



Comparative study of the physicochemical and functional parameters of chitosan prepared from *Schistocerca gregaria* (Desert Grasshopper) and *Cambarus baratonii* (Crayfish)

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Abstract

Chitosan a versatile biopolymer was prepared from *Schistocerca gregaria* (Desert Grasshopper) and *Cambarus baratonii* (Crayfish). The physicochemical and functional parameters of the two prepared chitosan were analysed. The structural differences between desert grasshopper Chitosan and Crayfish Chitosan were studied using FTIR and SEM analysis, where the FTIR spectra was used to determine the degree of deacetylation (DD). The results of the physicochemical and functional parameters revealed that, the two chitosan prepared shows N-H stretching of primary amines at 3257cm^{-1} indicating successful deacetylation. There were variations in fat binding capacity, Water binding capacity, moisture content and ash content between Chitosan prepared from Desert Grasshopper and the one prepared from Crayfish. However, they are both soluble in 2% acetic acid and have a pH of 6.7 and 7.0 respectively. The variations in the physicochemical and functional parameters of the two prepared chitosan were due to the differences in the DD and the morphological structures revealed by SEM.

Keywords: *Schistocerca gregaria*, *Cambarus baratonii*, desert grasshopper, physicochemical parameters, chitosan, degree of deacetylation (DD).

1. Introduction

Fishes and grasshoppers serve as potential sources of protein in the diet, which are abundantly found in seas and desert regions of the world. Both fishes and grasshoppers have numerous varieties used for different purposes. Grasshoppers lives as pest in most areas where they exist and contribute largely to the destructions of farm produces while fishes especially the cray fish exist as pests in the environment where they live. Both grasshoppers and cray fishes are important sources of protein in human diet (Bough, 1976).

Over few decades, cray fish were considered only as source of protein and grasshoppers as source of protein and pests, but can no longer be considered as sources of food/pests with minimal economic value, but should be considered as profitable alternatives leading to valuable products of commerce (No *et al.*, 2007). Similar research studies by Lee (2011) demonstrated that the asataxanthin-rich from crayfish waste is a valuable natural resource for commercially feasible pigment which is marketed as a fish food additives in aquaculture, especially for Salmon (Bough, 1975).

Chitin and chitosan are polysaccharide polymeric materials; chitin is the second most abundant renewable polysaccharide after cellulose. Shrimp, crab, squid, lobster, insect cuticle, fungi and yeast are the naturally occurring sources of chitin. Chitin and its derivatives are biomolecules of great importance, having versatile

biological actions and they exhibit biocompatibility and biodegradability (Akila, 2014). As a result, chitin and its derivatives are extensively used in pharmaceutical industries (Muzzarelli *et al.*, 2014), cosmetics, feed additives (Bough, 1975), Agriculture (Zohra, 2017), semi-permeable, food and textile industries and waste water treatment (Asano, 1978; Knor, 1984).

Like cellulose, chitosan is a fibre (Pillai *et al.*, 2009; Dutta *et al.*, 2004; Kurita, 2006) However, unlike plant fiber, chitosan possesses unique properties including the ability to form films, optical structural characteristics and much more. Chitosan also possesses a positive ionic charge, which gives it the ability to chemically bind with negatively charged fats such as lipids and bile acids (Suneetha *et al.*, 2017).

Chitosan is prepared by alkaline deacetylation of chitin (Kumar *et al.*, 2004). During this reaction, the acetamido groups (-NHCOCH₃) of chitin are converted into amino groups (-NH₂) leading to chitosan formation. Depending on the source from where it was extracted, chitin can be found in three polymeric conformations (α , β and γ) (Jang *et al.*, 2004). Chitosan and chitin have become materials of great interest not only as an under-utilized resource but also as a new functional biomaterials of high potential in various fields. Chitosan is insoluble in water and it is soluble in acidic solutions due to the protonation of its amine groups (Aranaz *et al.*, 2009; Rinaudo, 2006). Due to the presence of NH₂ group, chitosan potentially has high availability of attraction properties to absorb pollutants such as heavy metals and dyes (Peng *et al.*, 2013; Wan Ngah *et al.*, 2011). Crystallinity and availability of amine groups affect the adsorption capacity of chitosan (Wang *et al.*, 2014).

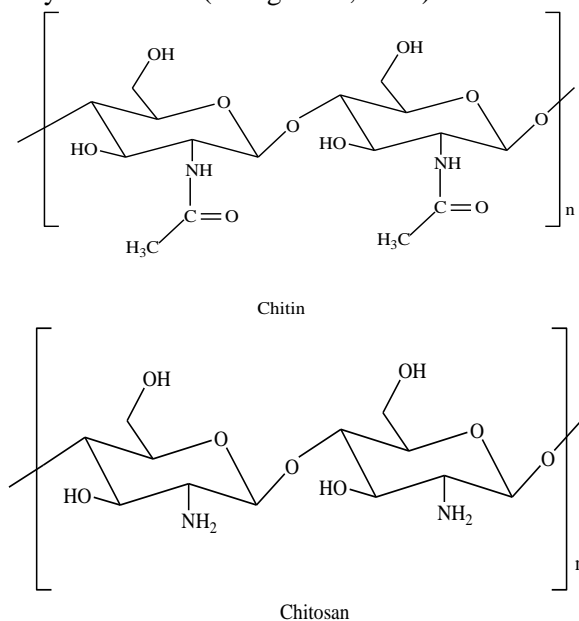


Figure 1: Structure of chitin and chitosan

Naturally occurring polysaccharides such as cellulose, pectin, alginic acid and carrageenan is neutral or acidic in nature (Wanule *et al.*, 2014). Chitin and chitosan are examples of highly basic polysaccharides (Acharyulu *et al.*, 2013). Chitosan is known for its properties such as being biocompatible with animal tissues, non-toxic, odorless, and enzymatically biodegradable (Rawda, 2015) most important properties of chitin and chitosan include polyoxysalt formation, ability to form films, chelation with metal ions and optical (Dash *et al.*, 2011). The degree of deacetylation and molecular weight of chitosan has strong impact on its physical properties (Muzarelli, 1985). The objective of the present work is to prepare chitosan from two local sources; desert grasshopper and crayfish using alkaline deacetylation method. However, there are no or few comparative investigations on the physicochemical and functional parameters of the two chitosan.



2. Materials and Methodologies

Materials

Crayfish was obtained from Sabon Gari market, Kano and Desert grasshoppers from Rimi market, Kano, Nigeria. Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Acetic acid and Distilled water from Chemistry Laboratory, Bayero University Kano. Scanning Electron Microscopy (SEM) from Umar Musa Yar' adua University, Katsina, Katsina State, Nigeria

Methods

Desert grasshoppers' wings were removed separately and both the Crayfish and the Grasshoppers were further dried in an oven at 105⁰C for 2hrs and later removed and then grounded into small paste/powder. Modified methods were used for preparation of chitosan which included demineralization, deproteination and deacetylation processes. Initially, the Crayfish/Grasshopper exoskeleton powder was demineralized with 5% HCl, with solid to solvent ratio of 1:15(w/v) with constant stirring for 30mins at ambient temperature followed by vacuum filtration. The residue was washed in a running tap water and rinsed with distilled water and oven dried at 60⁰C for 24hours. The demineralized powder was deproteinized with 3.5% NaOH solution in the ratio 1:15(w/v) for 30-40mins with constant stirring. The mixture was vacuum filtered and the residue was washed in a running tap water, rinsed with distilled water and oven dried at 60⁰C for 24hours, chitin is obtained. The removal of the acetyl group from the chitin was achieved by autoclaving for 2hrs at 121⁰C using 50% NaOH with a solid to liquid ratio of 1:15(w/v).The resulting chitosan obtained was washed and neutralized with tap water followed by rinsing with distilled water and oven dried at 60⁰C for 24hours (Shilratan *et al.*, 2014).

Chitosan purification

According to Signini and Campana Filho (1999), chitosan sample was dissolved in 2% acetic acid and left at least 12hrs. The solution was then filtrated through cheesecloth to remove contaminants and undissolved particles. Chitosan was then precipitated with 5% Sodium hydroxide, collected and washed in a running tap water and rinsed with distilled water to remove the excess of alkali until it reached neutrality and dried in a vacuum oven for 24hrs at 60⁰C.

Determination of Degree of Deacetylation

0.1g of the chitosan from the two samples were weighed and put in a desiccator for 12hrs. The samples were then removed from the desiccator and covered immediately to avoid air entry. The spectra of the chitosan samples were obtained using an Infrared spectrophotometry with a frequency range of 4000-650cm⁻¹. The degree of deacetylation (DD) of the Chitosan samples was determined by comparing the absorbance of the measured peak to that of the reference peak (Muzzarelli, 1985).

Ash Content

The ash content values of the chitosan were determined by weighing 1g of the prepared samples into previously ignited, cooled and tarred crucibles. The samples were heated in a muffle furnace preheated to 650⁰C for 6hrs.The crucibles were allowed to cool in the furnace to less than 20 to 30mins and then placed into desiccators with a vented top. The ash content of the two prepared chitosan was calculated using the following relationship proposed by (Shilratan *et al.*, 2014).

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

Moisture Content

The moisture content of the prepared chitosan was determined by the gravimetric method reported by Wang and Kinsella (1976). 1g of the each sample was weighed and dried to constant weight in an oven at 105⁰C for 24hrs. The moisture content was determined using equation (ii) as proposed by (Sneha *et al.*, 2014)

$$\text{Moisture content(\%)} = \frac{w_1 - w_2}{w_1} \times 100 \dots \dots \dots \text{(ii)}$$

Where W_1 = weight (g) of the sample before drying and W_2 after drying.

Fat Binding Capacity of Chitosan

Fat binding capacity (FBC) of the chitosan samples were measured using a modified method reported by Wang and Kinsella, (1976). Fat absorption was initially carried out by weighing 0.5g of the samples in pre-weighed centrifuge tube. To this 10ml of groundnut oil was added by mixing on a vortex mixture for 1min to disperse the sample. The content was left at ambient temperature for 30mins with intermittent shaking every 10mins and was centrifuged at 2000 rpm for 30mins. After that, the supernatant was decanted the tube was weighed again. FBC was calculated using the following relationship:

$$\text{FCB(\%)} = \frac{\text{Fat bound (g)}}{\text{Sample weight (g)}} \times 100 \dots \dots \dots \text{(iii)}$$

Solubility Test

0.1g of the chitosan sample was placed in a centrifuge tube of known weight and dissolved in 10ml of 1% acetic acid for 30mins using an incubator shaker operating at 240rpm and 25°C. The mixture in the centrifuge was heated for 10mins using boiling water and then cooled to 25°C and centrifuge at 5000rpm for 10mins. The supernatant was discarded and undissolved particles were washed in distilled water (25ml) and then undissolved matter was dried at 60°C for 24h and weight of the mass particles was recorded. The percentage solubility was determined using the following equation (Suneetha *et al.*, 2017)

$$\text{Solubility (\%)} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight - Weight of Empty tube}} \times 100 \dots \dots \dots \text{(iv)}$$

Water Uptake/Water Binding Capacity

Water uptake (%) estimation was performed by placing a weighed sample of 1.0g of chitosan previously dried in 25mls of distilled water using an incubator shaker operating at 240rpm and 25°C for 6hrs. The samples were then filtered off, carefully bolted with a filter paper and weighed. The water uptake was calculated by applying the following equation

$$\text{Water uptake \%} = \left[\frac{(M - M_o)}{M_o} \right] \times 100 \dots \dots \dots \text{(v)}$$

Where M is the weight of the swelled sample at time t and M_o is the weight of the sample at time t_o (Mohy Eldrin *et al.*, 2015).

Scanning Electron Microscopy (SEM)

The surface morphology of the two chitosan samples were observed using Scanning electron microscopy to verify the compatibility of the mixtures chitosan. To analyze the chitosan samples, the samples were cut into pieces of various sizes and wiped with a thin gold-palladium layer by a sputter coater unit (UG-microtech, UCK field, UK), (Shashikala and Shafi, 2017).

2.1 Results

Chitosan was prepared from *Schistocerca gregaria* (Desert grasshoppers) and Crayfish were characterized from physico-chemical points of view. The results of the physico-chemical and functional properties of the prepared chitosan were given in Figure 2, Figure 3 and Table 1.

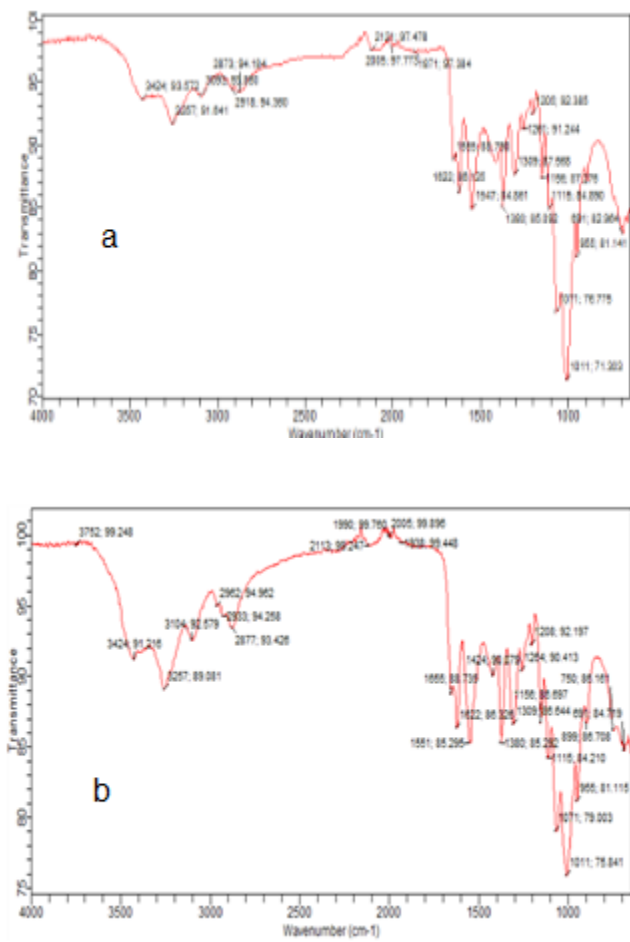


Figure 2: (a) FTIR of chitosan from crayfish and (b) FTIR of chitosan from desert grasshopper

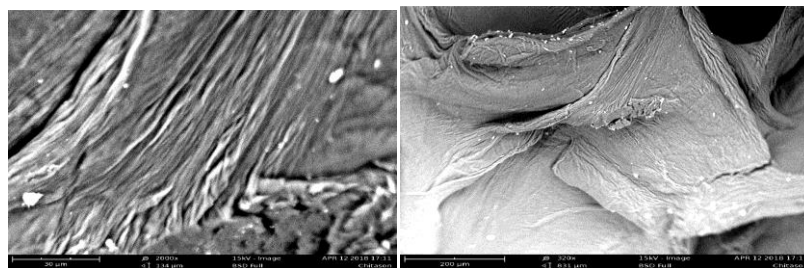


Figure 3: (a) SEM of chitosan from crayfish and (b) SEM of chitosan from desert grasshopper

Table 1: Physico-chemical and Functional parameters of Prepared Chitosans

Physico-chemical parameters & Functional	Chitosan from Grasshopper	Chitosan from Crayfish
Yield	25.23%	16.33%
Fat binding capacity	399.91%	466.88%
Moisture content	1.72%	1.32%
Ash content	3.25%	10.79%
pH	6.7	7.0
Solubility	2% acetic acid	2% acetic acid
Degree of deacetylation	97.68%	98.28%
Water binding capacity (WBC)	638.10%	1345.09%

2.2 Discussion

Structural Confirmation by Infrared Spectroscopy

The FTIR of Crayfish chitosan and Desert grasshopper chitosan was shown in Figure 2. The two Spectra exhibits strong peak at 3257cm^{-1} and 3424cm^{-1} which can be assigned due to primary amine and coxial stretching vibration of O-H superimposed to the N-H stretching band respectively. The C-H stretching band arises at 2873cm^{-1} in both Spectra. Weak absorption peaks of amides at 1655cm^{-1} , 1551cm^{-1} in desert grasshopper Spectra and 1655cm^{-1} , 1547cm^{-1} in crayfish corresponded to amide (I) and amide (II) respectively, which indicates that the prepared chitosan have high degree of deacetylation. Besides, the bands at 1115cm^{-1} and 1071cm^{-1} appears in both Spectra that was due to C-O stretching of (1-4)- linked- β -D- glucosamine unit (Vadivel *et al.*, 2015).

Ash Content

Ash content is an important parameter that affects chitosan stability, viscosity and other important characteristics. The obtained ash content for the prepared chitosan (Table 1) were 3.25% for Grasshopper chitosan and 10.79% for Crayfish chitosan, These are indicating the effectiveness of the demineralization step in removing minerals (Shilratan *et al.*, 2014).

Moisture Content

The moisture content of the two prepared chitosan (Table 1) was found to be 1.72% for Grasshopper chitosan and for Crayfish chitosan 1.32%, this could be due to the nature of the dryness of the exoskeletons of the two species. The mirror ranges could be attributed to the extent of the drying process and exposure to the atmosphere. Chitosan is hydroscopic in nature; it is likely that chitosan can be affected by small moisture adsorption during storage. The lower the moisture content of chitosan, the better the shelf stability and hence quality (Sneha *et al.*, 2014)

Solubility and pH

The prepared chitosans (Table 1) were found to be soluble in 2% acetic acid solution and insoluble in water and most organic solvents. The result of the solubility of the two chitosan was achieved by weighing after drying which were found to be 0.001g and 0.003g when compared to the initial gram 0.1g used previously. The pH of the prepared chitosan was in the value of 6.7 for grasshopper chitosan and 7.0 for crayfish chitosan, which indicates the neutrality of the prepared chitosan (Lee *et al.*, 2011).

Degree of Deacetylation (DD)

The degree of deacetylation (DD) is an important parameter affecting solubility, chemical reaction and biodegradability. DD may range from 30-90% depending on the available source and preparation procedure. It



was calculated by using FTIR (infrared spectroscopic of prepared chitosan), using the equation proposed by Domszy and Roberts (1985).

$$DD = 100 - \left[\frac{A_{1655}}{A_{3424}} \times \frac{100}{1.33} \right] \dots \dots \dots (iv)$$

Where A1655 and A3450 were the absorbance at 1655 cm⁻¹ of the amide-I band as a measure of the N-acetyl group content and 3424 cm⁻¹ of the hydroxyl band. The factor ‘1.33’ denoted the value of the ratio of A1655 / A3424 for fully N-acetylated chitosan (Domszy and Roberts, 1985). The DD of the prepared chitosan were found to be 97.68% for Grasshopper and 98.28% for Crayfish chitosan (Table 1), this variation of the degree of deacetylation may be due to differences in the source and origin of the species used. 100% DD is very rarely obtainable. Commercial chitosan with various DD in the range of 75-85% is generally found (Shilratan *et al.*, 2014).

Fat Binding Capacity (FBC)

Fat binding capacity signifies how the chitosan easily bind or adsorb fat. Fat binding capacity (FBC) of cray fish chitosan and grasshopper chitosan samples were measured using Ground nut oil. FBC values depend on chitosan production and its sources. Roul (2001) reported that the average fat binding capacity of commercial crab chitosan and crawfish chitosan for soya bean oil were 706% and 587% respectively, regardless of the type of the oil used. The prepared chitosan samples showed desirable FBC values, 399.9% for grasshopper chitosan and 466.88% for cray fish chitosan (Table 1). It was found that cray fish chitosan (466.88%) had higher fat binding capacity as compared to grasshopper chitosan (399.9%) which is in agreement with those obtained 314% to 535% with an average of 417% reported by Roul, (2001). Conducting demineralization prior to deproteinization and deacetylation results in increase in FBC than deproteinization followed by demineralization and deacetylation (Moorjani *et al.*, 1975).

Water Binding Capacity (WBC)

The functional property analysed i.e water binding capacity for the prepared chitosan from Grasshopper (Table 1) was found to be 638.10% and that of Crayfish was 1345.09%. The results were supported by similar observations made by Roul, (2001) for chitosan samples, wherein, WBC ranged from 581%-1150% with an average of 702%. Also, Cho *et al.*, (2014) reported the WBC ranging from 458% to 805% for five commercial chitosan from shrimp and crab shells.

Scanning Electron Microscopy Analysis

The chitosan produced from grasshoppers and Crayfish were examined using SEM. The surface morphology of the chitosan from Crayfish Figure 3 (a) appears to have organized microfibrillar crystalline structure which was truant in chitosan, similar observations were reported by Yeng *et al.*, (2009), Arabia *et al.*, (2013) and Muzzarelli *et al.*, (2014)

The extracted grasshopper chitosan Figure 3 (b) was observed to have layers of flakes and porous nature could be seen in some areas. The differences observed in the surface morphologies of the two extracted chitosan could be attributed to different species of the organisms used for the extraction (Suneetha *et al.*, 2017).

3.1 Conclusion

This study showed that chitosans from two local sources, Desert Grasshoppers (*Shistocerca gregaria*) and Crayfish (*Cambarus baratonii*) were successfully prepared. The results of the physicochemical and functional properties indicated that Crayfish and Desert Grasshoppers are good source of chitosan. The production of chitosan from desert grasshoppers will reduce much of its environmental destruction effect on agricultural produce. Finally, research revealed the variations in the physicochemical and functional parameters of the two chitosan that become of good advantage to Scientist and Technologist in terms of their applications.



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