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Comparative Study of Chitin Extraction from Waste Crab Shell Using Biochemical Method

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ABSTRACT:

After cellulose, chitin is the most prevalent natural polymer. It can be found in the exoskeletons of insects, plankton, fungi, and crustaceans. The biochemical method of extracting chitin from crustacean shells is a sophisticated and novel process that yields the long chain carbohydrate polymer chitin. It involves two important phases that are essential for the removal of protein, calcium carbonate, and other minerals found in the shells. Qualitative tests give confirmatory tests for presence of carbohydrates. In order to take a confirmatory test of derived chitin, qualitative and phytochemical tests of chitin were conducted. Even though chitosan is thought to be safe and environmentally benign, the harsh chemicals used to produce it from chitin must be handled cautiously to prevent harm to the environment.

Keywords – Chitin extraction, chitosan, Waste Crab Shell, Demineralization, Deproteinization.

INTRODUCTION:

The largest of the six classes of crustaceans, Malacostraca has over 40,000 extant species spread across 16 orders. The diverse range of body forms exhibited by its members, the malacostracans, includes woodlice, prawns, shrimp, krill, crabs, lobsters, and crayfish. Tongue-eating lice, mantis shrimp and numerous more uncommon creatures. They have taken over freshwater and terrestrial ecosystems and are widespread in all marine settings. These are segmented animals that are separated into the head, thorax, and abdomen and are connected by a common body plan with 20 body segments. The class Malacostraca comprises the decapods order of crustaceans, which includes several well-known species including crayfish, lobster, crabs, and prawns. Decapods are mostly scavengers. It is believed that there are about 15,000 species in the order, distributed among about 2,700 genera and 3,300 species. Crabs make up over half of these species; the majority of the remaining species are shrimp and Anomura, which includes hermit crabs, porcelain crabs, and squat lobsters. Up to 38 appendages can be seen on decapods, with one pair on each segmented body.

After cellulose, chitin is the biopolymer that is most prevalent in nature. Strong acids and bases are typically used in chemical procedures to isolate it from the exoskeletons of invertebrates, insects, marine diatoms, sponges, molluscs, coralline algae, cell walls of some fungi, and crustaceans like crabs, prawns, and lobsters. Chitin has a light yellow to brown tint and resembles flocculence or a filiform solid in appearance. In addition, water cannot dissolve chitin. Chitin has a variety of chemical structures instead of just one. It contains a number of polysaccharides made up of D-glucosamine and N-acetyl-D-glucosamine units. Chitin is structurally defined as a straight-chain polymer made of 1,4-N-acetylglucosamine. There are three main natural polymorphs of chitin: α-, β-, and γ-chitin, with α-chitin being the most prevalent in nature and having a structure of antiparallel chains, usually isolated from the exoskeleton of crustaceans. β-chitin can be obtained from squid pens. It has intra-sheet hydrogen bonding by parallel chains. Meanwhile, γ-chitin, found in yeast and the cell walls of certain fungi, has not been completely identified. [S. Fadlaoui, O. El Asri ,et al[2019]

One antiparallel chain and two parallel chains have both been postulated as its possible composition. Have proposed that rather than being a separate polymorph, γ-chitin could be a mix of α- and β-structures. Chitin has a compact structure, which makes it insoluble in the majority of solvents. Therefore, chitin undergoes chemical modifications. Chitosan, a hydrophilic, natural, cationic, nontoxic biopolymer formed from partial N-deacetylation of chitin, is the most widely used derivative. It is a straight chain polymer of glucosamine and N-acetylglucosamine.. Chitosan and chitosan oligomers are also known for their biological activities, such as their antimicrobial, antitumor, and hypocholesterolemic functions. [L. Mohammed, A. Sihame, A. Omari, et al;[2019].

The carapace waste of crustaceans is constituted mainly of 30–50% calcium carbonate, 30–40% protein, and 20–30% chitin. Nevertheless, these constituents are changeable, depending on the species and seasons.[M. Melhaou, et al;[2019]. The separation of chitin from various sources depends on the source, and the amount of chitin present in the source varies depending on the source's origin. Due to its effectiveness as absorbent, chitosan has received special attention. Due to its lower price as compared to activated carbon and its high amino and functional groups with strong potential for adsorption, this biopolymer is a desirable alternative. Due to its physic-chemical properties, chemical stability, strong reactivity, and good chelation, this biopolymer offers a compelling alternative to other biomaterials. Organic chitosan has undergone numerous modifications either physically or chemically for the purpose of increasing the adsorption on capacity for the eradication of various contaminants in wastewater and water.[Kishore Kumar Gadgey and Dr. Amit Bahekar [2017]. Protein and chitin combine in the exoskeleton tissue to generate a protein-chitin matrix, which is then heavily calcified to produce hard shells. In addition to lipids from the muscle waste, the waste may also include carotenoids, primarily astaxanthin and its esters a conventional process for extracting chitin for

commercial use from two fundamental steps make up a crustacean's exoskeleton: (A) protein separation, or deproteinization by alkali demineralization through acidic treatment and (B) calcium carbonate (and calcium phosphate) separation treatment at a high temperature, followed by a chemical bleaching procedure to produce a colourless product. Alkaline treatment is typically used to deproteinize. Generally speaking, demineralization accomplished using the acids HCl, HNO3, H2SO4, CH3COOH, and HCOOH; nevertheless, HCl seems to be the most effective to be chosen as the reagent.

The versatile biopolymer chitosan is mainly obtained from the shells of crustaceans, which include prawns, crabs and lobsters. The process involves deacetylating chitin, the second most prevalent natural polymer worldwide. The biocompatibility, biodegradability, and non-toxicity of chitosan make it useful in biotechnology, medicine, agriculture, and water treatment. Chitosan is a linear polysaccharide made up of N-acetyl-D-glucosamine (acetylated unit) and randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit). It comes from chitin, which is present in the cell walls of fungus and the exoskeletons of crustaceans including crabs, prawns and lobsters. Because of its unique antibacterial, non-toxic, biodegradable, and biocompatibility qualities, chitosan finds extensive use in a wide range of industries. The main process that produces chitosan is chitin deacetylation. This entails processing chitin at high temperatures with a strong alkali solution, typically sodium hydroxide, as was indicated in earlier comments. The procedure converts chitin into chitosan by removing acetyl groups from the molecule, raising the proportion of deacetylated units. The solubility and other physical characteristics of chitosan are influenced by its degree of deacetylation; in general, chitosan that has undergone more than 50% deacetylation is soluble in acidic solutions and insoluble in aqueous and alkaline conditions.

Even though chitosan is thought to be safe and environmentally benign, the harsh chemicals used to produce it from chitin must be handled cautiously to prevent harm to the environment. In order to reduce the impact of these substances, appropriate waste management and treatment protocols should be put in place.Chitin extraction from freshwater crab shells is an important procedure, especially considering how common this biopolymer is and how many uses it has in water treatment, agriculture, and biomedicine. Chitin extraction proceeds similarly to the first steps of chitosan extraction, with the exception of the deacetylation step.

MATERIAL & METHODOLOGY:

1. Biochemical Method of Chitin Extraction: Specimens of freshwater crab *Barytelphusa cunicularis* and marine water crab *Charybdis feriata*_were collected from the local fish market [Sai Fish Merchant] Gandhi Peth, Pimpri-Chinchwad]. Hydrochloric acid and sodium hydroxide were used in chitin preparation process. Additionally, distilled water was used to make the chemical solution at the necessary concentration and to wash the sample. The resulting chitin obtained was filtered, washed, dried, and weighted. The yield of chitin extracted from crabs was then calculated.

Collection of crab species

Shell separation from freshwater and marine water crabs

Sun drying of shells

Demineralization & deproteinization of shells

Filter & washed shells

Raw chitin

Flowchart of chitin extraction :

Sun dried 5gm crab shells

↓

250ml 1N NaOH at 80°C for 1 hour [Deproteinization]

↓

Filter and washed with distilled water and dried

↓

100ml 1N HCL Room temperature for 3-4 hours [Demineralization]

↓

Filter and washed with distilled water

↓

Dried in oven for 4 hours at 60°C

↓

CHITIN

2. Biochemical Method Of Chitosan Extraction:

For chitosan extraction, deacetylation of purified crab chitin was done. Chitin was treated with NaOH followed by stirring at 120°C for 2 hours. The resulting chitosan was filtered, washed, dried, and weighted. The yield of chitosan extracted was then calculated.

Raw chitin

Deacetylation of chitin

Chitosan

Flowchart of chitosan extraction:

1gm dried chitin

↓

100ml NaOH

↓

Stirring at 120°C for 2 hours

↓

Filtered, washed, dried

↓

CHITOSAN

QUALITATIVE TESTS FOR CARBOHYDRATES:

Molisch Test: Specific for carbohydrates, monosaccharide gives a rapid-fire positive test, disaccharides and polysaccharides reply slower.

1. 2 ml of a sample result is placed in a test tube.
2. 2 drops of the Molisch reagent is added.
3. Concentrated Sulphuric acid should be added to the test tube's sidewall.
4. The acid sub caste forms a sub caste at the bottom.
5. If there's a confirmation of violet ring also the presence of carbohydrate is verified

Fehling's Test: This test is given by reducing sugars.

1. 2 ml of a sample result is placed in a test tube.
2. Add 2 ml of Fehling's result A and Fehling's result B to it.
3. Keep the result in a scorching water bath for about 10 twinkles.
4. If there's the conformation of red precipitate also the presence of carbohydrate is verified.

Benedict's Test: This test is given by reducing sugars.

1. Take the given sample result in a test tube.
2. Add 5 ml of Benedict's reagent to it.
3. Boil the result for about 2 twinkles and cool it.

4. Still, red or unheroic precipitate also there's presence of reducing sugars, If there's conformation of green.

Tollen's Test: This test is administered by reducing sugars.

1. Take the given sample result in a test tube.
2. Add 2- 3 ml of Tollen's reagent to it.
3. Keep the test tube in a scorching water bath for 10 twinkles.
4. If there's appearance of candescent tableware glass confirms the presence of reducing sugars.

Phytochemical Tests of Chitin:

Methodology of Harborneset.al (1998)

1. Saponins: about 1 ml of chitin result was introduced into a tube containing 1 ml of distilled water, the admixture was roundly shaken for 2 min, and conformation of head indicated the presence of saponins.

2. Terpenoids: 5 ml of chitin result were mixed in 2 ml of Chloroform B and 3 ml Concentrated sulphuric acid was precisely added to form a subcaste. A sanguine browen colour at the interface indicates the presence of terpenoids.

3. Tannins To 2 ml of chitin result was added 2- 3 drops of 5 ferric chloride results. Conformation of black colour showed the presence of tannins.

4. Flavonoids: 2 ml of sodium hydroxide was added in 2 ml of chitin result. Appearance of unheroic color wasregarded as the presence of flavonoids.

5. Alkaloids: A little quantum of picric acid result was added in 2 ml of chitin result. Conformation of orange color showed the presence of alkaloids.

6. Phenols: 2 ml of ferric chloride result was added in 2 ml of chitin result. Conformation of blue, green or violet colour indicates the presence of phenolic composites.

7. Phlobatannins: Many drops of 1 N HCL were added in 1 ml of chitin result and boiled. Red precipitate was formed which indicated the presence of phlobatannins.

RESULTS:

Species confirmation of freshwater and marine water crabs was done Zoological Survey of India, Western Regional Office, Akurdi. and freshwater species *Barytelphusa cunicularis* and marine water species *Charybdis feriata* were confirmed. Chitin yield obtained from *Barytelphusa cunicularis* was 1.25gm whereas chitin yield obtained from *Charybdis feriata* was 1.38gm. Chitin yield obtained from both the crabs shows difference of0.13gm. Freshwater crab gives chitosan yield of 0.39gm and marine water crab gives chitosan yield of 0.49gm. Chitin gives positive result for Molisch test indicating presence of carbohydrate. Phytochemical tests shown negative results.

Chitin extraction from freshwater [*Barytelphusa cunicularis*] crab:

Sr. No	Concentration of deproteinization at 80°C	Concentration of demineralization at room temp.	Yield
1.	1 N NaOH	1 N HCL	1.25gm

Table.1.Chitin yield from freshwater crab

Chitin extraction from marine water [*Charybdis feriata*] crab:

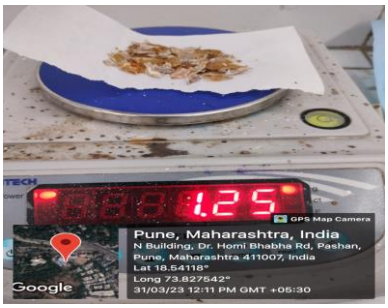
Sr. No	Concentration of deproteinization at 80°C	Concentration of demineralization at room temp.	Yield
1.	1 N NaOH	1 N HCL	1.38 gm

Table.2.Chitin yield from marine water crab

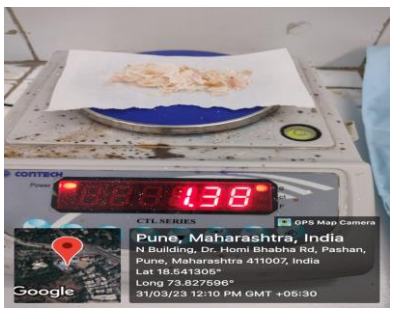
Comparison of chitin yield:

Sr. No.	Species	Yield
1.	*Barytelphusa cunicularis*	1.25gm
2.	*Charybdis feriata*	1.38gm

Table.3.Chitin yield from freshwater and marine water crab



Chitin of freshwater crab

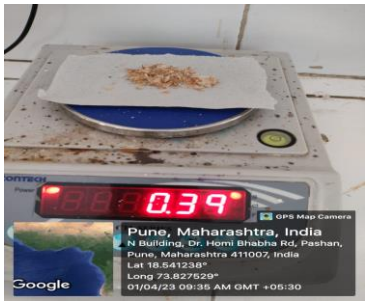


Chitin of marine water crab

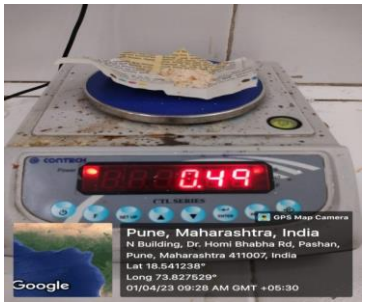
Comparison of chitosan yield:

Sr. No	Species	Yield
1.	*Barytelphusa cunicularis*	0.39gm
2.	*Charybdis feriata*	0.49gm

Table.4.Chitosan yield from freshwater and marine water crab



Chitosan of freshwater crab



Chitosan of marine water crab

Qualitative tests of chitin :

Test	Result
Molisch test	Present
Fehling's test	Absent
Benedict's test	Absent
Tollen's test	Absent

Phytochemical tests of chitin :

Test	Result
Saponin	Absent
Phlobatannins	Absent
Terpenoids	Absent
Tannins	Absent
Flavonoid	Absent
Alkaloid	Absent
Phenol	Absent

Conclusion :

In this study, the two same experiments were carried out simultaneously on two different species of crabs i.e freshwater crab [*Barytelphusa cunicularis*] and marine water crab [*Charybdis feriata*] to extract chitin. The concentration of NaOH and HCL were kept constant for both the experiments and comparative study was done on yield of chitin. The chitin yield obtained from freshwater crab was 1.25gm and marine water crab was 1.38gm. So the highest yield obtained as compared to freshwater crab was that of marine water crab with just 0.13gm difference. So we can conclude that the chitin yield obtained from both the crabs is almost same with negligible difference.

Chitosan extraction was done by deacetylation process on chitin where again two same experiments were carried out simultaneously on two different species of crabs i.e freshwater crab [*Barytelphusa cunicularis*] and marine water crab [*Charybdis feriata*] to extract chitosan. The concentration of NaOH was kept constant for both the experiments and comparitive study was done on yield of chitosan. The chitosan yield obtained from freshwater crab was 1.39gm and marine water crab was 1.49gm. So the highest yield obtained as compared to freshwater crab was that of marine water crab with just 0.10gm difference. So we can conclude that the chitosan yield obtained from both the crabs is almost same with negligible difference.

Qualitative tests were performed on chitin to confirm that the extracted chitin is a pure polysaccharide. The sample tested positive for Molisch test which cnfirmed that the given sample was a polysaccharide. As Chitin showed negative tests for Fehling's test, Benedict's test and Tollen's test, it was concluded that no reducing sugars were present in the sample. Phytochemical tests were performed on chitin to see the presence of Saponin, Phlobatannins, Terpenoids, Tannins, Flavonoid, Alkaloids and Phenol in chitin. All the tests gave negative results for the sample. Hence its was concludes that no phytoconstituents were present in the sample.

In conclusion, chitin derived products like chitosan can be made using the extracted chitin with the maximum yield. This study shown that a significant amount of wasted crab shells have significant nutritional and economic potential and can be used as high-quality nutritious ingredients in the food and health industries in the future. It is possible to conclude that the current work has successfully synthesised chtin from waste raw materials, such as crab shell, by using the "Fourier transform infrared" [FTIR] technique. Chitin has the advantages of being biocompatible, biodegradable, and an efficient biomedical interface material. Chitin that has been removed from crab shells has a wide range of industrial, medicinal, and pharmacological uses. To substantiate, however, a greater number of tests must be conducted and supported by suitable analytical techniques.

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