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Review Article

Sources And Applications Of Chitin: A Review

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ABSTRACT

Chitin, a naturally occurring biopolymer, has attracted a lot of interest because of its special qualities and broad range of uses. Chitin is a second-most prevalent biopolymer following cellulose. Chitin is a polysaccharide and very strong in natural state. Chitin is crystalline in nature include three morphological types like α-chitin, β-chitin and γ-chitin. Chitin is mostly present in crustaceans, fungi, insects, some microorganisms and also present in vertebrates. Chitin extraction was done in two methods, chemical or biological methods. The chitin extraction using chemical methods is done by deproteinization, demineralization and decolorization. The biological method uses enzymes or microorganisms. Chitin can be deacetylated to produce chitosan. This review paper offers a thorough summary of the sources, extraction techniques, and applications of chitin along with insights into current research trends.

INTRODUCTION

In recent years, the chitin is commonly used polymer next to cellulose. Chitin and chitosan have increasing attention among researchers. The majority of aquatic and terrestrial species, as well as certain bacteria and vertebrates, include chitin. The chitin is extracted in two different methods like biological and chemical methods. The extraction of chitin varies based on the source selected. The extraction of chitin includes three main steps, they are deproteinization, demineralization and decolorization. The chitin is produced in large scale, that is used in different application. Chitin is mostly used in the fields of medicine, agriculture, food, textiles, and cosmetics. Chitosan can be extracted from chitin. Chitin can be deacetylated to produce chitosan. The chitosan also has several applications.

CHITIN

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Chitin is a polysaccharide. After cellulose, chitin is the second most common biopolymer found in nature. When in its natural state, chitin is pellucid, elastic, flexible, and extremely strong(Cabib et al.,1981). Chitin is made up of Glucosamine, a significant precursor of lipids and proteins. The β1-4 glycosidic linkages bind the glucosamine units together, and their structural similarities to cellulose are observed, with the exception that acetyl amine groups substitute the hydroxyl (OH) group in cellulose. Chitin's content and percentage of n-acetylated units determine its structure and function. The degree of acetylation in macromolecules is defined as the quantity of n-acetylated units(DA), where the term "degree of deacetylation" refers to the proportion of units in the polymer(DD)(Buliyaminu Adegbemiro Alimi et al.,2023). It is a homopolymer of acetyl-glucosamine in theory. It is commonly found in the cell wall of fungi, exoskeletons, shells of crustaceans, and in other lower eukaryotic organisms. Chitin is composed of three distinct structures, designated as α, β, and γ, which vary in the orientation of their chains. These chains are either bounded in sheets or heaps and are joined by hydrogen bonds through different amide groups(Jang et al.,2004). Chitin has great economic value due to their characterization such as biocompatibility, non-toxicity, biodegradability and thermally stable. The main commercial sources of chitin are shells of crabs and shrimp and now a days the extraction of chitin from fungal source has greatly increased. There are various benefits of removing chitin from mushrooms. Primarily, fungal chitin is devoid of allergens such arginine kinase, myosin light chains, and tropomyosin which usually found in crustacean(Muñoz et al.,2015). Furthermore, the mushroom has fewer of the mineral impurities linked to chitin, making it easier to remove chitin from them(Hassainia et al.,2018). Furthermore, it is possible to regulate the physiochemical characteristics of fungal chitin. Finally, mushroom is easy to cultivate and the extraction of chitin from mushroom is milder compared to other source (Wu et al.,2004). Chitin, also known as γ chitin, is a linear chain polymer found in the cell walls of fungi like mushrooms. It is made up of 1,4 linked 2-acetamido-2-deoxy-β-D-glycopyranose units(Sandra Patricia Ospina Álvarez et al.,2014). A long-chained linear polymer called chitin is utilized to provide physical and mechanical barriers that maintain structural stability.

HISTORY OF CHITIN AND CHITOSAN

The word "chitin" is retrieved from the Greek etymology, meaning "tunic" or envelope. Firstly, Hatchett extracted the chitin from mollusks (crabs or lobster), prawns and crayfish in 1799 using mineral acids. Hatchett noted that although they resembled cartilage and had a slight effervescence, they were flexible(Hatchett et al.,1799). In 1811, Professor Henri Bracannot made the initial discovery of chitin from mushrooms. Henri Bracannot who called the chitin as "fungine" as it was from the cell wall of mushroom (Odier et al.,1823). The chitosan was approximately reported after 40 years from the discover of chitin in the year of 1859 by Charles Rouget's study. Felix Hopp-Seyler first used the term "chitosan" in 1894. Chemical structure of chitosan was discovered in 1950(Ruiz et al.,2017). The commercialization of both chitin and chitosan was begun in the year of 1970's (Hahn et al.,2020).

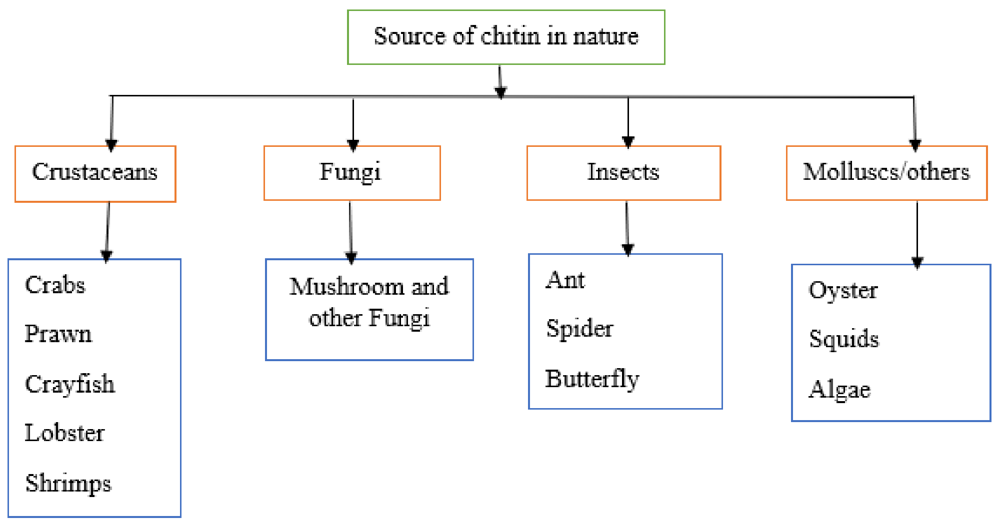
The chitin and chitosan are commercially isolated from crustaceans and also extended to fungi. Over thousands tones of chitin is produced annually. The chitin and chitosan have created a great interest among researchers to use the chitin in their research due to their diverse biological, physical and chemical characteristic (Bhuiyan et al.,2013). For a long time, prawn and crab shells were the primary source of chitin and chitosan.

STRUCTURE OF CHITIN AND CHITOSAN

Since chitin and chitosan are collected from marine sources, they are regarded as important marine renewable materials. Chitin is naturally crystalline composed of three distinct structures, designated as α, β, and γ. α-chitin contain anti-parallel chain, where it is a primary form responsible for the polymer's rigidity. α-chitin is mostly found in crustaceans. On another side, the components of β-chitin are parallel chains that combine to produce monocyclic crystals with both intra- and intermolecular interactions. γ-chitin, which is present in fungi, insects, and yeast, is a blend of anti-parallel and parallel chains with characteristics resembling those of α- and β-chitin(Iber et al.,2022).

SOURCE OF CHITIN

Chitin is the second most common polymer made worldwide, after cellulose. The chitin is not produced in large scale because it is difficult to separate from the pure state.



CHITIN FROM FUNGAL SOURCE

Mushroom is rich in nutrition content; it is consumed in all over the world. A mushroom is the fleshy fruiting body of a fungus that contains spores. Typically, found above the land or on its food source. The mushroom is of two types, one is edible mushroom and other one is toxic mushroom. The word "mushroom" is commonly linked to fungi that possess gills (lamella), a cap (pileus), and a stem (stipe or stalk). They are macro fungi in the families of basidiomycetes and Ascomycetes. A fungus is a type of eukaryotic creature that takes in nutrients from the outside of its cells through external digestion. Fungi may be single cellular or multi-cellular. The multicellular fungi consist of a network of long hallow tubes called hyphae. The mycelium, or dense network made up of hyphae, is frequently formed. Fungi's vegetative portion is called mycelium. There are many types of mushrooms. Some are with stem and without stem. Among recent research, the white button mushroom (Agaricus bisporus) is the leading mushroom for the extraction of chitin. Compared to crustaceans, mushrooms have the benefit of being cultivated and extracted year-round, meaning they are not subject to seasonal changes when used to produce chitin. The species of mushroom used for the extraction of chitin include Agaricus bisporus, Pleurotus sajor-caju, Boletus bovinus, Laccaria laccata, Lentinula edodes, Auricula-judea, Armillaria mellea, Ttrametes versicolor, Pleurotus eryngii and Pleurotus ostreatus. These sources provide more than 30 mg/g of chitin and chitosan overall(Kim et al., 2011). The extraction of chitin from mushroom is mainly done by acid extraction method. However, chitin complexes with glucans or other polysaccharides make it difficult to extract from mushrooms and result in a lower yield than from other sources. Chitin from the fungus Ganoderma lucidum was found to have reduced heat stability compared to other common sources by Ospina Alvarez et al. The fungal chitin has advantages based on the uniformity of particle size, antimicrobial effect and environmentally friendly (Ospina Alvarez et al.,2014). The deacetylation stage is not necessary for the extraction of chitosan from a fungal source; instead, the chitin from the mushroom can be removed by treating it with HCL or a decolorizing agent. After extraction, a chitin-β-glucan complex is produced by fungal chitin sources, because fungal chitin has a low number of chitin-associated impurities. So, Chitin is extracted in a milder manner than from other sources. It is possible to restrict the amount of native chitin's chemical structure breakdown and lower the extraction cost. For, these advantages mushrooms are used for the production of chitin (Navard et al.,2012). Chitin was first isolated from mushroom. Chitosan isolation from fungal mycelium was first developed by the scientist white et al (Odier 1823), followed by several other researchers to develop adaptations for improving the process efficiency. Hu and colleagues (1999) created a methodology for the extraction of fungal chitosan. This protocol describes steps for successful cultivation and extraction (Hu et al 1999). Araki and Ito (1974) made the initial discovery of chitosan production in fungal cell walls. Due to its many benefits, including seasonal independence, ease of extraction, lack of demineralization treatment, and low cost, the synthesis of chitin and chitosan from fungal sources has garnered attention from all over the world in recent years(Mostafa M. Abo Elsoud et al.,2019). Chitosan can be easily extracted from microorganisms (Synowiecki and Al-Khateeb 1997: Yokoi et al 1998). The fungal classes include absidia blakesleeana, absidia coeurulea, absidia glauca, mucor rouxii, aspergillus niger, phycomyces blakesleeana, Trichoderma reesei, colletotrium lindemuthianum, Lentinus edodes (Hu et al.,1999, Teng et al.,2001, Chatterjee et al.,2005). The amount of fungal chitin depends on several factors include the environmental conditions, species and age. Among fungal species, the fungus Aspergillus Niger is utilized to produce chitin. The Aspergillus Niger, as a waste material from citric acid industry, provide raw material for chitosan production (Wu 2004). Solid-state fermentation (SSF) and submerged fermentation (SmF) are two methods for producing fungal biomass. Mostly, solid state fermentation is used because this method provides easier control of parameters. The SSF produce large quantities of biomass than SmF. It has higher potential to produce large quantities of chitosan (Nwe et al., 2002). The fungal mycelia are grown in fermenter under the controlled condition. Solid media such as potato dextrose broth (PDB), molasses salt medium (MSM), or yeast peptone glucose broth (YPG) can be used to cultivate fungus chitin Chatterjee et al., It needs various ingredients as a nutritional source for fungal growth. Recently, studies focused on the utilization of inexpension carbon sources, such as biowastes for culturing fungi for chitosan production (Kannan et al, 2010). Chitin and chitosan are found in the cell walls of fungi in two different forms: free amino glucoside and covalently bound to β-glucan(Nwe et al.,2010). Traditionally, the fungus's cell wall is extracted in two steps to make chitosan. Mycelia is treated in an alkaline environment to create chitin glucan. This causes the chitin-glucan to separate, releasing the chitin, which then undergoes a deacetylation process to become chitosan. The cell wall is rich in "fungal chitin". This procedure does not include deproteinization process (Huq et al.,2022).

CRUSTACEANS SOURCE

Chitin is present in the shell of exoskeletons of crustaceans include crabs, prawn, shrimp, lobster.

The chitin is produced from the crustacean's shell waste. The shell of crustaceans have a complex hierarchical structure made up of α-chitin nanofibers and numerous protein and minerals (yang et al, 2020). Chitin is part of a complex network that is deposited alongside calcium carbonate to form the hard outer layer. Considering that the commercial harvest of crustaceans starts in the spring following the breeding season(Soibam Ngasotter et al.,2023). Marine biomaterials have become increasingly popular because to their uniqueness in the areas of separation, characterisation, and application. Effective recycling of crustaceans biowaste into useful polymers like chitin and the development of chitosan is cost-effective. The primary source of industrial chitin is shellfish industry wastes, such as lobster, prawn, and crab, which have chitin contents ranging from 8 to 40%. crustaceans produce more amount of chitin than fungal source. The crustacean's chitin is season dependant. There are two ways to obtain chitin from the waste of crustaceans' shells: chemically and biologically.

INSECT SOURCE

Insect have long been regarded as a reliable food source. When it comes to extracting chitin, insects show promise in addition to fungi and crustaceans. Insects are used for the extraction of chitin because of its cultivation possibilities, constitutes and percentage of dry matter. The overall composition of insects is 10-15% chitin, 25-40% fat, and 30-45 percent protein(Spranghers et al.,2017) . The extraction of chitin from the cuticles of insects are comparable to crustaceans. Insects are season independent, it can breed based on their fertility and reproductive rate. Chitin and chitosan content has been studied in arthropods, such as woodlice and centipedes. The inner soft layer of insect is covered by hard shell to protect it. The protective layer is called as exoskeletons. The exoskeletons of insects are rich in chitin. Procuticle contain chitin. Chitin naturally forms into microfibres, which are encased in a matrix of proteins. The purification stage is carried out to get rid of the attached protein, pigments, minerals, and lipids before extracting chitin from insect cuticles. The entire body of the insect has between 5 and 25% chitin, 30% to 50% calcium carbonate, and between 30% and 40% protein. About 45–50 different species of insects are employed in the chitin extraction process(Shamshina et al.,2019). The chitin from insect cuticles can be extracted by physical, biological or chemical method. Insect derived chitosan is less effective than crustaceans derived. Deacetylation of chitin in the exoskeletons of insects are used to make chitin in large scale. Chitosan from insects is necessary since they are a dependable supply of protein. The insect chitin was identical for their thermal stability, degree of acetylation (less than 87%) and also have crystallographic structure(Marei et al.,2016).

EXTRACTION METHODS OF CHITIN

CHEMICAL METHOD

In chemical method, chemicals are used for the extraction of chitin. The two basic processes of chemical extraction are the acidic treatment for the removal of minerals and the basic treatment for the removal of proteins. The production of chitin and chitosan from the sources should be economical, simple, time saving process and environmentally friendly. Recently, the chemical method is used in commercial scale. The chemical treatment process consists of the following steps: demineralization by acidic treatment, deproteinization by alkali treatment, and decolorization for the removal of pigments and colours.

1. CHEMICAL DEPROTEINATION

The first step in chitin extraction is deproteination includes the disruption of chemical bonds between the chitin and protein. In medical application the protein should be separated from the chitin. Human allergies are primarily caused by protein content. The protein from chitin can be separated by using NaOH at the concentration of 5.0M. NaOH also leads to the hydrolysis of biopolymer.

2. CHEMICAL DEMINERALIZATION

The demineralization leads to the removal of minerals, impurity and firstly remove calcium carbonate. The demineralization is commonly including acid treatment. It includes boric acid, hydrochloric acid, formic acid and sulphuric acid (Percot et al 2003). Among the acids, hydrochloric acid is commonly used. It entails the release of carbon dioxide as calcium carbonate breaks down into calcium chloride.

3. CHEMICAL DECOLORIZATION

The decolorization is the additional step to remove color and pigments. Pigment removal involves the use of acetone or a mixture of organic solvents. After chitin extraction, the process of deacetylating chitin can yield chitosan. Usually maintained between 60-90℃ to prevent chitin breakdown.

BIOLOGICAL METHOD

The biological method is recently developed for chitin extraction. These methods use either microorganism and purified enzymes. The use of biological method is ecofriendly, economical, cleaner. The main enzyme used in chitin extraction is proteases, which breaks protein bonds to give amino acids and peptides. The enzymatic method is less efficient compared to chemical method.

ENZYMATIC DEPROTEINIZATION

The chitin can be extracted using proteolytic enzymes. Various proteases include pepsin, alcalase, trypsin, papain, pancreatin. It is possible to employ both crude and refined enzymes in enzymatic deproteinization. The cost of the refined enzymes is high. The viscera of fish and microorganisms are used to extract the crude enzymes (Rao et al., 1998).

APPLICATION OF CHITIN

ENVIRONMENTAL APPLICATION OF CHITIN

Chitin is eco-friendly, for the use in environmental sector. Chitin was recently reported for protecting marine environment by protecting coralline algae from ocean acidification. The chitin has large surface area based on the size of biopolymer. Chitin is used for dye removal, removal of inorganic contaminants and organic contaminants, waste water treatment etc.,

1. DYE REMOVAL

Water pollution is the primary environmental issue that has an impact on living things. The water pollution is mostly caused for pharmaceutical, pesticide and dying industry. According to a recent study, because of the high surface to volume ratio and functional groups connected to the nanomaterial, modifying the structure of chitin-to-chitin nano whiskers offers a viable way to remove dye (Druzian et al.,2019). The chitin nano whiskers are used for the removal of dye like crystal violet and carmine in the aqueous solution (Meshkat et al.,2019).

2. REMEDIATION OF ORGANIC CONTAMINANTS

The organic contaminants like pesticides, pharmaceutical drugs, steroids, flame retardants and aromatic hydrocarbon are commonly present in water (Saxena et al.,2020). The organic contaminants combine and lead to bioaccumulation. Scientists are currently searching for new environmentally friendly bio-adsorbents that can remove contaminants just as well as traditional methods. Pharmaceutical drug is different from normal water contaminants, as they are biologically active. It was identified that the chitin/ lignin when used as adsorbents, bound potentially to the non-steroid anti-inflammatory drugs. The pH of the solution, which ranges from 2 to 6, determines the rate of absorption (Gwenzi et al.,2018).

3. REMEDIATION OF INORGANIC CONTAMINANTS

Majority of water pollution is caused by heavy metals. Heavy metals are mostly chromium from stainless steel manufacturing, leather tanning, pigment producing industry. These heavy metals accumulate in the aquatic organisms. The chitin being the alternative source for flocculants and coagulants used for the removal of metal ions from contaminated water (Nasrollahzadeh et al.,2021).

MEDICAL APPLICATION OF CHITIN

Chitin and chitosan have many applications related to medicinal sector because of their biocompatibility. Its medical application includes bone formation, artificial skin, immune-stimulant, treatment of tumors and leukemia, adjuvant properties and spermicide. Scientists are using the chitin and its derivatives for tissue regeneration compared to other polymer due to low isolation cost, enabling industrial use, fast production.

1. TISSUE ENGINEERING

The tissue engineering is the regeneration of the damaged tissue. The process of creating biomaterial scaffolding to give a cell the appropriate structure or shape is known as tissue engineering (Wan et al.,2013). The chitin is combined with small biomolecules like the sugar and peptides in recent years, the development of tubes based on chitin for tissue engineering has gained a lot of attention (Madhumathi et al.,2009).

2. WOUND DRESSING

The Chitin is having potential application in wound healing. Wound healing is a continual and intricate process. There are five important stages: homeostasis, inflammation, migration. proliferation and maturation (Ahmed et al.,2016). Bacterial infections are a major factor in wound healing and need to be appropriately managed. Chitin and its derivatives have the ability to heal wounds by regulating the inflammatory response via prostaglandin E, interleukin 8, and interleukins 1β (Jayakumar et al.,2010).

3. CANCER DIAGNOSIS

The nanotechnology being the emerging field in cancer treatment. Using nanoparticles is both biocompatible and has the ability to mark certain compounds. When applied to mannose-bearing KB tumor cells, zinc sulfide nanoparticles functionalized with D-mannose and encased in chitosan can produce intense fluorescence. The regular cells do not bind to these nanoparticles (Jayakumar et al.,2010).

4. DRUG DELIVERY

Chitin has a promising application in drug delivery. Drug delivery chitin products, such as polylactic acid (PLA)/chitosan (CS) nanoparticles and carboxymethyl chitin (CMS), have been produced. Drug delivery to various cell types and locations within the cell can be accomplished creatively with CMS nanoparticles (Wojtowicz et al.,2014).

AGRICULTURAL APPLICATION OF CHITIN

Chitin is used in agricultural sector because chitin is water soluble in nature. Chitin regulates anti-stress agents, growth regulators, growth stimulants, and elicitors for secondary metabolite production, all of which might strengthen a plant's defense mechanism.

1. CROP GROWTH

Chitin is a biopolymer, used for crop growth. Instead of using chemical fertilizers, eco-friendly biopolymer is used for crop growth. The scientific community believes that the greatest natural, non-invasive plant growth stimulant is water-soluble oligosaccharide fragments that are produced by grinding chitin and its derivatives (Li et al.,2020).

2. PATHOGEN RESISTANCE

An essential component of pathogen resistance is chitin. Chitin is essential to the pathogenicity of fungi. Chitin is not directly produced by plant; they do not secret enzymes to break the chitin. Based on the characters of chitin, the host plant secretes more amount of chitinase to resist against pathogens. The release of chitin fragments from the fungal cell wall triggers the host defense mechanisms, thereby aiding in the protection of the plant against fungal infections (Wan et al.,2008).

OTHER APPLICATION OF CHITOSAN

- The US Food and Drug Administration has authorized chitosan for use as a food additive.
- Chitosan is used in wine production for de-acidification, clarifying, and the removal of enzymes and other unwanted substances.
- Most research has focused on chitosan and its derivatives as an excipient in drug formulation and drug delivery.
- In veterinary medicine, chitosan has antibacterial, analgesic, wound-healing, and bone-regenerating properties.
- In cosmetics, chitin/ chitosan is used for hair or skin care by lotions and creams.
- Chitosan is used in the paper industry to increase paper's wet strength.
- In the field of Biotechnology, chitosan is used as biosensors.
- In food industry, it is used for the preservation of food (Nadia Morin-Crini et al.,2019).

CONCLUSION

In conclusion, this review paper has highlighted the diverse sources and wide-ranging applications of chitin. From natural sources like crustacean shells and fungal cell walls to synthetic methods, the availability of chitin is abundant. Its applications in various industries such as medicine, agriculture, food, and wastewater treatment showcase its versatility and potential. Future research could delve deeper into optimizing extraction methods, exploring new applications, and enhancing the sustainability of chitin-based products for a greener and more resource-efficient future.

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