EXTRACTION AND CHARACTERIZATION OF CHITIN AND CHITOSAN FROM LOCAL IRAQI FISH SCALES

Shemaa A. Soud[1*] Maysaa A. Ali[1] Wedean G. Alhadban[2] Ali A. Taha[1]
Assist Prof. Lecturer Lecturer Prof.
[1]Baiotechnology Division, Applied Science Department, University of Technology, Baghdad,Iraq
[2]Department of Biotechnology, College of Science, University of Baghdad, Iraq
E-mail: Shemaa.A.Soud@uotechnology.edu.iq

ABSTRACT

Both chitin and deacylated form of chitosan are considered the second most biopolymer available in nature after cellulose. Chitin and chitosan have an economic value related to their industrial, biological and biomedical applications. In this study, chitin was extracted from of Iraqi carp fish scales (*Cyprinus carpio L.*) and then deacylated into the chitosan. The obtained samples have been distinguished through Fourier transforms infrared spectroscopy (FTIR) and Thermo gravimetric analysis (TGA). The FTIR analysis for chitin showed the characteristic spectra at 1541 cm^{-1} and 1647 cm^{-1} associated to N-H bending vibration and C=O stretching vibration respectively. Thus, the FTIR spectra of chitosan gave characteristics bands of 1647 cm^{-1} for a carbonyl group. Thus, thermogravimetric analysis suggests that chitin had been found to be more stable than chitosan. Moreover, study of physiochemical properties for obtained chitosan was identified the molar mass (MW) of prepared chitosan to be 2.5 x 10^5(g/mol) with 4.5%moisture content, 0.050% ash and insoluble content about 0.59%. The degree of deacetylation (DD) was also studied based on potentiometric titration and FTIR were reached to 57% and 63% respectively. It has been confirmed by the functional properties of the prepared chitosan that it can be commercially used in different fields. Preparing chitosan from fish waste would be minimise the pollutants to the environment.

Key words: biopolymer, chitin, decoloration, demineralisation thermo gravimetric analysis, recycling

استخلاص وتوصيف الكايتين و الكايتوسان من قشور الاسماك العراقية المحلية

شيماء عبد الستار سعود[1] ميساء عادل علي[1] وديان غسان الهدبان[2] علي عبدالرحمن طه[1]
مدرس مدرس مدرس أستاذ
الجامعة التكنولوجية ،قسم العلوم التطبيقية، فرع التقنيات الاحيائية
جامعة بغداد،كلية العلوم ، قسم التقنيات الاحيائية

المستخلص

يعتبر كل من الكايتين والكايتوسان ثاني أكثر البوليمرات الحيوية المتوفرة في الطبيعة بعد السليلوز. الكايتين والكايتوسان لهما قيمة اقتصادية مرتبطة بتطبيقاتهما الصناعية والبيولوجية والطبية الحيوية. في هذه الدراسة ، تم استخلاص مادة الكايتين من قشور اسماك الكارب العراقية (*Cyprinus carpio L.*)، ومن ثم أزالة مجاميع الاستيل وتحويله الى الكايتوسان. تم تمييز العينات التي تم الحصول عليها من خلال التحليل الطيفي للأشعة تحت الحمراء (FTIR) والتحليل الوزني الحراري (TGA). حيث أظهر تحليل FTIR للكايتين الأطياف تشخيصيه له عند 1541 سم $^{-1}$ و 1647 سم $^{-1}$ المرتبطة باهتزاز الانحناء N–H و اهتزاز التمدد C=O على التوالي. في حين، أعطت FTIR للكيتوسان طيف امتصاص لمجموعة C=O عند 1647 سم $^{-1}$. اوضحت نتائج TGA أن الكايتين وجد أنه أكثر استقرارًا بامقارنة مع الكايتوسان.علاوة على ذلك ، حددت دراسة الخواص الفيزيوكيميائية للكيتوسان المتحصل عليه . اوضحت الدراسة ان الكتلة المولية للكايتوسان المحضر هو 2.5 × 105 (جم / مول) ، 4.5٪ محتوى رطوبة ، 0.050٪ رماد و كان المحتوى الغير قابل للذوبان مقارب الى 0.59 ٪. كما تمت ايضا تحديد درجة ازلة مجموعة الاستيل (DD) بناءً على كل من معايرة الجهد و FTIR واظهرت النتائج انها كانت مساوية الى 57٪ و 63٪ على التوالي. تم التأكيد من خلال الخصائص الوظيفية للكايتوسان المحضر أنه يمكن استخدامه تجاريًا في مجالات مختلفة. كما ان تحضير الكايتوسان من مخلفات الأسماك سيقلل من الملوثات البيئة.

الكلمات المفتاحية: البوليمر الحيوي، الكايتين، إزالة اللون، نزع المعادن، التحليل الحراري الوزني، اعادة تدوير
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INTRODUCTION

Most of industrial fish processes produce wastes in different forms, including bones, scales and meat. Such waste materials generate pollutants and pollute the environment and coastal areas (5,18).Fishery waste appears to be easily ruined via bacteriological processes and enzyme activity that attract different vermines, rodents and flies. However, about 30-40% from the solid waste result from the fish processing industry (10).This fish waste contains a large quantity of proteins, minerals, fats and chitin (22, 23, 24). Both chitin and deacylated form chitosan are considered the second most biopolymers available in nature after cellulose (11,12,14). Chitosan is a polycation heteropolysaccharide of both N-acetyl-glucosamine and D-glucosamine, which can differ in relative amounts over a wide range, resulting in chitosan with varying degrees of deacetylation from 75% to 95% (16, 20). Since chitin biodegradation is very sluggish, the accumulation of significant quantities of discards from the processing of crustaceans has become a major problem in the processing of seafood. Thus, recycling those by-products is necessary (2, 3, 6, 25). Chitin and its derivatives are very important biomolecules with versatile biological activities and they demonstrate biocompatibility and biodegradability. These results in widespread use of chitin and its derivatives in the medical, cosmetics, food ingredients, membranes, agriculture, fabric industries and wastewater treatment (4, 17, 28, 19). The objective of the present work is synthesis chitosan by extraction chitin from local Iraqi fish scales as a recycling way for fish west. Acid and alkaline additions were used for chitin extraction, then potassium permanganate and oxalic acid used for decoloration and for chitosan preparation sodium hydroxide was added for further N-deactylation. The extract chitosan was characterized by FTIR, TGA and both physiochemical properties and degree of deacetylation (DD) was also studied.

MATERIAIS AND METHODS

Materials: About 5 kg from Iraqi carp fish (*Cyprinus carpio L.*) were obtained from the local Iraqi fish market in Baghdad. Then 100 g from pre-cleaned dry fish scales was used in our study. HCL, Potassium permanganate, Oxalic acid and NaOH were purchased by Sigma, Germany.

Extraction of Chitin and Chitosan

Methods: Fish scales from (*Cyprinus carpio L.*) were scraped free from loose tissue in the first step, followed by washing and drying as shown in figure 1. Chitosan was prepared from fish scales using a general process consisting of demineralisation, decolourisation and deacetylation. Dried scales were soaked in 1% w/v of HCL solution for 36 hrs. After that scales were washed and kept for 48 hr in oven at 30°C for drying, then placed for 36 hrs in (2N) NaOH solution for demineralization. Subsequently, for decolourization process the fish scales were kept for 1hr in 1% of KMnO4 solution, then in 1% w/v of oxalic acid. At the end of this step chitin was obtained, which was additional soaked for 6hrs in 50 % w/v of NaOH for deacetylation leading to chitosan as the end product (15).

Characterization

Fourier transforms infrared spectroscopy (FTIR): Infrared spectra for both prepared chitin and chitosan thoroughly mixed with KBr pellets were achieved using IR Prestige-21, FTIR Spectrometer Shimadzu, over the frequency range 4000-400cm^{-1}.

Thermo gravimetric analysis (TGA)

TGA was done using Shimadzu Simultaneous TGA/DTA Analyzer. About 1 mg from prepared chitin and chitosan were weighed and heated at (10 °C -1000°C) with 10°C/min temperature ramping.

Figure 1. (a) Carp Iraqi fish (*Cyprinus carpio L.*) and (b) washed-dried fish scales.

Physiochemical properties

Viscosity average molar mass: Ubbelohde Viscometer was used to measurement the viscosity and efflux time of chitosan which was determined at a 25 ± 0.1°C. The solvent mixture of 0.3 M acetic acid/0.1 M sodium acetate was used as a solvent for chitosan samples. Mark- Houwink relationship was using to estimate the viscosity average molar mass (MW) (19).

$$[\eta] = K [MW]^{a}$$

Where $K = 0.76 \times 10^{-1}$ cm^3/g and a = 0.76

The mean of three replicates was used from measurements of the viscosity.

Insoluble content: The solubility test was done according to study done by De Queiroz Antonino et al. (7) When 1% of acetic acid was used to identify the solubility of each sample at a 25 ± 0.1°C, then the solution was filtered. In triplicate manner the insoluble content was determined from the weight for both dissolving chitosan and insoluble particles on the filter.

Ash content: The ash value for each sample was estimated according to method of (16,25). About 1g of samples were put in a porcelain crucible that had previously been ignited and weighted. In muffle furnace the samples were preheated to 650°C for 4 hrs. Then the crucibles were cooled and subsequently were placed into desiccators. Measurements were done in triplicate and the % ash was calculated according to the following equation:

$$\%Ash = [(W1) \times 100]/W0$$

Where W_0 and W_1 are the sample and residue weight respectively**.**

Moisture content: Moisture content was identified based on the method of Huthman et al., using the gravimetric method (9). Firstly, the sample was dried to constant weight; then the water mass was calculated by taking the difference of sample weigh before and after drying. Also the calculations were made in triplicate and the weight mean value was used to calculate the moisture content of sample by the following equation:

$$\text{Moisture content \%} = [W2\ (g) - W1\ (g)] \times 100 / W2\ (g)$$

Where W_1 and W_2 are the sample weights before and after drying.

Degree of deacetylation (DD): The Degree of deacetylation (DD) for chitin was determined in triplicate based on the FTIR (8) and potentiometric titration (PT) (18). According to (PT) method 25 ml of 0.1 M HCl was used to dissolve 0.5 g from chitosan. The chitosan solution was then diluted to 100 ml with D.W, and to adjust the ionic strength to 0.1 calculated amount from KCl was added. The titration was done against 0.05M of NaOH until the value of the pH reached 2.0. Then NaOH was then added stepwise by recording the solution's pH values and a curve with two inflection points was established. The acid consumed by the amine groups in chitosan for salification corresponding to difference in volumes of NaOH between these points, which allows the calculation the DD% from the equation below: (1)

$$DD\% = (1 - 161Q)/ (1 + 42Q)$$

$$\text{Where } Q = N_{(NaOH)} \Delta V/m$$

N = 0.05 mol/l, ΔV = the consumed volume (in l) of NaOH between the two inflection points, and m is the dry weight of chitosan (in gm). The DD value was also determined in triplicate. The DD of the chitosan was estimated using FTIR which was done by (8) from the following equation:

$$DD = 100 - [(A1/A2) \times 100/1.33$$

Both A1 and A2 are the absorbance spectra for the amide-I band and the hydroxyl bond respectively.

Statistical analysis

All study data are shown as mean values ±SD and analyses statistically with the Graph pad Prism version 8.4.2.

RESULTS AND DISCUSSION

FTIR for Chitin and Chitosan

FTIR spectrum for both chitin and chitosan was shown in Figure (2) .For chitin the characteristic absorption spectra are found at 1541 cm^{-1} and 1647 cm^{-1} associated to N-H bending vibration and C=O stretching vibration respectively. Spectra at 3417 cm^{-1} as well as 2926 cm^{-1} corresponded to O-H and –CO-CH3 respectively. Spectra ranged between 871 cm^{-1} and 1037 cm^{-1} are represented the content of polysaccharide.

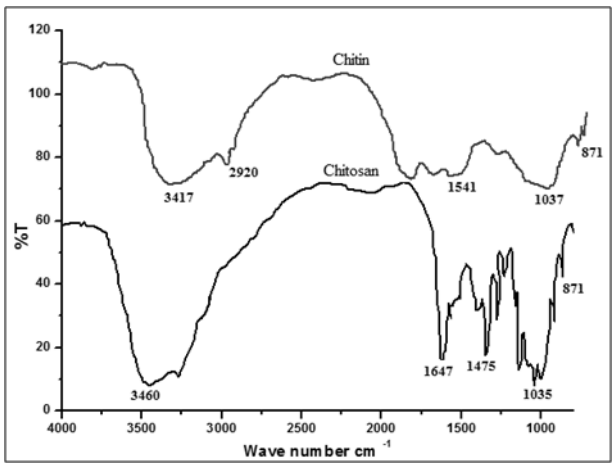

Figure 2. FTIR spectra for extracted chitin and chitosan

According to the spectrum of chitosan, spectra was found at 3466 cm^{-1} and 1647 cm^{-1} matching to O-H and C=O, group respectively. Bands between 871 cm^{-1} and 1037 cm^{-1} are also related to polysaccharide. Furthermore, as we see in figure 2 in chitin the longer intensity of bands at 2926 cm^{-1} and 1541 were observed than the intensity of chitosan; the discrepancy is evidence of deacetylation (27).These result reported also by Suneeta et al (15).

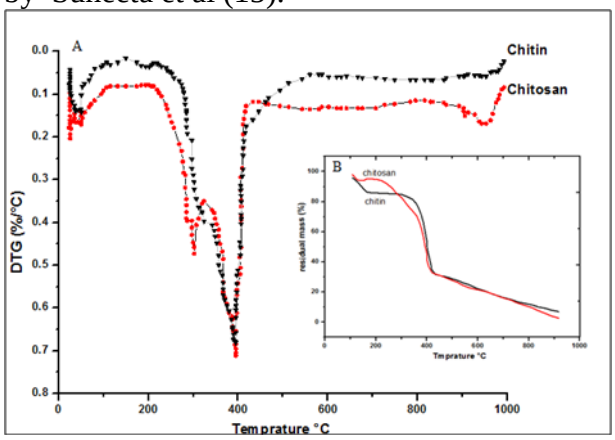

Figure 3. Thermo gravimetric analysis (TGA) for chitin and chitosan DTA (A) and TG (B)

Thermos gravimetric Analysis (TGA)

In figure 3 the thermogravimetric analysis for both chitin and chitosan are presented. According to our TGA result only two endothermal peaks for chitin are observed while, three endothermal peaks for chitosan are shown from the thermogravimetric curves. The first weight loss was observed at 50°C–150°C related to water loss for both chitin and chitosan. While the second endothermal peaks between 300°C–420°C related to the weight loss as a result of thermal decomposition of saccharide structure for chitin and chitosan (15).The third endothermal peak in thermogravimetric curves of chitosan that observes around 300°C relate to thermal decomposition in a part of the deacetylated molecule. It suggests that chitin had been found to be more stable than chitosan. Thus was in agreement with a study done by Kumari et al; (15) Abdou et al; (1) and Andrade et al (20). Moreover, as shown in figure (3) B after heating at 1000 °C the % residual mass was found about 35%, which proposed the presence of non-extracted minerals in the acidic step.

Physiochemical properties

Table 1 shows the physicochemical properties of the extracted chitosan. The moisture content of chitosan varied according to season, intensity of sunlight and relative humidity (26). The moisture content % of prepared chitosan was found about 4%. Our result which is in the range, according to KFDA, that identified the amount of moisture content of chitosan powder must be < 10% (14). Moreover, in 1% (v/v) acetic acid a transparent solutions from chitosan was observed with insoluble content about 0.59% and the ash content was found lower than 0.1%. Moreover the molecular weight of prepared chitosan was found 2.5 x 10^5 (g/mol) as showed in Table 1. Thus, both parameters are essential for biomedical application of chitosan (4).

Table1. Physiochemical properties of extract chitosan sample

Physiochemical properties	Chitosan
Intrinsic viscosity (η) (100 mL/g) ± SD	962 ± 2.3
Molecular weight (g/mol)	2.5 x 10^5
Moisture content % ± SD	4.5 ±0.41
Ash (%) ± SD	0.050 ±0.010
Insoluble content (%) ± SD	0.59 ± 0.110

SD=stander deviation

Deacetylation Degree (DD): Molecular weight (MW) and deacetylation degree (DD) are important for their biological and physical characteristics for chitosan. Hence, It is necessary to find a precise and fast method to determine the DD (13).Thus, in this study potentiometric titration (Pt) (1) and FTIR (5)are used for determinating the DD. Results

obtained from FTIR and potentiometric titration were found to be 57% and 63% respectively. A study done by Sagheer et al. identified that the DD was strongly dependant on the temperature and pH of solution. Furthermore, the chitin origin sources and isolation procedure have wide effect on DD (21).

CONCLUSIONS

The chitin obtained from fish scales could be used in a number of applications, particularly when converted to chitosan, the most useful compound. The approximate analyses in this study showed that the prepared chitosan has 0.050% ash content and 4.5% moisture content, with degree of deacetylation estimated as 63% by means of FTIR analysis. Moreover, in 1% (v/v) acetic acid a transparent solution from chitosan was observed with an insoluble content about 0.59%. The FTIR analysis for chitin showed the characteristic spectra at 1541 cm^{-1} and 1647 cm^{-1} associated to N-H bending vibration and C=O stretching vibration respectively. Thus, the FTIR spectra of chitosan gave characteristics bands of 1647 cm^{-1} for a carbonyl group. Thus, thermogravimetric analysis suggests that chitin had been found to be more stable than chitosan. It has been confirmed by the functional properties of the prepared chitosan that it can be commercially used in different fields. Preparing chitosan from fish waste would be minimise the pollutants to the environment.

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