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Extraction, Physicochemical Characterization and in Vitro Antioxidative Potential of Chitosan in Shrimp Shell Waste from Beni Saf Sea, Algeria

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Abstract: ***Chitin and its deacetylated derivative chitosan are natural polymers, they are oligosaccharides from exoskeleton, of crustaceans, insect shells and cell walls of fungi. The present study was undertaken to study the physiochemical parameters and the antioxidant activity from shrimp shell waste ; The intrinsic viscosity of chitosan with a molecular weight are 2164±40,37 cps and 1,718 x 10^6 ± 0,019 Da mol-1 respectively, the percent of Ash 0,345±0,040 %, moisture is 2,98 ±0,13 % , and protein is 0.3 ±0,041 %. The DPPH radical scavenging potential of chitosan ranged from 37.66% to 62.66% at varying concentrations (0.5 to 2%) chitosan produced was also characterized with Fourier Transform Infrared Spectroscopy (FTIR).***

Keywords: Shrimp shells, FTIR spectroscopy, molecular weight, deacetylation degree, antioxydant activity

1. Introduction

Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident, are chitin and its main derivative, chitosan. Chitosan is one of the most important derivatives of chitin, which is the second most abundant natural biopolymer found on earth after cellulose, and is a major component of the shells of crustaceans such as shrimps and crabs. Chitosan can be obtained by N-deacytlation of chitin and it is a co-polymer of glucosamine and N-acetylyglucosamine unit linked by 1-4 glucostatic bond (figure 1) [1]. Chitosan is a fibre like cellulose but unlike plant fibers, it possesses some unique properties including the ability to form films, optical structural characteristics, and much more. Chitosan is the biopolymer that has received much research interests due to their numerous potential applications in agriculture, food industry, biomedicine, paper making and textile industry. Recently, the antioxidant activity of chitosan and its derivatives attracted an increased attention [2]. The current research is to prepare chitosan from shrimp shell waste and to study the physiochemical parameters and characterized by biological activities such as antioxidative activities. FTIR spectra were also established for chitosan.

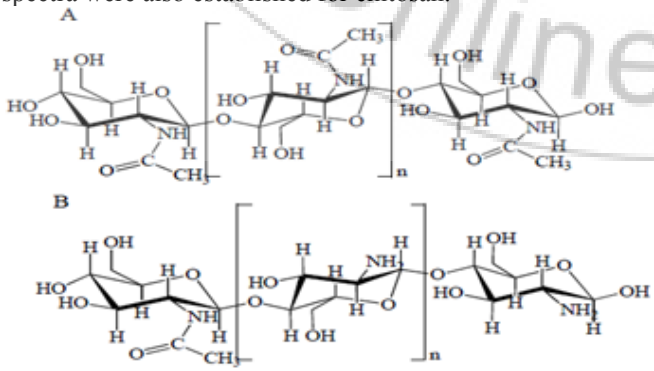

Figure1. Chemical structure of 100% acetylated chitin (**A**) and chitosan (**B**)

2. Materials and Methods

Chemicals acetic acid, hydro chloric acid and sodium hydroxide and all the other chemicals and reagents are purchased from Sigma Chemical Co.

2.1 Chitin and chitosan preparation

2.1.1 Sourcing and collection of samples

Shrimp shells were collected at BENI SAF fish market at Tlemcen in Algeria . For this research, shells were collected, in a stomacher bag and transported to the laboratory for preparation. The shells were scraped free of loose tissue, washed and dried at room temperature, for 10 days until it was well dried and crispy, and grounded to pass through a 0.5-0.8 mm sieve. Then they were subjected to demineralization, deproteinization and deacetylation.

2.1.2 Demineralization

Demineralization was carried out in 2M HCl, at ratio 1:15 (w/v)), at room temperature, stirred constantly overnight It was observed that the emission of CO2 gas depends upon the mineral content. The sample was then washed thoroughly with tap water several times to neutrality. The chitin was dried at ambient temperature (30 ± 2°C). The dried chitin was pulverised into powder using a dry Grinder

2.1.3 Deproteinization

Dried shell waste was washed with tap water and deproteinised by boiling in 3% aqueous sodium hydroxide for 15 min, after chitin was carried out using 2M NaOH at ratio 1:10 NaOH at 60 °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. Then the resulting solution was washed with water to neutrality. The purified chitin was dried at 50 °C to constant weight.

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2.1.4 Deacetylation of chitin

The chitin (10 g) was put into 50% NaOH at ratio 1:20 at 60°C for 8 h to prepare crude chitosan. After filtration, the residue was drained off and washed with tap water to neutrality. The crude chitosan was obtained by drying in an air oven at 50°C overnight. The chitosan was derived as a white powder.

2.2 Measurement of degree of N-deacetylation

The samples of chitin and chitosan produced were characterized by Fourier transformed infrared (FTIR) spectroscopy (Bruker Alpha-T) in the range of 400 to 4000 per cm. Samples of chitin and chitosan (10g) were mixed with 100 g of dried potassium bromide (KBr) and compressed to prepare a salt discs (10 mm diameter) . The disks were conditioned in a desiccator placed in an oven at 80°C for 16 hr before analysis for reading the spectrum.

The absorbances at 1655 and 3450 cm^{-1} were used to calculate the DD according to the following equation **[3]**:

$$DD = 100 - \frac{\frac{A1655cm^{-1}}{A3450cm^{-1}}}{1,33} \times 100 \quad (a)$$

Where DD is deacetylation degree; $A1655cm^{-1}$ and $A3450cm^{-1}$ are absolute heights of absorption bands of amide and hydroxyl groups. The factor 1.33 denoted the value of the ratio of *A*1655 / *A*3450 for fully Nacetylated chitosan.

2.3 Viscosity and Molecular Weight measurements

For the determination of viscosity-average molecular weight the chitosan was dissolved in a mixture of 0.5 M acetic acid with 0.5 M Sodium acetate buffer , Ubbelohde capillary viscometer (Relative Viscometer Model Cat #9721-R56, Cannon instrument Corp., State College, PA. USA) was used to determine intrinsic viscosity in a constant-temperature water bath at 25 ± 0.01 ◦C in triplicate. The viscosity-average molecular weights of chitosan were calculated using the classical Mark-Houwink equation $[\eta]=KM^a$ Where [η] is the intrinsic viscosity of the depolymerized chitosan, K and a are constants for given solute–solvent system and temperature **[4]** .Before charging the chitosan solution into the viscometer, the solution should be filtered using hydrophilic membrane filter of 0.45 micron size.

2.4 Moisture determination

Moisture content of the sample was determined by gravitational method **[5].**The water mass was the difference between the weights of the wet and oven dry samples expressed in percentage

$$\%\,\text{Moisture content} = \frac{\text{Wet weight (g) - Dry weight}}{\text{Wet weight (g)}} \times 100$$

2.5 Ash Content

Chitosan ash content was determined by combustion using a constant weight crucible **[6].** The crucible was repeatedly placed into an oven at 550 °C ± 20 °C for 30 min and then removed, cooling for 30 min in dessicator, and then weighed until a constant weight .To determine the ash value of chitosan 2.0 g of chitosan sample was combusted in the constant weight crucible in an oven at 550 °C ± 20 °C for 3 h until constant weight is achieved. Ash is then calculated in %.

$$\%\text{Ash} = \frac{\text{Weight of residue (g)}}{\text{Sample Weight (g)}} \times 100$$

2.6 Protein content

The protein content in the chitosan sample was determineted using Lowry method **[7]** with bovin serum albumin (BSA). Chitosan sample solution concentration was 10 mg/mL in 0.1 M acetic acid for the protein assay. The solutions were filtred and aliquot 5ml of waste water stored for protein analysis , from waste water 0,2 ml were taken and read at 750 nm UV-visible spectrophotometer (Model : Pd-303s Company : Apel, Japan) after adding the lowry solution .

2.7 Antioxidant Determination Assay

The free radical scavenging effect of chitosan at different concentrations 0.5, 1, 1.5 and 2 % (w/v) was estimated using the modified method described by Blois (1958) **[8]** .The chitosan sample (1 mL) was added into the 1 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma–Aldrich Co., St. Louis, MO). The mixture was shaken and incubated for 30 min in the dark at room temperature and the absorbance was then measured at 517nm using an UV-visible spectrophotometer (Model : Pd-303s Company: Apel, Japan) Ascorbic acid, were used as standard.

DPPH radical-scavenging activity was calculated as follows:

$$\text{Scavenging activity} = \frac{\text{Abs of control} _ \text{Abs of Radical- sample}}{\text{Abs of control}} \text{ X } 100$$

2.8 Statistical analysis

Statistical analysis was conducted using ANOVA analysis (StatBox logiciel, GrimmerSoft; version 6.4, France). Comparisons were made using Student–Newman–Keuls test for multiple comparisons. A $P<0.05$ was considered statistically significant. All data presented are mean values of triplicates obtained from three separate runs (n = 5).

3. Results and Discussion

3.1 Degree of Deacetylation (DD)

The degree of deacetylation (DD) was calculated by using the equation **(a)** and FT-IR (infrared spectroscopic analysis) of the prepared chitosan**.** The DD is an important parameter affecting solubility, chemical reactivity, and biodegradability. Depending on the source and preparation

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procedure, DD may range from 30% to 95% **[9].** This study revealed that, DD of the prepared chitosan is 75%. It is rare that the production of chitosan with 100% degree of deacetylation is achievable. Therefore, commercial chitosan with various degree of deacetylation in the range of 75–85% is commonly found.

3.2 FT-IR spectral analysis

The FT-IR spectrum of the chitosan sample from the shell recorded 16 peaks in the range of 689.40/cm and 3430.02 /cm (Figure 2). The FT-IR spectra of shell extracted chitosan showing the absorbance band at 3430.02cm- 3258.97/cm, 3100.03/cm, 2877.11/ cm, 2325.01 /cm, 1168.38/cm,1652.30/cm,1629.99/cm, 1556.30/cm, 1377.02/cm,1380.11/cm,1258.98/cm, 1155.03/cm, 1009.11/cm, 951.78/cm, and 689.40/cm. The region between 3000/cm and 3500/cm indicates the hydroxyl stretching vibration. This band is broad because of the hydrogen bonds.The peak at 2877.11/cm represents the characteristic -CH- stretching vibrations. The OH band overlaps the stretching band of NH. Another significant change is observed in the region from 1000 cm-1 to 1200cm-1. In this region chitosan presents a broad band centered it 1155.03/cm associated with the stretching of C=O. It is also observed It is interesting that the absorption peak of chitosan at1629.99cm-1 corresponding to the chitosan NH2 band , and the band at 1652.30 cm-1 corresponds to the amide I stretching of C = O.

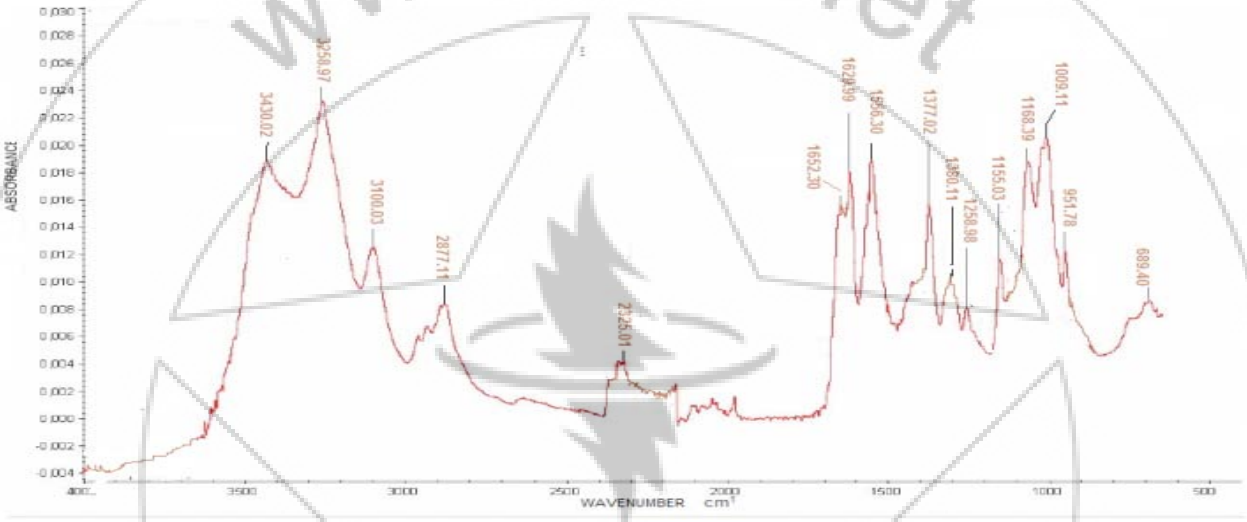

Figure 2: FTIR of prepared chitosan

3.3 Viscosity and Molecular Weight measurements

Average molecular weights of chitosan were calculated from measured intrinsic viscosities using the classical Mark-Houwink relationship $[\eta]=KM^a$ The molecular weight of chitosan in this study was found to be 1,718 x 10^6 ± 0,019 Da this indicated that the quality of the chitosan used in the present work was good, this value was relatively same of chitosan from crab shells reported in the literature **[10].** The intrinsic viscosity of chitosan with a molecular weight of 1,718 x 10^6 ± 0,019 Da mol-1 was 2164±40,37cps this is acceptable, however, since the molecular weight of chitosan depends on such factors as source of raw material and method of preparation the chitosan viscosity is considerably affected by physical treatments (grinding, heating, autoclaving, ultrasonicating, but not freezing) and chemical treatments. Viscosity is an important factor in the conventional determination of molecular weight of chitosan and its commercial applications. High viscosity chitosan has various applications, for example, as an emulsifying agent, or dietary ingredient, for metal reduction, and drug delivery **[11]**

3.4 Ash content

The prepared chitosan had an ash content of 0,345±0,040% as shown in Table 1 below; the ash content in chitosan is an important parameter that affects its solubility, viscosity and also other important characteristics **[12].** Low ash content was achieved because shrimps do not have high calcium carbonate in their shells.

3.5 Moisture content

The moisture content of chitosan obtained from shrimp shells was measured to be 2,98 ±0,13 % as shown in Table 1, which is in agreement with other authors who reported moisture content in the range of 7-13% obtained from different species **[13].**

3.6 Residual protein content

Protein assay results showed that residual protein content of chitosan ranges from 0.3 ±0,041 % The protein content in a sample depends on the source of the sample and, especially, on the method of preparation. . In the presence of NaOH the amide is hydrolyzed and the protein content in the chitin product is reduced. Chitin occurs naturally in association with protein (Chitinoprotein). Some of this protein can be extracted by mild methods, but other portion is not readily extracted, suggesting strong covalent bonding to chitin **[14].** Deproteination with NaOH get a low protein content.

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Table 1: Physicochemical and functional properties of chitosan

Parameter	***Result***
Degree of Deacetylation (DD)	75%.
Viscosity	2164±40,37cps
Molecular Weight	1,718 x 10^{-6} ± 0,019 Da
Ash	0,345±0,040%
Moisture	2,98 ±0,13 %
Residual protein	0.3 ±0,041 %

3.7 Scavenging ability on DPPH radicals

The scavenging ability of chitosan from on DPPH radicals was reported at 62.66 %at 2%. Ascorbic acid showed moderate scavenging abilities of 68.33%. Thus the antioxidant activity of chitosan was found to be a moderate scavenger for DPPH radicals. The DPPH radical scavenging potential of chitosan ranged from 37.66% to 62.66% at varying concentrations (0.5 to 2%). Ascorbic acid was used on standard (Figure 3). It is generally considered that the inhibition of lipid peroxidation by an antioxidant can be explained by various mechanisms. One is the free radical-scavenging activity. Park, Je, and Kim (2004) **[15]** suggested that chitosan may eliminate various free radicals by the action of nitrogen on the C-2 position of the chitosan.

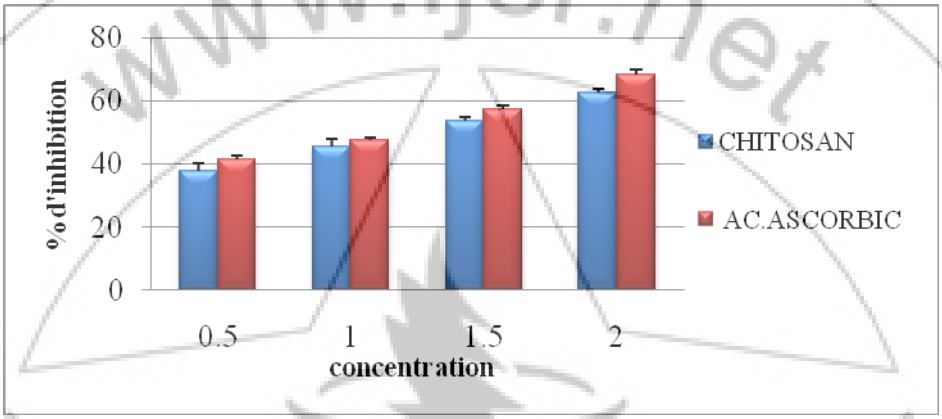

Figure 3: 1-1-Diphenyl-2-picrylhydrazyl (DPPH radical scavenging activity of chitosan.

4. Conclusion

Conclusion In this study chitosan has been successfully prepared from shrimp shell waste. By employing FTIR spestroscopy, all functional groups in chitosan macromolecules are elucidated characterization of the prepared chitosan showed that it can be used commercially. Antioxidative properties of the chitosan extract is of great interest in food industry, since it possible use as natural additives emerged from a grow-ing tendency to replace synthetic antioxidants by natural ones.

5. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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