



Preparation, Chemistry and Applications of Chitosan

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ABSTRACT

Chitosan is an amino- polysaccharide derived from chitin that comprises of linear amino-polysacchride including N-acetyl D-glucosamine and D-glucosamine components. Chitosan has gathered largely interest starting with industry and academic as a result of its biological activities. Chitosan may be fitting biopolymer that could be employed in a variety of industries. Currently the major sources of chitosan are crustacean shells, shrimp, crabs and crayfish. The present review interested on the recent sources of chitosan particularly insects such as grasshopper. Insect like grasshopper are considered to be pest and harmful species to agricultural land and they are in abundance especially in the northern part of Nigeria and it can serve as possible source of chitosan and their scientific benefit has not been tapped, also different method may be used to prepare chitosan such as chemical and biological method, and it has many application. The present review provided the best insight into the sourcing the chitosan from insect such as grasshopper as alternative to crustaceans shells, shrimp and crabs which may also address food scarcity related challenges by reducing the population of the pests and thereby their infestation. The future direction of research should focus on the destruction of catastrophic species into a value-added product that could replace the existing biopolymers sources and hence increase the opportunities in this field, also studies are required to optimize the production process for higher yield using modern technology such as electrochemical methods or ultrasonication and microwave. Further innovative insect rearing methods would also produce a constant supply of insects for industrial uses i.e specific species/stages. Cost-effective method and straightforward synthesis approaches could be required for large-scale production of insect chitosan. Hence, up-scaling efficiency, insect species selectivity, and stability in real-time applications need to be explored. Also, further research is required for understanding the chitosan mechanisms of action and bioactivity.

Keywords: Chitosan, sources, preparation, chemistry, application

1.0 INTRODUCTION

The Biopolymer Chitosan has emerged as an essential material in numerous scientific discipline and biotechnology sector and hence driven by its verifies of biological activities (Abiodu *et al.*, 2020). Chitosan having being a biodegradable and eco- friendliness polymer holds immense potentials for diverse applications. In addition to that, its full potential remains untapped as a result of incompleted research (Pooja *et al.*, 2022). Chitosan's molecular structure comprises of linear chain of glucosamine molecules linked by (1, 4)-bonds, thereby intercepted with-N-acetyle glucosamine units (Adeleke *et al.*, 2003). Chitosan demonstrates remarkable properties for improving useful firms suitable for food packaging, leveraging its biological properties including antioxidant and anti inflammatory benefits (Backshire *et al.*, 2002). Chitosan is the most available naturally occurring cationic biopolymer characterising amino groups (-NH₂). It however dissolves readily in acidic solutions PH<6) to both organic and inorganic amino groups. This process imparts polycationic character enabling Chitosan to form polyelectrolyte complexes with polyanions (Adeleke *et al.*, 2023). Chitosan exhibits outstanding firm forming properties and can be formulated into firms, fibers, hydrogels and micro/nano

particles (Amelia *et al.*, 2021). Chitin, the precursor to Chitosan is the world's second most abundant polymer, surpassed only by cellulose. This rigid, while polyschride is primarily found in the cellular walls of fungi, exoskeletons of crustaceans and exoskeleton of insects .The present review provide best insight into the chemistry, source, history, method of extraction and application of chitosan.

1.2 History of chitosan

Research on the extraction and characterization of chitin began in 1811 with the work of French chemist Professor Braconnot, who identified an alkali-resistant fraction from fungal isolates and named it "fungine" (Prasanna *et al.*, 2020). In 1823, another chemist, Odier, discovered a material with similar properties in the cuticle of beetles and named it "chitin," derived from the Greek word "chiton," meaning "coat of mail." In the following years, researchers continued to study chitin. In 1843, Lassaigne demonstrated the presence of nitrogen in the structure of chitin using silkworms. Later, in 1859, Rouget boiled chitin in potassium hydroxide, making it soluble in organic acids and leading to the discovery of chitosan (Rouget, 1859). By 1878, Ledderhose identified compounds such as glucosamine and acetic acid within chitin, and in 1894,

Gilson confirmed the presence of glucosamine units. That same year, German chemist Felix Hoppe-Seyler officially named the material “chitosan” (Hoppe, 1894). The chemical structure of chitosan was first determined in 1950. Between 1930 and 1950, numerous scientific articles were published exploring its potential applications. Over the recent decades, researchers have increasingly focused on chitosan as a bioactive material (Amelia *et al.*, 2021). The first reports of chitosan production emerged in the 1970s, primarily in Japan and the United States. By 1986, the marketing of chitosan was established, and by 1999, Japan had at least fifteen industries commercially producing chitin and chitosan. Today, Japan and the United States are recognized as global leaders in chitosan production (Prasanna *et al.*, 2020).

1.3 Chemistry of Chitosan

Chitosan, a derivative of chitin obtained through partial deacetylation, exhibits a degree of deacetylation (DDA) between 50% and 99%, with varying molecular weights (Mati-Baouche *et al.*, 2014). It is a copolymer composed of two sugars: glucosamine and N-acetylglucosamine. Its structure includes reactive functional groups such as an amino group at C₂, a primary hydroxyl group at C₃, and a secondary hydroxyl group at C₆, represented by the molecular formula (C₆H₁₁O₄)_n. Chitosan stands out due to its nitrogen content, making it commercially significant and suitable for various applications (Luo *et al.*, 2019). Under acidic conditions (pH below 7), chitosan is water-soluble and acts as a cationic polyelectrolyte due to the positive charge on its amino groups (Patricia *et al.*, 2021). The molecular weight (MW) and degree of deacetylation (DDA) are key parameters influencing its biological and chemical properties (Aranaz *et al.*, 2021). Chitin, closely related to cellulose in structure, has hydroxyl groups replaced by acetyl amine groups (NHCOCH₃), forming poly-(N-acetyl D-glucosamine) or poly-β-[1,4]-N-acetyl-d-glucosamine. The degree of acetylation (DA) refers to the proportion of N-acetylated units, while the degree of deacetylation (DD) reflects the content of β-1,4-D-glucosamine units (Younes and Rinaudo, 2015). The structure of chitosan is depicted in Figure 1.

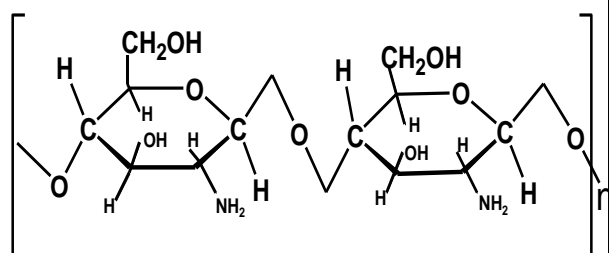


Figure 1: Structure of Chitosan

1.4 Origin of Chitosan

Chitin is produced by a variety of organisms and is commonly extracted from the cell walls of fungi and algae, the exoskeletons of insects, the internal skeletons of cephalopods, and the shells of mollusks and crustaceans (Chik *et al.*, 2023). It can also be obtained from natural sources such as crustaceans (e.g., lobsters, shrimp, crabs, krill, barnacles, crayfish), insects (e.g., grasshoppers, scorpions, ants, cockroaches, spiders, beetles, brachiopods), invertebrate animals, and mollusks (e.g., octopus, cuttlefish, clams, oysters, geoducks, squids, snails). Additionally, chitin is found in algae (e.g., diatoms, brown algae, green algae) and certain microorganisms like fungi and bacteria. According to Dima, *et al.*, (2017), crustacean waste contains approximately 10–25% chitin on a dry weight basis. Insects are emerging as promising alternative sources of chitin due to their high nutritional content (rich in proteins, fats, and biopolymers), their ability to be cultivated on organic waste, and their substantial dry matter content (Aranaz *et al.*, 2021). This review highlights that insects can serve as viable alternative sources for chitin and chitosan production. Furthermore, insects can reproduce rapidly under suitable environmental conditions, potentially leading to an abundant supply for extraction (Aranaz *et al.*, 2021). Compared to crustaceans, which are the primary source of chitin, insect exoskeletons contain lower levels of inorganic materials, making the demineralization process faster and more efficient (Chik *et al.*, 2023).

2.0 CHITOSAN PRODUCTION METHODS

Chitosan can be synthesized using biological or chemical approaches. The chemical process remains the most commonly employed method for extracting chitin and preparing chitosan on a commercial scale. This is largely due to its advantages, which include shorter processing times, high degrees of deacetylation (DD) in the final chitin product, suitability for industrial-scale operations, and the effective removal of organic salts. However, these chemical methods pose significant environmental concerns. The extracted minerals and proteins are unsuitable for human or animal consumption, and the safe disposal of chemical waste substantially increases production costs in the chitin industry (Andressa *et al.*, 2021). Proteins can be removed enzymatically using digestive proteolytic enzymes such as papain, pepsin, trypsin, and pronase. Alternatively, microbial methods offer a more efficient approach for recovering chitin compared to chemical techniques. In this method, organic acids (e.g., lactic acid produced by probiotic bacteria) are used for the demineralization process. Enzymatic and microbial approaches provide several benefits, including higher yields and quality of chitin (due to reduced depolymerization and deacetylation), the possibility of using protein by-products as animal feed, and the elimination of chemical pollution. However, these methods also have limitations, such as inconsistent

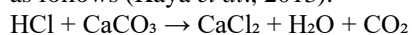
research outcomes, higher overall costs, and the lack of large-scale industrial implementation, requiring further study and development (Andressa *et al.*, 2021). During the demineralization reaction, calcium carbonate (CaCO_3) decomposes into water-soluble calcium salts, such as calcium chloride (CaCl_2), along with the release of carbon dioxide (CO_2), as shown in the reaction below: $\text{HCl} + \text{CaCO}_3 \rightarrow \text{CaCl}_2 + \text{H}_2\text{O} + \text{CO}_2$. The amount of CO_2 emitted depends on the mineral content of the shrimp shells and the extent of acid penetration. Based on stoichiometric calculations, two molecules of HCl are required to convert one molecule of CaCO_3 into CaCl_2 . A schematic diagram illustrating the stages of chitosan production from extracted chitin is provided in Figure 1 (Andressa *et al.*, 2021).

2.1 Chemical Extraction Method of Chitosan

The chemical extraction method is the most widely used technique for chitin isolation and chitosan production on a commercial scale (Hou *et al.*, 2020). This process consists of three main steps: 1. Demineralization: The removal of inorganic materials, such as calcium carbonate (CaCO_3) and calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), using an acidic medium, typically hydrochloric acid (HCl) solutions. 2. Deproteinization: The extraction of proteins using an alkaline medium, commonly sodium hydroxide (NaOH) or potassium hydroxide (KOH) solutions. 3. Deacetylation: The removal of acetyl groups to produce chitosan (Hamed *et al.*, 2016).

2.1.1 Demineralization

Demineralization refers to the extraction of minerals, primarily calcium carbonate. In 1978, a patented method for commercial demineralization of chitin from crustacean shells was introduced. This process is typically carried out using acid treatments, including sulphuric, hydrochloric, nitric, acetic, oxalic, and formic acids (Hou *et al.*, 2020). Among these, hydrochloric acid (HCl) has proven to be the most effective for chitin extraction from insects. The process involves the conversion of calcium carbonate into calcium chloride, accompanied by the release of carbon dioxide (Barbosa *et al.*, 2019). Specifically, the reaction results in water-soluble calcium salts (such as calcium chloride, CaCl_2) and the emission of carbon dioxide gas (CO_2). The amount of CO_2 released depends on the mineral content of the exoskeleton and the degree of acid penetration. The chemical reaction is represented as follows (Kaya *et al.*, 2013).



2.1.2 Deproteinization

Deproteinization is a challenging step due to the disruption of the chemical bonds linking chitin and proteins. The process typically begins with chemical treatments to eliminate proteins. A variety of chemicals, including sodium hydroxide (NaOH), potassium hydroxide (KOH), potassium carbonate

(K_2CO_3), and calcium hydroxide ($\text{Ca}(\text{OH})_2$), are used for this purpose. NaOH and KOH are widely applied for deproteinizing chitin from sources like shrimp, crab, lobster, and krill. Reaction conditions, such as chemical concentrations and durations, vary significantly between studies. Complete protein removal is critical, especially for biomedical applications, as shellfish-derived proteins can trigger allergic reactions in some individuals. NaOH is the preferred reagent, often used in concentrations ranging from 0.125 M to 5.0 M (Kaya *et al.*, 2014). The process may involve temperatures up to or exceeding 160°C and treatment times ranging from a few minutes to several days (Luo *et al.*, 2019).

2.1.3 Deacetylation

Deacetylation is the process of removing acetyl groups from chitin while introducing reactive amino groups in their place. The degree of deacetylation (DDA) measures the percentage of free amino groups in the structure, which helps distinguish between chitin and chitosan. DDA plays a crucial role in defining chitosan's physicochemical and biological characteristics, such as its acid-base balance, electrostatic properties, biodegradability, self-aggregation, sorption abilities, and metal ion chelation potential (Andressa *et al.*, 2021). Chitin can be converted into chitosan through enzymatic or chemical methods. Although enzymatic methods show good efficiency, they are costly and result in low productivity, limiting their use to laboratory-scale production of low molecular weight (Mw) and amorphous chitin (Hou *et al.*, 2020). In contrast, traditional chemical methods, widely used in industries for large-scale production, offer higher practicality but face challenges like inconsistency in molecular weight and degree of deacetylation, along with environmental concerns. These inconsistencies arise due to uncontrolled hydrolysis and chemical modifications that generate undesired by-products and significant aqueous waste (Pooja *et al.*, 2022). N-deacetylation, the simplest modification of chitin, involves removing acetyl groups to convert it into chitosan. Alkaline treatments, often used for this purpose, enhance the solubility of chitin by breaking its main chains and disrupting its crystalline structure (Hamdi *et al.*, 2020). Typically, deacetylation is achieved through severe alkaline hydrolysis at elevated temperatures using concentrated sodium hydroxide (NaOH) or potassium hydroxide (KOH) solutions (40–50%) (Hou *et al.*, 2020). Alkaline methods are preferred over acidic treatments because glycosidic bonds are highly sensitive to acid, making alkali more suitable for deacetylation (Bakshi *et al.*, 2020). Several factors influence the properties of the resulting chitosan, with temperature and reaction time being the most significant in determining the DDA and molecular weight (Liu *et al.*, 2020). The structural diagram

illustrating the preparation of chitosan is shown in Figure 2 below.

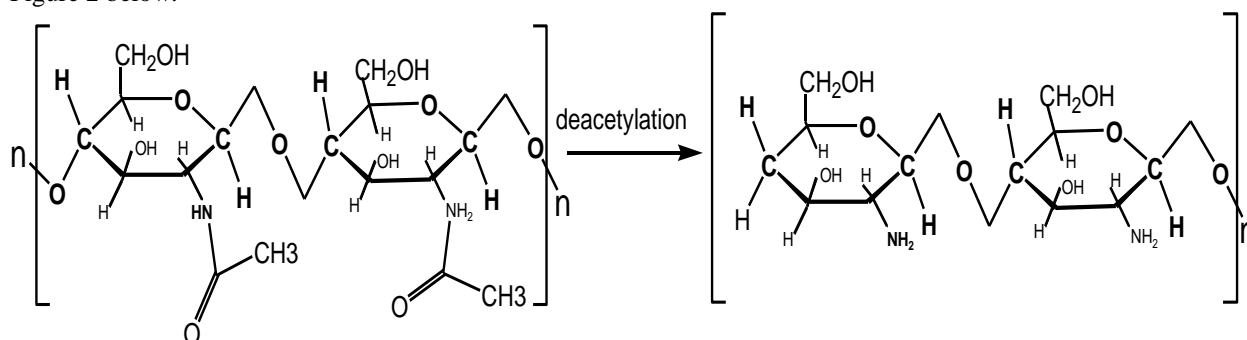


Figure 2: Structural Diagram of Chitosan Preparation

2.2 Characteristics of Chitosan

Chitosan possesses a broad spectrum of unique characteristic, such as its ability to chelate metal ions, exhibit variable viscosity, dissolve in different solvents, form films, adhere to mucosal surfaces, and display polyelectrolyte behavior along with polyoxysalt formation (Amelia *et al.*, 2021). Its linear, unbranched structure contributes to its remarkable viscosity, which can be adjusted by modifying deacetylation conditions. Structurally, chitosan features linear polymine chains, reactive amino and hydroxyl groups, and the capacity to chelate various transition metal ions. Chitin, the precursor to chitosan, is a white, rigid, nitrogen-containing polysaccharide found in the exoskeletons and internal structures of invertebrates (Amelia *et al.*, 2021). The structure of chitosan is crucial as it directly influences its physicochemical properties. A key feature of chitosan is its high degree of deacetylation and minimal crystalline regions, making it highly suitable for numerous applications, especially in the fields of pharmacy and biotechnology. The physicochemical properties of chitosan are shaped by factors such as its degree of deacetylation (DD), crystallinity, molecular weight (MW), and methods of degradation (Pooja *et al.*, 2022). Chitosan and its derivatives demonstrate remarkable biological properties, including anti-inflammatory, antibacterial, antitumor, antifungal, hemostatic, and analgesic activities (Patricia *et al.*, 2021). Studies show that chitosan exhibits a strong antibacterial effect against a variety of pathogens due to its cationic nature, which allows interactions with the negatively charged lipids and proteins of bacterial cell walls. This interaction, along with the penetration of chitosan into the cell membrane, disrupts membrane permeability, causing leakage of cytoplasmic contents and bacterial cell death. Furthermore, *in vitro* experiments suggest that the antibacterial activity of chitosan may also stem from its ability to bind DNA, thereby inhibiting mRNA synthesis when in contact with bacterial nuclei (Hou *et al.*, 2020). Chitosan's antifungal activity is attributed to its electrostatic interactions, which disrupt fungal cell processes and enhance its overall bioactivity.

2.3 Applications of Chitosan

The growing significance of chitosan is evident from the extensive body of research highlighting its applications. This polycationic, linear amino-polysaccharide is highly valued for its biodegradability, biocompatibility, non-toxic nature, and remarkable adsorption capacity. Its wide-ranging applications span the cosmetic, food, and pharmaceutical industries due to its anti-inflammatory, antitumor, antifungal, anticancer, antioxidant, and antibacterial properties (Amelia *et al.*, 2021). The versatility of chitosan makes it an essential material across various industrial domains. Chitin and its deacetylated form, chitosan, have been documented in numerous studies as raw materials for synthetic fibers. Fibers derived from chitin and chitosan serve crucial roles, such as absorbable sutures and wound-dressing materials. Moreover, chitosan fibers exhibit potential for wastewater treatment, particularly for the removal of heavy metal ions through chelation, which has garnered significant attention. Additionally, substances that absorb harmful UV radiation or bind with dyes can be covalently attached to chitosan's amino groups. Innovative formulations combining chitosan with other hydrocolloids and plant-based antioxidants, anti-allergic, or anti-inflammatory agents further expand its industrial utility. Below is a summary of chitosan's diverse applications across various industries (Chik *et al.*, 2023). As there is an increase in the demand for chitosan biopolymer materials for healthcare, personal care, packaging, and coating materials, and this emerging situation has increased the demand.

for biomaterials for biomedical, food, and pharmaceutical applications. The projected statistics show the market size for chitin/chitosan will grow up to 162.7 thousand MT, mainly derived from 15.6% of chitosan with a growth rate of 17.6% in the following years (Newswire, 2019).

2.3.1 Adsorption Capacity

The amino (NH_2) and hydroxyl (OH) groups in the structure of chitosan enable it to form complexes with metal ions through coordination mechanisms,



chelation, or electrostatic interactions (Negm *et al.*, 2020).

2.3.2 Antibacterial Activity

Chitosan exhibits exceptional antibacterial properties against a broad spectrum of pathogenic bacteria. Its cationic nature facilitates interactions with the negatively charged components of bacterial cell membranes, lipids, and proteins in the cell wall. This interaction disrupts vital cellular functions, leading to membrane damage, leakage of intracellular contents, and ultimately bacterial cell death (Zaghibba *et al.*, 2022).

2.3.3 Antifungal Activity

Chitosan's antifungal effect arises from its electrostatic interaction with the negatively charged phospholipids on fungal cell surfaces. This interaction damages the cell membrane, allowing chitosan to penetrate the cell. Inside, chitosan binds to fungal DNA via its protonated amino groups, inhibiting microbial DNA synthesis and leading to cell death (Chik *et al.*, 2023).

2.3.4 Antioxidant Activity

Chitosan functions as an antioxidant by neutralizing reactive oxygen species such as hydroxyl radicals, superoxide radicals, alkyl radicals, and stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. It acts as a hydrogen donor to interrupt oxidative chain reactions (Hou *et al.*, 2020).

2.3.5 Applications in the Food Industry

Chitosan has proven to be a valuable material for antimicrobial food packaging. It is used in the production of paper for food packaging to inhibit microbial growth. Additionally, studies have shown its potential to extend the shelf life of bread by preventing microbial spoilage (Harathi *et al.*, 2020).

2.3.6 Cosmetic Applications

Chitosan is widely applied in the cosmetics industry, particularly for skincare products. When applied to the skin, it forms a protective, elastic, and moisturizing film, making it an essential ingredient in creams and lotions. This film smoothens and shields the skin from environmental damage. Furthermore, chitosan is used in deodorants and hair care products due to its antimicrobial properties, which inhibit the activity of odor-causing enzymes produced by bacteria (Cheengku *et al.*, 2023).

3.0 CONCLUSION AND FURTHER WORK

Chitin is synthesized from many different organisms such as cell walls of fungi and algae, the exoskeleton of insects, endoskeleton of cephalopods and shells of molluscs and crustaceans). the present review provide best insight into the sourcing the chitosan from grasshopper as alternative to crustaceans shells, shrimp and crabs which may also address food scarcity related

challenges by reducing the population of the pests and thereby their infestation. Chitosan can be prepared via chemical method as the most common method in commercial scale. Chitosan is known for its wide range of distinct properties like the biodegradability, biocompatibility, non-toxicity, adsorption capacity, cosmetic and food industry applications antiinflammatory antitumour its antifungal, anticancer, antioxidant, and antibacterial properties and versatility of Chitosan favor its usage in several industrial sectors. chitosan fiber have potential applications in wastewater treatment, where the removal of heavy metal ions by chitosan through chelation has received much attention chelation, viscosity, solubility in different vehicles, film forming and mucoadhesivity, polyelectrolyte behaviour and polyoxysalt formation. chitosan has Linear polyamine, Reactive amino groups, Reactive hydroxyl groups, A substantial number of research publications on Chitosan is a testimony to the growing importance and application of this polycationic linear amino-polysaccharide compound.. The future direction of research should focus on the destruction of catastrophic species into a value added product that could replace the existing biopolymers sources and hence increase the opportunities in this field, also studies are required to optimize the production process for higher yield using modern technology such as electrochemical methods or ultrasonication and microwave. Further innovative insect rearing methods would also produce a constant supply of insects for industrial uses i.e specific species/stages. Cost effective method and straightforward synthesis approaches could be required for large scale production of insect chitosan. Hence, up scaling efficiency, insect species selectivity, and stability in real-time applications need to be explored. Also, further research is required for understanding the chitosan mechanisms of action and bioactivity.

For example, several studies report the antimicrobial activity of CS. However, the exact mode of action is not fully understood.

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