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Preparation of Chitosan from Iraqi Shrimp Shell by Autoclave, Studying Some Physiochemical Properties and Antioxidant Activity

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Abstract
The aim of this study was preparing chitosan from Iraqi shrimp shell by autoclaved chitin with concentrated sodium hydroxide (50%) at 121c°for15min.Some physiochemical characteristics and antioxidant activity was investigated. The yield of chitosan was (64.5%) as dry weight of chitin. The moisture content was (6.2%) and Ash was (0.72%). Chitosan showed low viscosity it was 19 cp and molecular weights 102.5 KDa. The degree of deacetylation chitosan was 52%.The chitosan showed higher ability for fat binding that was ranged from 174.2% – 226.8%.The water binding ability was 197.4%. The antioxidant activity of chitosan in linoleic acid system was estimated .The result showed high activity of oxidative suppression with increaced chitosan concentration for 6 days from storage. The high antioxidant activity of chitosan was at concentrated 10 mg compared to control.
Keywords : Chitosan , Autoclave , Antioxidant , Iraqi shrimp shell ,physiochemical characteristic.

INTRODUCTION:

Chitin is found in shells of exoskeletons of insects, shells of crustaceans and fungal cell wall (5). Chitosan is a bio copolymer prepared from chitin by enzymatic treatment or alkalin treatment at different condition for partially or totally deacytalated group from polymer (1,2).Chitosan is a linear unbranched polymer made up of mixed subunit , N-acetyl D-glucoseamine and glucosamine which are joined by β(1-4)glycosidic bonds. The Subunits are reached to 2000-3000 unit (16, 26) Figure (1).Chitosan reported to be biodegradable, biocompatible and nontoxic, dissolves as a poly cation in dilute acids (9).It is possess three functional groups amino group at the C2 carbon atom, primary and secondary hydroxyl groups at the C3 and C6 carbon respectively that make it appropriate for used widespread in many different industries like food industry and medical field(4). It is therefore active in metal chelat, dyes adsorbed ,flocculent protein(25).Chitosan has been used as antioxidant (12)antimicrobial(7)The degree of deacetylation (DD) and molecular weight (MW) are two fundamental parameters that can affect the properties and functionality of chitosan (2).

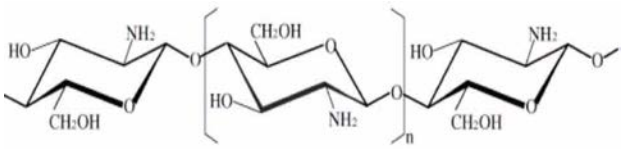


Figure 1. Chemical structure of chitosan.

During chitosan deacetylation, the degradation of the polymeric chain takes place. At the same time, the crystallinity of chitosan can be damaged by using harsh reaction conditions(22). Taking these two facts into account, the reaction conditions must be controlled when preparing chitosan (27) When deacetylated chitin we mast avoiding high degradation involve using heterogeneous conditions with NaOH 75% (w/v) and a temperature of 110ºC (10)The type of crustacean and the chitin isolation process are also factors that affect chitosan quality (11). Since deacetylation process of chitosan involved the breakage of C-N bond by nucleophilic substitution, it required a high activation energy which can be provided by high temperature and pressure . Therefore, the present study aims to extract chitin from shrimp shell and used it to produced chitosan by autoclave method (121c°/15min), the autoclave method lead to reduction the deacetylation time as compared to several hours when using the boiling method. Some characteristic and antioxidant activity of chitosan was studied also

EXPREMINTAL

The study was done in agriculture college / University of Baghdad.

Chitin preparation :

Fresh shrimp shells was collected from Basra fish market , washed under tap water and then dried. The dried shells were ground through grinding mill and stored at ambient temperature. .Chitin was extracted from shrimp shell powder by two main steps according to(15): deproteinizing with dilute sodium hydroxide (3.5%) for two hours at 65c° with solid to solvent ratio of 1:10(w/v). Sample filtered under vacuum and the filtrate washed with tap water and oven dried. The second step was demineralised shell with hydrochloric acid (1N) for30min at room temp with solid to solvent ratio 1:10(w/v). The sample filtered under vacuum and the filtrated was washed and with tap water and dried. The shell was discoloration by adding acetone then sodium hypochlorite (0.1315%)for 5min at room temperature at ratio 1:10(w/v). The product was washed by distilled water and dried at 60C°for 25min.

Chitosan preparation:

Chitosan was prepared from chitin accoding to Zakaria et al(29).Chitin was treated with concentrated sodium hydroxide (50%) and autoclaved at 121c°for 15 min .The solid to solvent ratio was 1:10(w/v).The chitosan was washed under vacuum by hot distilled water until neutrality. The sample was dried in oven at 60c° for overnight.

Proximate analysis: The Moisture content was calculated after drying 1g of chitosan in an oven at 60c°for 24h. The ash content was calculated after heating the sample at 650°C for 3 h.(3) .

Determination the degree of deactylation:

Fourier Transform Infrared Spectroscopy (FTIR) was used to determin the degree of deacytelation of chitosan with frequence of 4000-400 cm-1.Chitosan was grounded with potassium bromide and compressed to a semi-transparent dish to thickness 0.5 - 1mm. The DD of chitosan was calculated using the aquation (8) :

$DD = 100-(A1655/ A3950 \times 100/ 1.33)$

Viscosity and Molecular weight (MW)Determination :

For the determination of viscosity and average molecular weight of chitosan , chitosan was dissolved in 1% acetic acid .The viscosity of chitosan solution was determined with ostuled tube visco meter at 25c°.The values reported in centipoises (cPs),and MW was calculated using equation[19]:

$[\eta sp] = (\eta - \eta s) / \eta s$

$\eta = \eta sp/c$

$Mv = [\eta/k]^{1/a}$

ηs , η solvent and sample viscosity

ηsp -specific viscosity; c -concentration of solution

1.81×10^{-5} cm^3/g and 0.93 the values of K and a respectively.

Water binding capacity (WBC):

Water binding capacity of chitosan was measured according to method of No and lee. (19). The centrifuge tube with chitosan 0.5g was weighing then adding 10ml of water ,mixing on a vortex mixer for 1 min to disperse the sample. The content were left for 30 min at ambient temperature with shaking for 5s every 10 min and centrifuge at 3000rpm for 25 min.The supernatant was decanted and the tube was weighed again. The WBC was calculated as follows:

WBC% = Water Bound(g)/ Sample weight(g) × 100

Fat binding capacity(FBC):

Fat binding capacity of chitosan was measured according to the method of No and lee (19) . The centrifuge tube with chitosan 0.5g was weighing then adding 10ml of sesame oil , castor oil , soybean oil , coconut oil sun flower oil and corn oil,mixing on a vortex mixer for 1 min to disperse the sample. The content were left for 30 min at ambient temperature with shaking for 5s every 10 min and centrifuge at 3000rpm for 25 min.The supernatant was decanted and the tube was weighed again. The WBC was calculated as follows:

WBC% = Fat Bound(g)/ Sample weight(g) × 100

Determination of antioxidant activity :

The inhibition of outoxidation in linolic acid system by chitosan was determined according to the ferric thiocyanate method (7). A volum of 0.5ml of chitosan solubilized in acetic acid 1% at concentration 1,2,4,6,8,10 mg/ ml, add to 1ml sodium phosphate buffer (0.1%) pH 7,and 1 ml of 50 mM linoleic acid in ethanol (95%) . The mixture was incubated at 50c° for 6 days to accelerate oxidation .To determination antioxidant activity 50 µl from the reaction mixture was add to 2.35ml ethanol (75%),50 µl ammonium thiocyanate and 50 µl ferrous chloride solution (20mMin 3.5%HCL),mixed for 3 min by stirrer and the peroxide value was determined spectrophotometry at 500 nm. The analyses sample was dane in duplicate.

RESULT AND DISCUSSION

Chitosan characterization are shown in table (1).The result showed high yield of chitosan when prepared from chitin by autoclave it was 64.5% as dry weight. Salman and Khaleel (24)reported the chitosan yield was about 72.8% and 69.8% of chitosan prepared by treating chitin with alkalin solution at 100c°/4 hour and 20hour respectively. The yield of chitosan may be effect by loss of sample mass or weight by removal of acetyl groups from the polymer during deacetylation or due to depolymerization of chitosan polymer because thermal degradation during prepared of chitosan from chitin by utilized high temperature(121c°) with alkalin treatment. The proximate analysis in table (1) showed that chitosan had a moisture content about 6.2%.

This result indicates the ability of chitosan to absorb moisture from the atmosphere. The chitosan is hygroscopic in nature because the chemical groups in chitosan stracture can be hydrogen bond with water (13, 28). Li et al.(18) indicated that commercial chitosan products contain less than 10% moisture content. The ash content in chitosan was 0.72% as dry weight and this result is an indicator of the effectiveness of demineralization step for removal calcium and phosphor carbonate during chitin preparation from shrimp shell. Knor(17) reported that shell of crustacean was consist of 30-50% of calcium and phosphate carbonate. No et al(20) indicated that ash content of good quality of chitosan must not be excess 1%. The chitosan density was 1.111cm^3. The deacetylation degree of chitosan was 52% as determined by FTIR spectra (table 1). Zakaria et al (29) reported the similar result when using an autoclave(121c°/30min) and sodium hydroxide at concentration 50% to prepared chitosan it was 52.9% . The N-deacetalytion of chitin is carried out by alkaline hydrolysis with NaOH at 120C° for 1-3 hr and this treatment produce 40- 80% deacetylated chitosan (21). There are several factors that affect on the ratio of deacetylation including temperature and time of deacetylation, alkali concentration, ratio of chitin to alkali solution(15).

The absorption of amid group approximately at 1655cm^{-1} which represent as the measurement of N-acetyl group while the band at 3450cm^{-1} represent the hydroxyl group=measurement(figur1).The ratio of deacetylation in chitosan structure had influence on their physiochemical and functional properties such as viscosity, solubility and molecular weight.

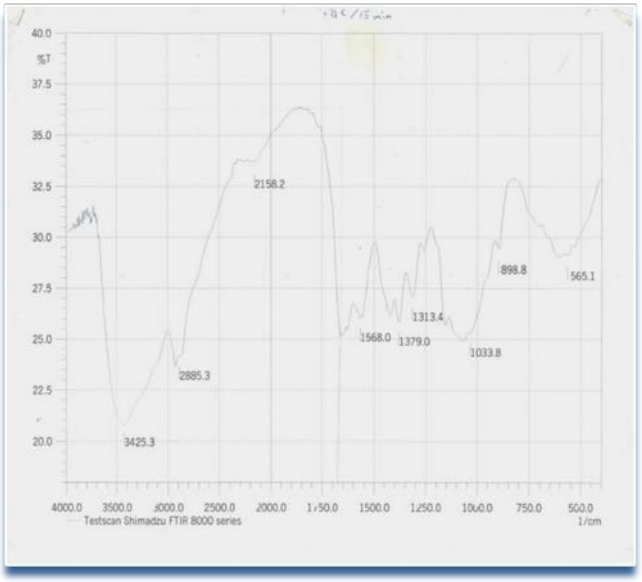


Figure1: The FTIR-Spectroscopy of chitosan

The chitosan showed decreased in viscosity reached 19cep. The decreased viscosity of the outoclaved chitosan may be due to the severe treatments (120C°/15min and50%NaOH) which may cause depolymirazation of chitosan structure and also cause low molecular weight 102.5 kd. Sjoholm et al. (26) determined that autoclaving chitosan in the presence of strong NaOH causes both depolymerization and even further deacetylation (from 80% to 98% deacetylation over 30 minutes). Rout(23)demonstrated that chitosan molecular weight depending on the temperature during processing for instance at temperature over 280c°,thermal degradation of chitosan occurs and polymer chins rapidly break down and lowering molecular weight. No et al.(19)indicate that the brake down of chitosan polymer and increase the deacetylation ratio lead to low molecular weight and viscosity and increase the solubility of chitosan. Zimmerman (30) indicated that autoclaving with concentrated NaoH can result bath deacetylate and depolymerize chitin.

Table(1).Physiochemical Properties of chitosan

Tretment	Yeild %	Moisture %	Ash %	Density g/cm^3	Viscosity cp	M.W Kd	Solubility %	W.B.C %	D.D %
Value	64.5	6.2	0.72	1.111	19	102.5	100	197.4	52

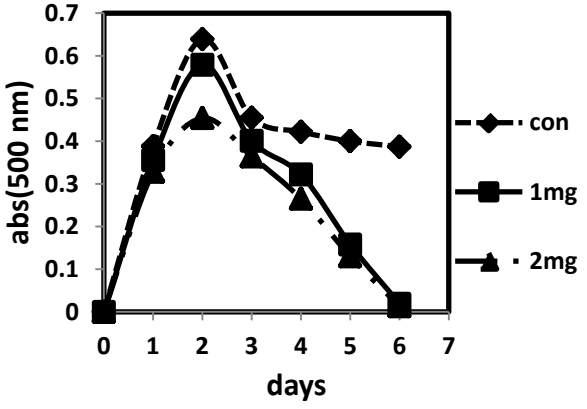
Table2. Fat binding capacity of chitosan with different oil.

Oil Type	Coconut oil %	Soybean oil %	Sesame oil %	Corn oil %	Sunflower oil %
Fat binding capacity	174.2	175.4	194	208.6	226.8

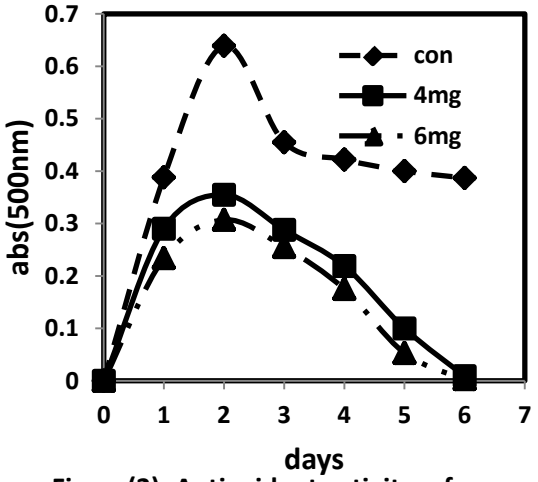
There are several factors tht influence the viscosity of chitosan during its preparation such as degree of deacetylation, molecular weight, concentration, ionic strength, pH and temperature(5). Kim (15) noted that there is appositive relationship between molecular weight and viscosity. The chitosan solubility was 100% in acetic acid (1%) (table 1)and this results due to the low molecular weight and low viscosity.

The different methods of preparation of chitosan such as change temperature, time and alkaline concentration affect the deacetylation degree and M.W of chitosan and then affect physiochemical and functional properties of chitosan including solubility and viscosity(20). Kim (15) explained the increased chitosan solubility to 95% in acetic acid(1%) as a result of chitin treatment with a high concentration of NaOH solution (40-50%) for 10-15min. Chitosan is insoluble in water, organic solvents and soluble in acids such as acetic, lactic, formic, hydrochloric and phosphoric acid(15), the acetic acid at concentration of 1% is the most common used. Chitosan reactive amino groups carries appositive charge in acetic media which increase the molecules infirmity and dispersion in the media. When the pH is higher than 6 the solubility of chitosan decreased and precipitate or become gel (15). The water binding ability of chitosan was 197.4% (table 2). The result showed there was a difference in fat binding ability with different source of oil ranged between 174.2-269.2%. The highest binding was with castor oil 269.2% and the lowest with coconut and soybean oil were 174.2,175.4% respectively, while sesame, corn and sunflower oil were 194,208.6 and 226.8% respectively. The chitosan ability to binding fat and water is important in its work as an emulsifier agent in various applications as food processing or pharmaceutical etc.

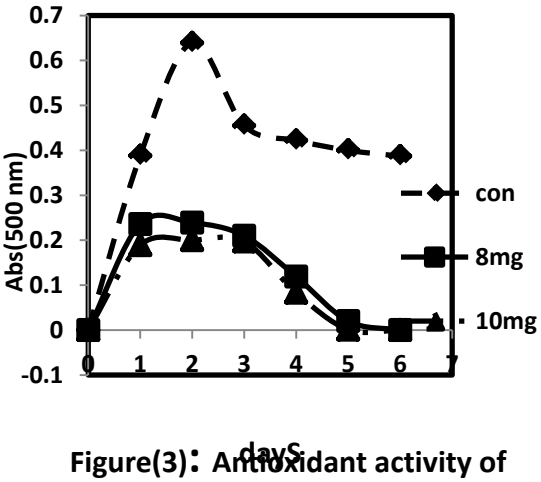
The antioxidant activity of chitosan was studied at concentration (1-10mg/ml) with linolic acid system. The result showed (figure 1,2,3) That chitosan was high ability to reduce development of peroxide during six days of storage at 40C° compared to control. The antioxidant activity of chitosan was increased with the increasing concentration. The hydroxyl groups (OH) and amino groups (NH) in chitosan are the key functional groups for its antioxidant activity. Chitosan inhibit the reactive oxygen species (ROS) superoxide anion, hydroxyl radical and hydrogen peroxide and prevent the lipid oxidation in food and biological systems. Several mechanisms about the antioxidant activity of chitosan have been proposed (14).



Figure(1):Antioxidant activity of chitosan at concantration 1mg,2mg



Figure(2): Antioxidant activity of chitosan at concantration 4mg,6mg



Figure(3): Antioxidant activity of chitosan at concantration 8mg,10mg

CONCLUSION:

The results of this work demonstrate that chitosan characterization and application was effected by its preparation techniques, Therefore the autoclave method is a good process to prepared chitosan with high antioxidant activity . From this result we conclude that Chitosan is a natural antioxidants can protect food products from oxidation and the human body from free radicals so its prevent many chronic disease .

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