solution ? was obtained by extrapolating the reduced and inherent viscosity versus concentration data to zero concentration and the viscosity average molecular weight of chitosan (Mw) was determined by Mark–Houwink–Sakurada's empirical equation, reported by literature[38], that relates the intrinsic viscosity to the polymer's molecular weight as follows:

$$? = kM^a \quad (4)$$

where M is the average molecular weight, (k) in mL/g and (a) dimensionless, are the constants that depend on the solvent–polymer system (k = 0.076 ml/g & a = 0.76)[39].

***X*–Ray Diffraction Analysis (XRD):**

The XRD patterns of the prepared chitin and chitosan samples were measured using Shimadzu XRD 6000 diffractometer with Cu target. The XRD runs were carried out over the 2? ranging from 10^o to 40^o at a scan speed of 8^o/min. The crystalline index (ICR) was calculated from normalized diffractograms was determined according to the method currently applied to polysaccharide diffraction studies[40, 41] after the mathematical treatment of the peaks corresponding to its deconvolution and application of the Lorentzian function. The intensities of the peaks at [110] lattice (I_{110}, at 2? ~ 20^o corresponding to the maximum intensity) and at 2? = 16^o (amorphous diffraction) were used to calculate ICR (Eq. (4)):

$$ICR = \left(\frac{I_{110} - I_{am}}{I_{110}} \right) \times 100 \qquad (5)$$

Thermal Gravimetric Analysis (TGA):

All thermal gravimetric analysis (TGA) for the investigated samples were performed under nitrogen atmosphere at a flow rate of pure nitrogen gas 50 ml/min., heating rate was 10°C /min. from ambient up to 600°C using Perkin Elmer Pyris 6.

RESULTS AND DISCUSSION

Preparation of chitin and chitosan from shrimp waste

Chitin was extracted from non irradiated and irradiated shrimp waste by chemical method which involves various major steps: extraction of protein (deproteination), followed by elimination of inorganic matter (demineralization)

1. Deproteination

Proteins are bound by covalent bonds to the chitin through aspartyl or histidyl residues or both, thus forming stable complexes such as glycoproteins[42]. Table (1) shows the effect of irradiation dose on the deproteination time of non-irradiated and irradiated shrimp shells powder. It is observed that irradiation at 25 kGy of shrimp shell powders allows reducing the time of deproteination from 6 h to 2 h and that may be due to irradiation of proteins at such dose causes their degradation[43] .Cost saving were achieved as a result of reducing the time of deproteination process.

2. Demineralisation

The most important minerals in shrimp shells are calcium carbonate and calcium phosphate[42]. The residual Ca in the shells treated with 1 M hydrochloric acid at room temperature with agitation to convert calcium carbonate to calcium chloride. Irradiation at 25 kGy of shrimp shell powders allows reducing the time of demineralisation from 10 h to 2 h as shown in Table (1) and this may be due to radiation affect minerals complexation.

Samples deproteinised, demineralised, were rinsed to neutrality, washed with hot ethanol and later boiled in acetone to remove any impurities and dried at 60^oC. The resultant white insoluble powder is a chitin.

3. Deacetylation of Chitin

Deacetylation is the process that allows converting chitin to chitosan by the removal of acetyl groups. Non-irradiated and irradiated chitin samples were N-deacetylated by refluxing in aqueous solutions of concentrated NaOH (40 wt%). To speed up the process, the chitin was steeped in the same concentrated sodium hydroxide solution for 24 h at room temperature before refluxing process[44]. The time of refluxing process differs from 8, 3, 2 h for chitin (non-irradiated and irradiated at 20, 25 kGy) samples, respectively as shown in Table (1). The irradiation of chitin at the dose 25 kGy causes their degradation. The obtained chitosan was soluble in diluted acids and the weight average molecular weight, degree of deacetylation was determined[45].

Table (1): Effect of irradiation dose on deproteination, demineralization and deacetylation time of non-irradiated and irradiated shrimp shell powder.

Dose (kGy)	Deproteination time (h)	Demineralisation time (h)	Deacetylation time (h)
0	6	10	8
20	4	3	3
25	2	2	2

Chitin and Chitosan Yield

Chitin and chitosan yield was calculated as the dry weight of chitin and chitosan obtained from 1500 g of dried non-irradiated and irradiated shrimp shell powder. Table (4) shows the chitin and chitosan yields extracted from both non-irradiated and irradiated shrimp shell powders. It was observed that the highest chitin yield was obtained from non-irradiated sample followed by samples irradiated at 20 & 25 kGy, respectively and this may be due to scission of glycosidic bonds by radiation leading to loss of sample mass/weight by degradation of polymer chain. Yield of the obtained chitosan is lower than that of the obtained chitin due to loss of sample mass/weight from excessive removal of acetyl groups from the polymer during deacetylation. It was also observed that yield of chitin and chitosan extracted from irradiated shrimp shell powder lower than that obtained from non irradiated one, but chitosan extracted from irradiated sample has more advantages is it has lower molecular weight and higher degree of deacetylation.

Table (4): Chitin and chitosan production yield from both non-irradiated and irradiated shrimp shell powder.

Sample	Weight of shrimp shell waste (g)	Chitin yield (g)	Chitosan yield (g)
Non irradiated sample	500	116	50
Irradiated sample at 20 kGy	500	83	33
Irradiated sample at 25 kGy	500	72	29

Characterization and Properties of Prepared Chitin and Chitosan

The samples of chitosan extracted from shrimp shell waste were characterized by determination degree of deacetylation (DDA%), average molecular weight. Chitin and chitosan were characterized by fourier transform infrared spectroscopy, thermogravimetry analysis (TGA) and X-ray diffraction.

1. Determination of the Degree of Deacetylation

Potentiometric Titrations

Typical pH-potentiometric titration curves are shown in Figure (1). At around a 70% degree of titration, precipitation occurred in the solutions and in this range the response of the glass-electrode became sluggish. However, after the second equivalence point, a reasonably fast electrode response was achieved, again indicating that proton-exchange processes including chitosan are already over in this pH-range. The procedure results in a titration curve with two inflexion points: the first corresponds to the excess of HCl, while the second to the protonated chitosan. The difference between the two inflexion points yields the moles of H+ required for the protonation of the free (deacetylated) amino groups and results in the amount of D-glucosamine (D-GlcN) in the titrant solution. Assuming that the rest of the sample is N-acetyl-D-glucosamine (D-GlcNAc), the DDA% value of the specimen can be calculated using Eq. (1) as mentioned in experimental part and resulting data were recorded in table (2)[46]. It was noted that chitosan sample obtained from irradiated sample at 25 kGy have high degree of deacetylation and this can be interpreted as a result of radiation induced controlled decrease in molecular weight which mainly due to scission of glycosidic bond by radiation [47]. It may be attributed that at high radiation dose, chain length of polymer are reduced which make it more flexible. Flexible chains of polymer facilitate mobility and removal of acetyl groups from the polymer leading to increase number of NH_2 groups thus DDA% increased.

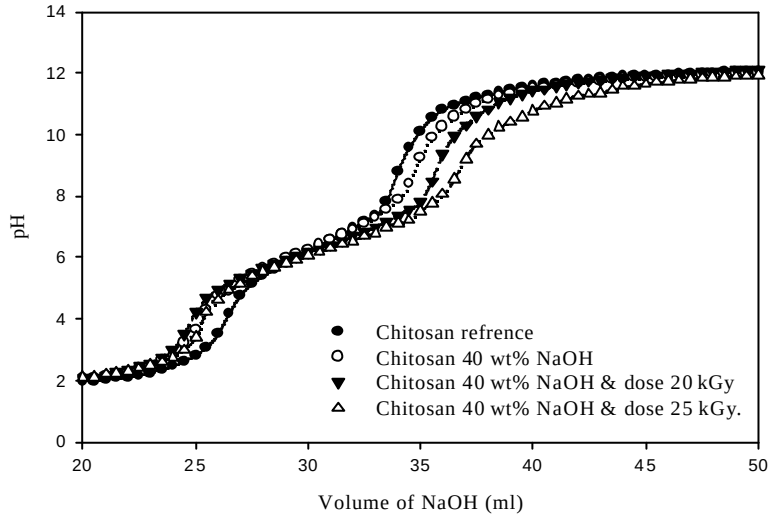

Fig. (1): Determination of the degree of acetylation (DDA%) from potentiometric titration of chitosan prepared from both non irradiated and irradiated shrimp shells powder.

Fourier Transform Infrared (FT-IR) Spectroscopy

Infrared spectroscopy can be used to determine DDA% and to characterize the structures of the polymer. The DDA% of the samples can also calculated from IR spectra. Several authors report on the possibility of determining the DDA% of chitosan by comparing a peak that is proportional to the DDA% (measurement peak) to one that is independent of the DDA (reference peak). The DDA% was determined using eq.(2) as mentioned in experimental part and resulting data were recorded in table (2). The degree of deacetylation calculated from infrared method was found to be very close to the values obtained by potentiometric titration method.

Table (2): DDA values of chitosan prepared from both non irradiated and irradiated shrimp waste powders determined by different methods.

Sample	Potentiometric method		IR method
	?V (ml)	DDA (%)	DDA%
Chitosan reference	7.5	65.7	62.0
Chitosan from non irradiated sample	9.5	80.4	77.0
Chitosan from irradiated sample at 20 kGy	11.0	90.7	88.0
Chitosan from irradiated sample at 25 kGy	11.5	94.0	92.0

Infrared spectroscopy can also be used to characterize the structure of the polymer. Figure (2) shows FTIR spectra of chitin reference and chitin prepared from both non irradiated and irradiated shrimp shells powder. The bands observed at 3271 and 2894 cm^{-1} correspond to the vibrational stretching of the hydroxyl groups and –CH– stretch chitin, respectively. Another absorption band appears at 1656 and 1420 cm^{-1} corresponds to the (amide I) stretching of C=O bonds of the acetamide groups and symmetric deformation of CH_3. The band at 1316 cm^{-1} corresponds to a CO–NH deformation and to the CH_2 group (amide III), due to the formation of CO–NH group, and at 1560 cm^{-1} corresponds to N–H deformation of amino group (amine II)[48,49]. The peaks observed at 1070 and 1029 cm^{-1} were the secondary hydroxyl group (characteristic peak of –CH–OH in cyclic alcohol, C–O stretch) and the primary hydroxyl group (characteristic peak of $–CH_2–OH$ in primary alcohol, C–O stretch), respectively. The absorption band at 1153 cm^{-1} was the asymmetric stretching of the C–O–C bridge[50]. It was also observed that chitin prepared from irradiated sample is more broader hydroxyl band at 3200-3500 cm^{-1} due to glycosidic bonds (C_1-O-C_4 group) decrease with increasing radiation doses leading to hydroxyl group formation[51].

Figure (3) shows an FTIR of chitosan, a strong and broad band due to the axial stretching of O–H and N–H bond observed between 3500 and 3100 cm-1, centered at 3400 cm^{-1}, the band at 1590 cm^{-1} has a larger intensity than at 1655 cm^{-1}, which suggests effective deacetylation. When chitin deacetylation occurs, the band observed at 1655 cm^{-1} decreases, while a growth at 1590 cm^{-1} occurs, indicating the prevalence of NH_2 groups[52]. When the same spectrum is observed in which the band from 1500 to 1700 cm^{-1} is stressed, indicated that there was an intensification of the peak at 1590 cm^{-1} and a decrease at 1655 cm^{-1},that suggests the occurrence of deacetylation. It was also noted that the intensity of the peak at 1590 cm^{-1} increases by increasing irradiation dose due to increasing the degree of deacetylation[53].

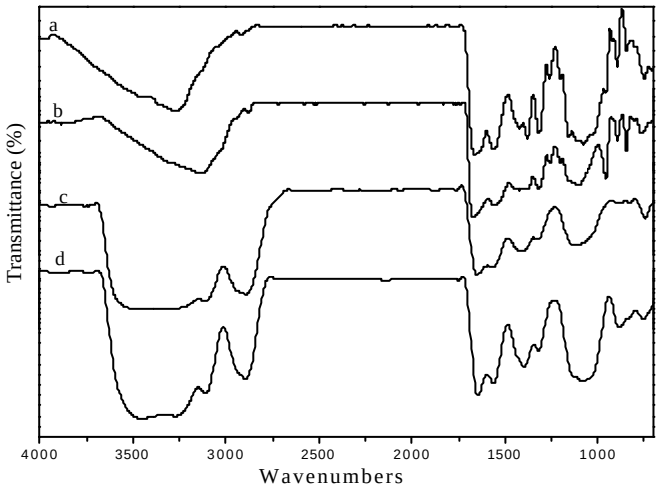

Fig. (2): FTIR spectra of (a) Chitin reference and chitin prepared from (b) non irradiated sample, (c) irradiated sample at dose 20 kGy, (d) irradiated sample at dose 25 kGy.

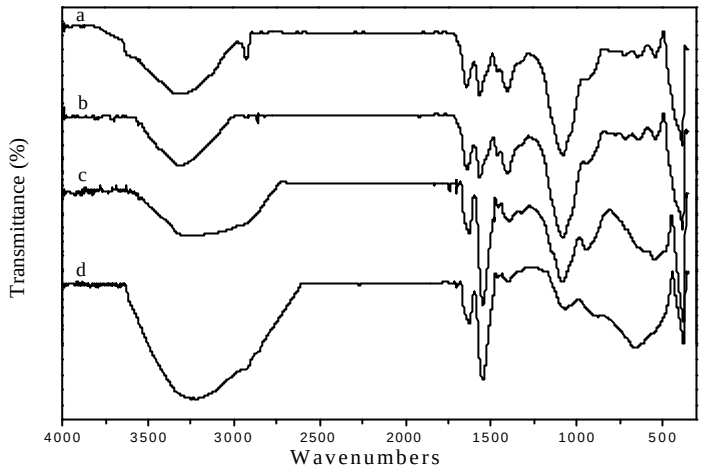

Fig. (3): FTIR spectra of (a) Chitosan reference and chitosan prepared from (b) non irradiated sample , (c) irradiated sample at dose 20 kGy, (d) irradiated sample at dose 25 kGy.

3. Nuclear Mmagnetic Resonance (NMR)

Nuclear magnetic resonance can be used to determine DDA% and to identify the structure of the polymer. The DDA% of the samples can also calculated from the ^{1}H.NMR spectrum using eq.(3) as mentioned in experimental part. The DDA% value was determined from the integral of $-CH_3$ signal at 1.97 ppm compared with the integral of H-1 protons considered as internal standard[54]. The evidence of higher degree of deacetylation of prepared chitosan could be seen in the ^{1}H.NMR spectrum (Fig. 4a, b) with lower intensity in the N-acetyl peak at 1.97 ppm than that of the reference chitosan Fig. (4a). The calculated value of DDA% was 67% for chitosan reference and for chitosan prepared from irradiated shrimp shell powder at 25 kGy was 95%. The degree of deacetylation calculated from ^{1}H.NMR spectrum method was found to be very close to the values obtained by potentiometric titration.

^{1}H.NMR spectra can also be used to identify the structure of the polymer. Fig. (4a,b) shows the ^{1}H.NMR spectra of chitosan reference and chitosan prepared from irradiated shrimp shells powder at 25 kGy, respectively. The peak around 1.97 ppm can be due to the methyl protons in the acetamide group, the peak at 4.9 ppm is assigned to C1 proton of glucosamine unit in chitosan and the peaks in 3-4 ppm due to C2-C6 protons of glucosamine and N-acetylglucosamine units. They also showed that C1 proton of N-acetylglucosamine unit appears around 4.6 ppm[55]. Fig. (4a) shows the peak resonance between 1.0-1.5 ppm which is assigned to methyl proton of protein and absence of this peak in Fig. (4b) gives a good indication of the purity of prepared chitosan sample[45].

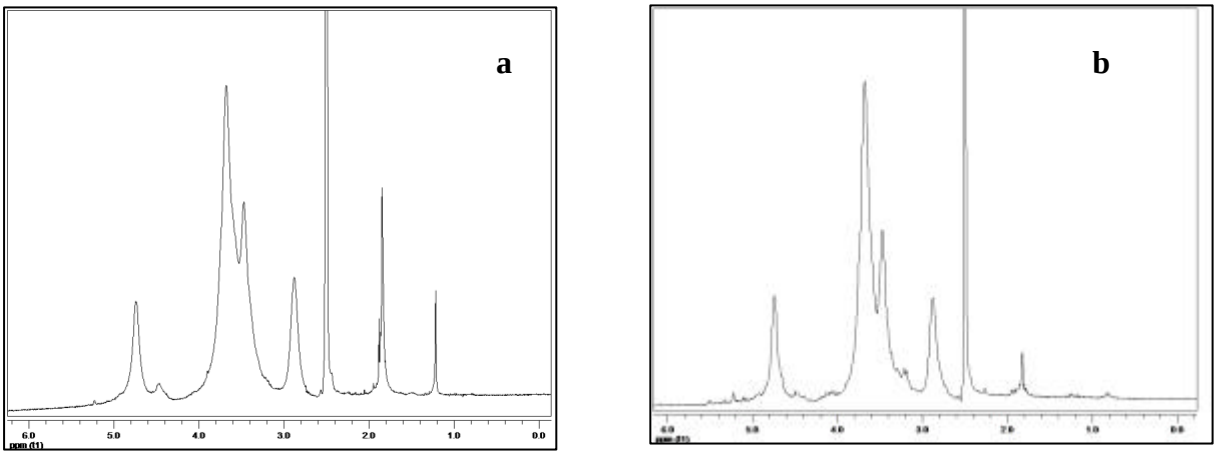

Fig. (4): ^{1}H.NMR spectra of (a) chitosan reference and (b) chitosan extracted from irradiated shrimp shell powder at dose 25 kGy.

Determination of the Intrinsic Viscosity and Average Molecular Weight:

In order to evaluate the molecular weight of polymeric chains, viscometry is commonly selected as it is one of the simplest and most rapid methods for determining their molecular weights. A well established correlation between viscosity and molecular weight is given by eq.(4) as mentioned in experimental part. Table (3) displays the changed in the intrinsic viscosities and corresponding molecular weights of chitosan obtained from non irradiated and irradiated shrimp shell powders. It is observed that the molecular weight of chitosan decreases continuously with increasing radiation dose from 1.2×10^{6} to 7.7×10^{4} mainly due to chain scission at 1,4-glycosidic bonds by radiation, the obtained product dissolved completely in acetic acid to a clear solution. This implies that ?-ray irradiation induces chain degradation[51].

Table (3): Average molecular weight and intrinsic viscosity data of chitosan.

Sample	Intrinsic viscosity, ? (L/g)	Molecular weight (g/mole)
Chitosan reference	19.7	1.5×10^{6}
Chitosan from non irradiated sample	18.5	1.2×10^{6}
Chitosan from irradiated sample at 20 kGy	14.3	8.7×10^{4}
Chitosan from irradiated sample at 25 kGy	13.1	7.7×10^{4}

Thermogravimetry Analysis (TGA)

Figures (5a, b) show TGA curves of chitin and chitosan samples, all curves show that weight loss occurs in two stages. The first stage starts around 90, 110 ^{o}C for chitin and chitosan respectively and the second stage starts around 280 ^{o}C for all chitin samples, chitosan reference and 251 ^{o}C for chitosan prepared from non irradiated and irradiated samples. The first stage is assigned to the loss of water because polysaccharides usually have a strong affinity for water and therefore may be easily hydrated. The second one corresponds to the thermal decomposition of main chain of chitin and chitosan, vaporization and elimination of volatile product[45].

The decomposition temperature of chitin reference is higher than that of prepared chitin samples and this may be due to different extraction sources, as chitin reference was extracted from crayfish while in this paper chitin extracted from shrimp shells. Also, the decomposition temperature of chitin

is higher than that of corresponding chitosan. This result indicates that chitin exists as a stable structure toward thermal decomposition than chitosan. Also there is no difference between the thermal stability for chitin and chitosan prepared from non irradiated and irradiated shrimp shells powder.

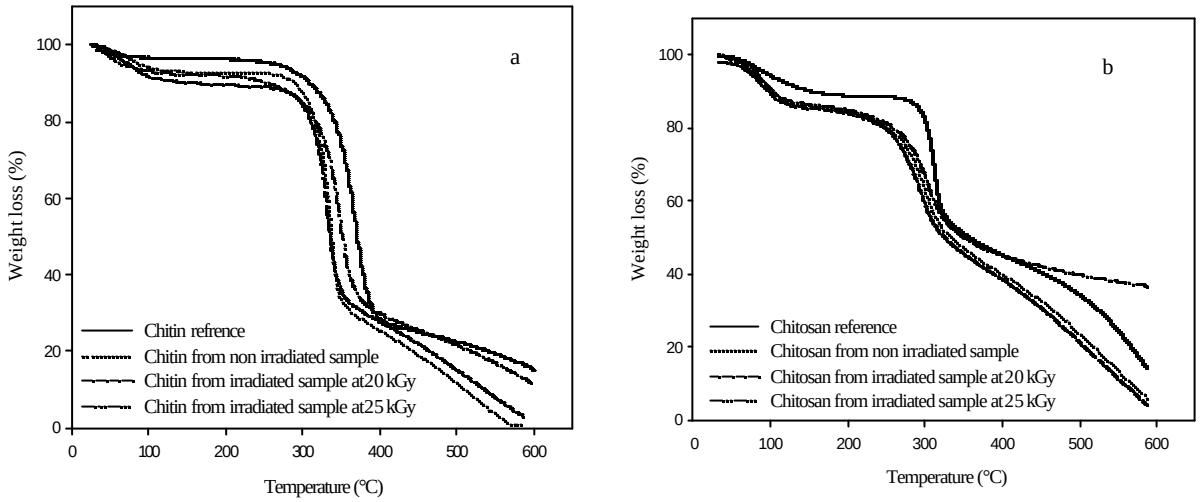

Fig. (5): TGA thermal diagram for (a) Chitin and (b) Chitosan those irradiated and non-irradiated samples.

X-Ray Diffraction

XRD analysis was applied to detect the crystallinity of the extracted chitin and chitosan. Figure (6) represents XRD pattern for chitin prepared from both non irradiated and irradiated shrimp shell powder. It is observed strong reflections at 2? = 9.3° and 2? =19.2°. The band at 9.6° is due to the incorporation of bound water molecules into the crystal lattice. The intensity of peak at 2? = 9.6° increases with increasing radiation dose due to hydroxyl group formation.

Figure (7) represents XRD pattern for corresponding deactylated chitosan. It is observed that, the sharpness of the bands are higher in the chitin samples than in their chitosan analogue with slight decrease in the crystallinity percent of chitin after deacetylation reaction. Peaks corresponding to the angle 2?=20.1° in XRD of chitosan were less resolved and shifted to higher 2?.

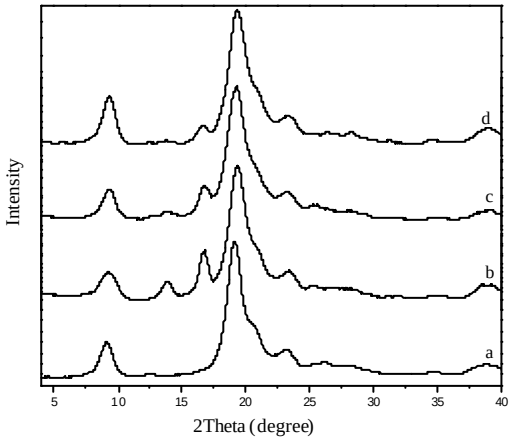

Fig. (6): X-ray diffraction patterns of (a) chitin reference, and chitin prepared from (b) non irradiated sample , (c) irradiated sample at dose 20 kGy, (d) irradiated sample at dose 25 kGy.

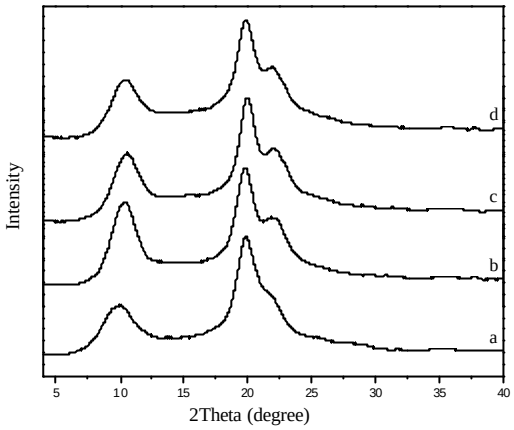

Fig. (7): X-ray diffraction patterns of (a) chitosan reference, and chitosan prepared from (b) non irradiated sample , (c) irradiated sample at dose 20 kGy, (d) irradiated sample at dose 25 kGy.

The crystalline index of chitin and chitosan was calculated from the X-ray diffraction data (Table 5) according to eq.(5) as mentioned in experimental part, confirming that chitin is more crystalline than chitosan samples. Chitin, chitosan prepared from non irradiated shrimp shell powder exhibits higher crystallinity than that prepared from irradiated one.

Table (5): Values of ICR for chitin and chitosan

Sample	ICR (%)	
	Chitin	Chitosan
Reference sample	73	56
Non irradiated sample	78	58
Irradiated sample at 20 kGy	76	50
Irradiated sample at 25 kGy	63	44

CONCLUSION

Chitin and chitosan were extracted from local shrimp waste by chemical method. The influence of ?-irradiation on the extraction processes before chemical treatment was investigated. It was found that the irradiation dose at 25 kGy, causes the reduction of time of deproteination, demineralization and deacetylation processes by a factor of three, five and four fold, respectively comparing with non irradiated samples. It was also noticed that, chitosan prepared from irradiated sample has higher solubility, DDA% and lower molecular weight.

REFERENCES

(1) M.S. Islam, S. Khan, and M. Tanaka ; Marine Pollution Bulletin ; 49, 103 (2004).
(2) P. Beaney, J. L.Mendoza, and M. Healy ; J. Chem Technol. Biotechnol; 80, 145(2005).
(3) E.L.Johnson, and Q.P. Peniston "Utilization of shellfish waste for chitin and chitosan production", In Chemistry and Biochemistry of Marine Food Products; Martin, R.E., Flick, G.J., Hebard, C.E., Ward, D.R., Eds.; AVI Publishing:Westport, CT; 19 (1982).
(4) J.H. Green, and A. Kramer. Food Processing Waste Management. AVI publishing Co.,Westport, CT; 214 (1984).

(5) J.H. Green, and J.F. Mattick. Fishery waste management. In "Food Processing Waste Management", Green, J.H. and Kramer, A. (Eds.). AVI Publishing Co.,Westport, CT; 202 (1979).
(6) M.G. Healy, C.R. Romo, and R. Bustos.; Resources, Conservation and Recycling; 11,139(1994).
(7) H. K. No, and S. P. Meyers; Journal of Aquatic Food Product Techn.; 4, 27 (1995).
(8) A. Percot, C.Viton, and A. Domard ; Biomacromol.; 4, 12 (2003).
(9) A. Gildberg, and E. Stenberg ; Process Biochem.; 36, 809 (2001).
(10) J. Synowiecki, and N. A. A. Q. Al-Khateeb, Food Chem.; 68, 147 (2000).
(11) O. Cremades, J. Parrado, M.C. Alvarez-Ossorio, M. Jover, L. C. Teran, J. F. Gutierrez, and J. Bautista ; Food Chemistry; 82, 559 (2003).
(12) M. S. Heu, J.S. Kim, and F. Shahidi; Food Chemistry; 82, 235 (2003).
(13) H. M. CHEN, and S. P. MEYERS; J. Food. Sci; 48,1516 (1983).
(14) J. Ferrer, G. Paez, Z. Marmol, E. Ramones, H. Garcia, and C.F. Forster; Bioresource Technology; 57, 55 (1996).
(15) A.T. Quitain, N. Sato, H. Daimon, and K. Fujie ; Ind. Eng. Chem. Res.; 40, 5885 (2001).
(16) C. R. Allan, and L. A. Hadwiger ; Exp. Mycol.; 3, 285 (1979).
(17) R.A.A. Muzzarelli. "Chitin", Pergamon Press, New York (1977).
(18) N.A. Ashford, D. Hattis ; Murray, A.E. Industrial prospects for chitin and protein from shellfish wastes; MIT Sea Grant Report MISG 77-3:MIT: Cambridge, MA. (1977).
(19) P.R. Austin, C.J. Brine, J.E. Castle, and J.P. Zikakis ; Science; 212, 749 (1981).
(20) M.G. Peter ; J. Macromol. Sci., Part A: Pure Appl. Chem.; A32, 629 (1995).
(21) J. Z. Knaul, S.M. Hudson, and K.A.M. Creber ; J. Polym. Sci.: Part B:Polym. Phys.; 72, 1079 (1999).
(22) V. Savant, and J.A. Torres ; American Chitoscience Society ; 1, 1 (1995).
(23) L. Illum.; Pharm. Res. 15, 1326 (1998).
(24) Y. Shigemasa, and S. Minami, Biotechnol. Genet. Eng. Rev.; 13, 383 (1995).
(25) Y. Shigemasa, K. Saito, H. Sashiwa, and H. Saimato, Int. J. Biol. Macromol.; 16, 43 (1994).
(26) R. J. Nordtveit, K. M. Varum, and O.Smidsrod; Carbohyd. Polym.; 29, 163 (1996).
(27) G. Bochard, and H.E. Junginger; Adv. Drug. Del. Rev.; 52, 103 (2001).
(28) J. Karlsen, and O. Skaugrud ; Manuf. Chem. 62, 18 (1991).
(29) N. Zydowicz, L. Vachoud, A. Domard, in: A. Domard, C. Jeuniaux, R. Muzzarelli, E. Roberts (editors), Advances in Chitin Science, Vol. 1, Lyon: Andre J; p.262 (1996).
(30) G. Peluso, O. Petillo, M. Ranieri, M. Santin, L. Ambrosic, D. Calabro, B. Avallone, and G. Balsamo; Biomaterials; 15, 1215 (1994).
(31) L. Y. Lim, E. Khor, and O. Koo; J. Biomed.Mater.Res 43, 282 (1998).
(32) T. U. Rashid, M. M. Rahman, Sh. Kabir, S. M. Shamsuddin, and M. A Khan.; Polym. Int. (2011)
(33) K.C. Gupta, and F. H. Jabrail.; Carbohyd. Polym.; 66, 43 (2006).
(34) T. A. Khan, K. K. Peh, and H. S. Chng ; J. Pharm. Pharmaceut. Sci.; 5, 205 (2002).
(35) J. G. Domszy and G. A. F. Roberts ; Die. Makromol. Chem.; 186, 1671 (1985).
(36) A. Baxter, M.Dillon, K.D.A. Taylor, and G. A.F. Roberts ; Int. J. Biol. Macromol; 14, 166, (1992).
(37) N. T. An, D. T. Thien, N. T. Dong, and P. L. Dung; Carbohyd. Polym.; 75, 489 (2009).
(38) G. A. F. Roberts, and J. G. Domszy; Int. J. Biol. Macromol.; 4, 374 (1982).
(39) M. Rinaudo, M. Milas, and P. L. Dung ; Int.J.biol.Macromol.,15,281(1993).
(40) B. Focher, P.L. Beltrame, A. Naggi, and G. Torri; Carbohyd. Polym. ; 12, 405 (1990).
(41) R. L. Lavall, O. B.G. Assis, and S. P. Campana-Filho ; Bioresource Tech.; 98, 2465 (2007).
(42) S. O. Fernandez-Kim, Master thesis; B.S. Seoul National University, (2004).
(43) J.G. Von Kooij, and P. Loharanu, IAEA-STI/DOC: 10, 114 (1982).
(44) K. Kurita ; Prog. Polym. Sci. ; 26, 1921(2001).
(45) F.A. Al Sagheer, M.A. Al-Sughayer, S. Muslim, and M.Z. Elsabee; Carbohyd. Polym.; 77, 410 (2009).

(46) N. Balazs, and P. Sipos; Carbohyd. Res.; 342, 124 (2007)
(47) D. Tahtat, C. Uzun, M. Mahlous, and O. Guven ; Nucl. Instr. and Meth. in Phys. Res. B; 265, 425 (2007).
(48) M.L. Duarte, M.C. Ferreira, and M.R. Marvao, J. Rocha; Inter. J. Biol. Macromol; 28, 359 (2001).
(49) R. Ravindra, K. R. Krovvidi, and A.A. Khan; Carbohyd. Polym. 36, 121 (1998).
(50) G. Ma, D.Yang, Y. Zhou, M. Xiao, J. F. Kennedy, and J. Nie ; Carbohyd. Polym.; 74, 121 (2008).
(51) R.Yoksan, M. Akashi, S. Biramontri, and S. Chirachanchai; Biomacromol.; 2, 1038 (2001).
(52) F. Bordi, C. Cametti, and G. Paradossi ; J. Phys. Chemi. ; 95, 4883 (1991).
(53) A. T. Paulino, J. I. Simionato, J. C. Garcia, and J. Nozaki ; Carbohyd. Polym. ; 64, 98 (2006).
(54) A. Tolaimate, J. Desbrieres, M. Rhazi, A. Alagui, M. Vincendon, and P. Vottero ; Polym.; 41, 2463 (2000).
(55) E. S. Abdou, K. S.A. Nagy, and M. Z. Elsabee ; Bioresource Technology; 99, 1359 (2008).