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Original Article

Production of Chitosan from Crab Shells

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ABSTRACT

The amino polysaccharide of chitin polymer happens to be the most commonly known polymer. Chitosan as a derivative of chitin is obtained through demineralization and deacetylation. Owing to the special properties of chitin and chitosan for instance their bio-compatibility, bio-biodegradability, non-toxicity and also capability to form films, have resulted in their suitability in chemical and thermal conductivity applications. In the field of nanotechnology, chitosan-based materials are currently being widely applied. The purpose of this review is to synthesize and characterize chitosan obtained from the crab shells. The absorption band obtained from FTIR between 1220 cm−1 and 1020 cm−1 represented free amino group (−NH2). The 40.000 grams of powdered crab shells resulted in the formation of 23.000g of chitin after undergoing the process of demineralization to eliminate the minerals available in the crab shells. The removed minerals from the crab shells constituted 17.000 g (39.53%) of the total dry weight in the crab shell. The water binding capacity (WBC) was found to be 69 %.

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INTRODUCTION

Chitosan (CS) is a polysaccharide that features a b-1-4- glycosidic bond between glucosamine and N-acetyl glucosamine (Iber et al., 2022). CS is a bio-polymer that is both harmless and biodegradable by enzymes found in the human body (Gîjiu et al., 2022). Crystallinity is a crucial factor in adsorption efficiency, and commercial CS are semi-crystalline polymers (Wang et al., 2023). Polymer CS has a broad-spectrum antibacterial effect because it binds to the negatively charged bacterial cell wall and then attaches to the DNA, preventing replication of the bacterium (Li et al., 2021). Chitosan is an antimicrobial bio-polymer (Olafadehan et al., 2021). Chitosan bio-activity can be enhanced by combining it with other bio-active compounds and applied in the medical field (Hao et al., 2021). The antimicrobial and healing functions of silver nanoparticles (AgNPs) are well-established (Abirami et al., 2021). They are highly effective against many different types of bacteria, and this is true even at extremely low concentrations (Ismail et al., 2023).

Experimental

All the experiments for this study were conducted in the following institutions' laboratories: Mount Kenya University, Kenya, Pwani University Kilifi County, Kenya, Kemfri and Government Chemistry Mombasa County, Kenya. The crustacean, crab shells, freezer (-16˚C), hydrochloric acid, sodium hydroxide and acetone, Glutaraldehyde (GA) solution 25% in water as a cross-linking agent and 100% (v/v) glacial acetic acid as a solvent, an oven, grinder. Crab shells were collected from the Malindi Marine Biotechnology Industry. The permit to collect the samples was obtained from the Department of Agriculture, Livestock Development and Fisheries Directorate (REF: KCG/MSC/FL/VOL.1/10) as shown in Appendix I. Crab shells were washed several times with distilled de-ionized water and then dried at 40 °C in an oven. Dried shells were powdered by a GRTA-2903 grinder and stored in a tightly closed glass bottle in Pwani University Research Laboratory at room temperature. The extraction process began through the demineralization procedures, where 40.000 g of powdered sample was reacted with 2.0 M hydrochloric acid solution for 24 h at 80.0 °C to eliminate all the minerals in the sample. Secondly, deproteinization procedures were done, whereby, the sample was reacted with 2.0 M sodium hydroxide at 110.0 ºC for 20 hours at a solid-to-solution ratio of 1:10 to remove all the proteins present in the sample. After demineralization and deproteinization, the sample was treated with acetone to remove colour. Finally, filtration was done to the sample, then washing with distilled de-ionized water severally and drying at 40.0 °C in an oven.

The extracted chitin was by applied with 50% concentrated sodium hydroxide at 150.0 °C after 4 hours at a solid to solution ratio of 1:10 (w/v). This process was used for the removal of the acetyl group attached to the extracted chitin. Therefore, this procedure allowed for the process of deacetylation to take place. The deacetylated sample was filtered using a Whatman filter paper of 0.35 mm thickness and thorough washing was done using distilled de-ionized (DD) water, this was done to obtain a neutral pH which is very fundamental in the extraction of chitosan. The extracted chitosan was the allowed to dry for further application. 2.00 mL of chitosan solution was prepared in (6%, v/v) acetic acid solution and mixed until the solution became homogeneous and clear. 1.00 mL of glutaraldehyde (GA) solution (1, 3 and 6%, v/v) was poured into the prepared chitosan solutions. The polymer and cross-linker mixture were immediately poured into a plastic syringe and placed into the cryostat. The mixture was incubated in the cryostat at -16.0 °C for 2 hours and stored in the freezer at the same temperature for 24 hours (D. Liang et al., 2023).

Characterization of chitin and chitosan Yield

Percentage production of chitin calculations were done by dividing the weight of extracted chitin to initial dry crab shell weight. On the other hand, the percentage production of chitosan was done by dividing the weight of yielded chitosan to dry

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chitin weight before deacetylation. Yield calculations were done as follow:

Production of chitin (%) = [yielded chitin (g)/Crab shells (g)] x100 …… equation 1(a)

Production of chitosan (%) = [yielded chitosan (g)/Chitin (g)] x100…… equation 1 (b)

FT-IR analysis of chitin and chitosan

The infrared spectral analysis of the extracted chitosan samples was measured using Fourier Transform Infrared Spectrometry, FT-IR (Frontier Spectrometer, Perkins Elmer, USA) in the wavelength range of 450 - 4000 cm^{-1} at a resolution of 4 cm^{-1}. For the preparation of pellet from the sample, the following were required; - a pellet holder, pestle, mortar, and drier. The 2.000 g of the extracted sample was washed with 80.00 mL alcohol at a 1:4 ratio. The dried sample was then centrifuged using a Remi C-854/6 centrifuge at 3500 revolutions per minute (rpm). Then KBr was ground using a pestle and mortar. The ground KBr was placed in FT-IR to hide the rock bottom in pellet disc and pressed at 0-10 Tones. The pressed sample was then carefully removed from the disc and placed in the sample holder for FTIR analysis.

Solubility of chitosan

A volume of 10.00 mL 1% acetic acid solution was added to a centrifuge tube containing 0.100 g of produced chitosan. The sample was centrifuged using a Remi C-854/6 centrifuge at 10,000 rpm for 30 minutes. The supernatant was poured away and the undissolved part of chitosan was washed with 25.00 mL of distilled de-ionized water and then centrifuged at 6,000 rpm. The supernatant liquid was poured away and the undissolved solid was dried at 60 °C for 24 h in an oven. The dried solid was stored in a dry petri dish. It was then weighed and the percentage of solubility determined. To determine the water uptake capacity (WUC), 10.00 mL of distilled water was put in a centrifuge tube containing 0.500 g of produced chitosan. The sample was mixed on a vortex about 5 minutes until the sample was dispersed. Then, the dispersed sample was vortexed for 5 seconds in every 10 minutes (for a total of 30 minutes) and centrifuged at 3500 rpm for 30 minutes. After centrifugation, the supernatant was poured into a glass petri dish and then the sample was weighed (Suryani et al., 2024). Water uptake capacity (WUC) was calculated as follows:

WUC (%) = [Bound water (g)/Initial chitosan weight (g)]x100…….. equation 1 (c)

Oil uptake capacity (OUC)

To determine the oil uptake capacity, 10.00 mL of sunflower oil was added into a centrifuge tube containing 0.500 g of produced chitosan. Then sample of sunflower oil and chitosan was mixed on a vortex for up to 5 minutes until the sample was dispersed. It was eventually vortexed for another 5 seconds every 10 minutes (for a total of 30 minutes) and centrifuged at 3500 rpm for 30 minutes. After centrifugation, the supernatant was poured away for disposal and the sample of was weighed to determine oil uptake capacity. OUC was calculated as follows: -

OUC (%) = [Bound oil (g)/Initial chitosan weight (g)]x100 ……...equation 1 (d)

Characterization

Both spectroscopic and microscopic techniques were applied in this study for the characterization of the synthesized samples. They included the following: - Furiour transform infrared (FT-IR), ultraviolet (UV-Vis) and liquid chromatography-mass (LC-MS) spectrometry, scanning electron microscope (SEM) and Motic light microscope. The samples were analyzed for functional groups by FT-IR spectroscopy, SEM analysis for visualizing the morphology. Images of the ITO electrode before and after embedment with silver nanoparticles (AgNPs) were viewed under Motic light microscope. The dyes were then analyzed for the levels of azo dyes before and after degradation using UV-Vis spectroscopy and LCMS.

FT-IR analysis

Furiour transform infrared spectroscopy was applied in the analysis of the chitosan, silver nano-composite film electrodes. The spectra were obtained over 16 scans covering the 4000-400 cm^{-1} wave-number range at 4 cm^{-1} resolution and at

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25.0 using a Thermo Scientific Nicolet iS10 FTIR spectrometer.

Results and analysis

Synthesis and characterization of chitosan

The 40.000 grams of powdered crab shells resulted in the formation of 23.000g of chitin after undergoing the process of demineralization to eliminate the minerals available in the crab shells. The removed minerals from the crab shells constituted 17.000 g (39.53%) of the total dry weight in the crab shell. The extracted chitin was further reacted with 50% concentrated sodium hydroxide at 150.0 ºC after 4 hours at a solid-to-solution ratio of 1:10 (w/v) in order to carry out deacetylation for the removal of the acetyl group attached to the extracted chitin. The deacetylated sample was filtered using a Whatman filter paper of 0.35 mm thickness and thorough washing was done using DD water resulting in the formation of 7.200 g (31.3 %) of chitosan. Similar results were obtained by Pakizeh in his previous studies on "Chemical extraction and modification of chitin and chitosan from shrimp shells" (Pakizeh et al., 2021).

Physicochemical properties of the chitosan

Solubility of the prepared chitosan was determined by dissolving 0.100 g of the extracted chitosan in (6%, v/v) acetic acid, 1.00 mL of glutaraldehyde (GA) solution (1, 3 and 6%, v/v) was poured to the prepared chitosan solutions to act as a crosslinker and then centrifuged using a Remi C-854/6 centrifuge at 10,000 rpm for 30 minutes. The supernatant was poured away and the undissolved part of chitosan was washed with 25.0 mL of distilled de-ionized water and then centrifuged at 6,000 rpm. The dried solid was stored in a dry petri dish, weighed and the percentage of solubility determined. The extracted chitosan solution was also noted to possess a yellow colouration which corresponded to the findings from the shrimp shell after the removal of the acetyl and mineral components (El-Araby et al., 2024). The water binding capacity (WBC) was found to be 69 %. The WBC % was determined by putting 10.0 mL distilled deionized water in a centrifuge tube containing 0.500 g of produced chitosan, mixed on a vortex for about 5 minutes until dispersed and then centrifuged at 3500 rpm for 30 minutes. The fat binding capacity (FBC) was found to be 56 %. The percentage FBC was determined by adding 10.0 mL of sunflower oil into a centrifuge tube containing 0.500 g of the extracted chitosan. After Centrifugation, sunflower oil and chitosan was placed on vortex for up to 5 minutes until the sample was dispersed and centrifuged again at 3500 rpm for 30 minutes. Chitosan obtained from these crab shells exhibited 89.5% degree of deacetylation (DD) which was noted to be effective and efficient in reducing and stabilizing silver nanoparticles from the Ag^+ ions because these are value trends that were also obtained in previous studies when shrimp shells were used in synthesis of silver nanoparticles (Jia et al., 2024). The wide range usage of chitosan in reducing the nanoparticles is due to its low levels of toxicity, biodegradability and capability of being biocompatible (Spósito et al., 2024)

Characterization of chitosan using Fourier Transform Infrared (FT-IR) Spectroscopy

Characterization of chitosan was accomplished using, FT-IR (Frontier Spectrometer, Perkin Elmer, USA). Analysis was carried out in the wavelength range of 450 - 4000 cm^{-1} at a resolution of 4 cm^{-1}). 2.000 g of extracted, washed, and dried chitosan was centrifuged. KBr was placed in FT-IR to hide the rock bottom in pellet disc, sample Pellets were using KBr salts due to their transparent nature, hence allowing greater resolution of spectra (Muñoz-Nuñez et al., 2023). The FT-IR spectrum for the characterization of 2.0 % chitosan was carried out and the signals obtained analysed as follows.

The signal shown at 526 cm^{-1} corresponds to the plane with N-H bends overlapping with C−O plane-out and bends in stretching bonds vibrations. The chitosan spectra showed a peak at 863 cm^{-1} corresponding to carbon - nitrogen (C−N) stretch, absorption spectral peaks between 1019 and 1073 cm^{-1} represented bending of C−O in glucose, whereas, 1422 cm^{-1} corresponded to C−H bend side chain. The signal shown at 1157

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cm^{-1} corresponded to C− O−C stretching vibrational bond, C-H stretching included 2873 cm^{-1} and 2916 cm^{-1}, a broader band at 3434 cm^{-1} for an overlap between the O−H stretching vibration and the N−H stretching vibration of the oligosaccharide was recorded. The amide, C=O bond showed its vibrational stretching mode at 1658 cm^{-1}. The signal at 1381 cm^{-1} corresponded to the C−O starch in the primary alcohols. The patterns of vibrational mode acquired from the FT-IR spectrum indicated that the functional groups available included carbon, nitrogen, and oxygen. The bands obtained from the FT-IR spectra occurred in the range between 4000 and 400 cm^{-1} frequencies, based on the fact that, it ranges within the electromagnetic spectrum where several molecules absorb electromagnetic radiation (Benamer Oudih et al., 2023). FT-IR is therefore, essential in the identification of the molecular groups in the organic compounds of both the chitin and chitosan found in crab shells (Javaid et al., 2023). Therefore, it is worth noting that the amino groups in the chitosan facilitate the formation of complexes with metallic ions, thus making it a super effective polymeric chelating agent with silver (Ag^+) ions (Ge et al., 2024). These results agree with those obtained from the studies carried out by azimi which indicated that FT-IR carried out on shrimp shells had its chitosan spectral lines showing at 1073 cm^{-1} confirming the presence of the amino groups located on the second carbon in the glucosamine (Azimi et al., 2023). Erdoğmuş et al., 2023 noted a major absorption band between 1220 cm^{-1} and 1020 cm^{-1} which represented free amino group ($-NH_2$) in the glucosamine in the same locant position earlier established.

Discussion

This section is often combined with the previous one to read Results and Discussion. If there are many major scientific or engineering novelties presented in the paper, it is recommended to have a separate discussion in order to eliminate a convoluted logical flow and confusing discussion of results.

Conclusion

This research illustrated that chitin and chitosan can be obtained from crab shells. Chitin and chitosan extracted from crab shells are supplementary to other sources obtained from other organisms such as crustaceans and fungi and can be applied for numerous research areas such as in nanotechnology, medicine, cosmetics and effluent treatment.

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Table 1 Percentage of extracted crab chitosan

Initial weight (g)	Final weight (g)	% of extracted Chitosan
23	7.2	31.3

Table 2 Physicochemical properties of the extracted chitosan

Properties	Chitosan in %
Water binding capacity (WBC)	69
Fat binding capacity (FBC)	56
Moisture content	59.611
Deacetylation degree	89.5

Figure 1: A graph showing FT-IR spectrum of 2.0 % chitosan