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A New Horizon in Chitosan Research: Silkworm-Derived Biopolymer and Its Applications

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Authors' contributions

This work was carried out in collaboration among all authors. Author AN designed the study and wrote the protocol. Author BRN wrote the first draft of the manuscript. Author KMS managed the literature searches. Authors AN and PC managed the analyses of the study. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Chitosan, a biodegradable biopolymer traditionally sourced from crustaceans, has growing significance in the food, biomedical and environmental sectors. However, concerns over allergenicity, heavy metal contamination and seasonal availability necessitate alternative sources. Silkworm pupae, an underutilized by-product of India's silk industry, offer a sustainable and efficient alternative. Chitosan extracted from silkworm pupae exhibits superior physicochemical properties, including high solubility (>98%), low ash content (<1%) and a high degree of deacetylation (>85%),

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along with excellent antimicrobial, film-forming and biocompatible characteristics. Compared to shrimp shell-derived chitosan, it demonstrates a higher molecular weight and purity, with potential applications in food preservation, pharmaceuticals, textiles and sericulture. This review highlights the extraction techniques, structural attributes and multi-sectoral applications of pupal chitosan, advocating for its commercial development as a viable, eco-friendly substitute for conventional chitosan sources.

Keywords: Silkworm; chitosan; biopolymer; silk industry.

1. INTRODUCTION

Chitosan, a deacetylated derivative of chitin, is a high-value biopolymer with diverse applications in medicine, agriculture, food preservation and environmental remediation. Traditionally, commercial chitosan production relies on crustacean shells, but concerns remain regarding batch variability, potential heavy metal contamination and residual shellfish allergens (Neelam et al., 2024). Silkworm pupae, an often-ignored by-product of the silk industry, offer a promising alternative. India generates approximately 40,000 metric tons per year of dry pupae, most of which goes unutilized (Anon., 2024).

Silkworm pupae-derived chitosan displays compelling characteristics, including a high degree of deacetylation (>98%), low ash content (<1%), excellent solubility (>99% in acid) and greater antimicrobial and antifungal activity compared to conventional standards (Xuli et al., 2021). Nutritionally, silkworm pupae are rich in protein, fat and minerals (Longvah et al., 2011). After extracting oil, defatted pupae are an excellent source of chitin, which can be processed into chitosan through deacetylation (Suresh et al., 2012).

Structurally, pupal chitosan has shown around 48 per cent crystallinity and supports NIH3T3 cell viability, pointing to strong biocompatibility for medical and food applications (Prajwal et al., 2020). While the absolute yield lags behind crustacean sources, the sheer scale of pupae generation makes this resource abundant and sustainable. Collaborations between ICAR-CIFT and startups like Ecogenie are adapting conventional crustacean extraction methods to pupae, optimizing the process to meet quality standards.

The contrast between crustacean-sourced and silkworm-based chitosan is clear: crustacean-sourced chitosan benefits from market ubiquity and high yield but suffers from allergenic risk and contaminant variability. Silkworm-based chitosan presents a renewable, insect-based alternative with high purity, potent bioactivity and low allergenic potential. Given its combination of superior antimicrobial efficacy, cytocompatibility and environmentally responsible origin, pupal chitosan appears promising for emerging domains like biodegradable packaging, seed coating and eco-friendly antibacterial agents. As regulatory and production protocols mature, pupae-derived chitosan may challenge crustacean dominance, particularly in sectors sensitive to allergy and contamination concerns.

As regulatory and production protocols mature, pupae-derived chitosan may challenge crustacean dominance, particularly in sectors sensitive to allergy and contamination concerns. This review will explore the biochemical properties, extraction techniques and industrial potential of chitosan derived from silkworm pupae, contrasting it with traditional aquatic sources, to highlight a pathway toward more sustainable and diversified chitosan supply chains.

2. SOURCES & STRUCTURE OF CHITIN AND CHITOSAN

Chitin: Chitin is the second most abundant biopolymer after cellulose, found in crustaceans, insects, mollusks and fungi. Its content varies: shrimp (30-40%), crab (15-30%), krill (20-30%), squid (20-40%), clams/oysters (3-6%), insects (5-25%) and fungi (10-25%) (Kurita, 2006). Structurally, chitin comprises β-(1→4)-linked N-acetylglucosamine units and exists in α-, β- and γ-forms based on chain orientation (Maniukiewicz, 2011). Its hydrogen-bonded crystalline matrix renders it insoluble, an obstacle to industrial use, though it finds applications in sutures, wound-healing sprays, gels and drug-delivery systems due to its excellent biocompatibility.

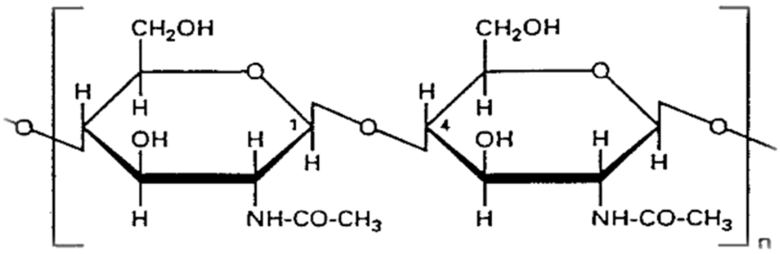

Fig. 1. Structure of chitin

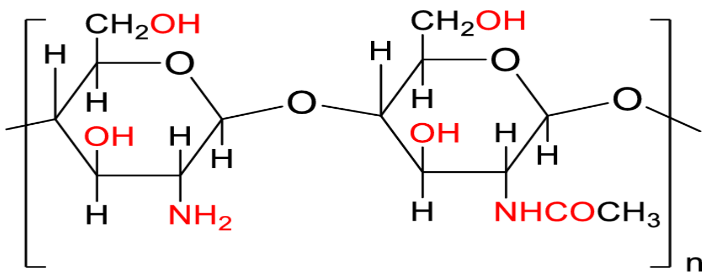

Fig. 2. Structure of chitosan

Chitosan: Chitosan results from partial deacetylation of chitin, yielding a copolymer of glucosamine and N-acetyl-glucosamine. Commercial chitosan typically has a deacetylation degree of 60-75 per cent. Its solubility, cationic nature and biological behaviour highly depend on its degree of acetylation (DA) or deacetylation (DD = 100 - DA) (Peniche et al., 2008). Unlike chitin, chitosan is soluble in acidic environments and can be precipitated, cast into films, fibres, beads, or sponges. Its versatile chemistry allows easy processing for biomedical and environmental uses.

3. FORMS OF COMMERCIALLY AVAILABLE CHITOSAN

Depending on the end use, chitosan is produced as:

Flakes: According to Verbych et al. (2005), chitosan flakes are a practical choice for removing heavy metals, such as copper (II) ions, from solutions, with an adsorption capacity of 127.0 mg/g dry mass. Additionally, chitosan flakes have shown potential in removing residual oil from palm oil effluent, highlighting their versatility in environmental applications.

Powder: Chitosan powder has been explored for its effectiveness in removing pollutants, including dyes, heavy metals and residual oil, due to its adsorptive properties. Its potential as a natural, biodegradable alternative to synthetic flocculants and coagulants makes it an attractive option for environmental remediation, offering a more eco-friendly solution.

Gel beads: Chitosan hydrogel beads, typically 2-3 mm in diameter, can be synthesized using chitosan powder and acetic acid. These gel beads have various applications, including catalysis, enzyme immobilization and adsorption of heavy metals and dyes, showcasing their versatility in different fields.

Chitosan fibres and resins: The production of chitosan fibers, first reported in 1926, was initially hindered by high costs. However, treating chitin with alkali can yield highly deacetylated chitin, which can be processed into fibers with strength comparable to viscose fibers. These fibers exhibit varying crystalline structures, crystallinity and crystallite sizes. Chitosan-based resins have potential applications in removing metal ions and dyes. Furthermore, quaternization can introduce cationic groups, enabling the creation of anion exchange resins as an alternative to existing options (Elwakeel, 2010).

Membranes: Research has explored the effectiveness of chitosan membranes in removing heavy metals from aqueous solutions. Studies have compared the performance of untreated chitosan membranes to those crosslinked with glutaraldehyde, revealing a specific order of permeability coefficients for various metal ions, which correlates with the metal ion's affinity for chitosan.

Nano fibres and resins: Nanofibers are solid particles with dimensions between 1-100 nm. Various methods, including sol-gel, chemical vapor deposition, electrospinning and thermal oxidation, can be used to prepare nanofibers. Chitosan nanofibers have shown promise in tissue engineering applications (Mahoney et al., 2016). Additionally, chitosan resins can be prepared, modified and utilized for removing contaminants like As(V) from solutions.

Nanoparticles: Nanoparticles, typically sized between 1-100 nm, have diverse applications, including medical uses, drug delivery and enzyme immobilization. Chitosan nanoparticles can be synthesized using various methods, such as ionic gelation and reverse micellar approaches, as demonstrated by Zhang et al. (2014).

Chitosan on supportive material: Chitosan's limitations, such as poor diffusion and mechanical properties, can be addressed by depositing it onto porous materials like silica beads or sand. This approach enhances chitosan's surface area and affinity properties, making it more effective in various applications. Chitosan-coated materials have been used as thickening agents in the food industry. Furthermore, modifying chitosan with compounds like 3,3-diphenylpropylimine methyl benzaldehyde (PPIMB) can create new binding sites, improving its adsorption capabilities (Shahraki et al., 2020).

Modifications of chitosan: Polymers with specific functional groups are gaining attention due to their unique properties. Biopolymers like alginates and carrageenans have anionic groups, while chitosan possesses cationic amino and hydroxyl groups. Chitosan's versatility allows for physical or chemical modifications, enabling its use in various applications.

4. EXTRACTION PROCESS OF CHITIN AND CHITOSAN

Extraction involves three main steps after pre-treatment (cleaning, drying, powdering) (Priyadharshini *et al*., 2018):

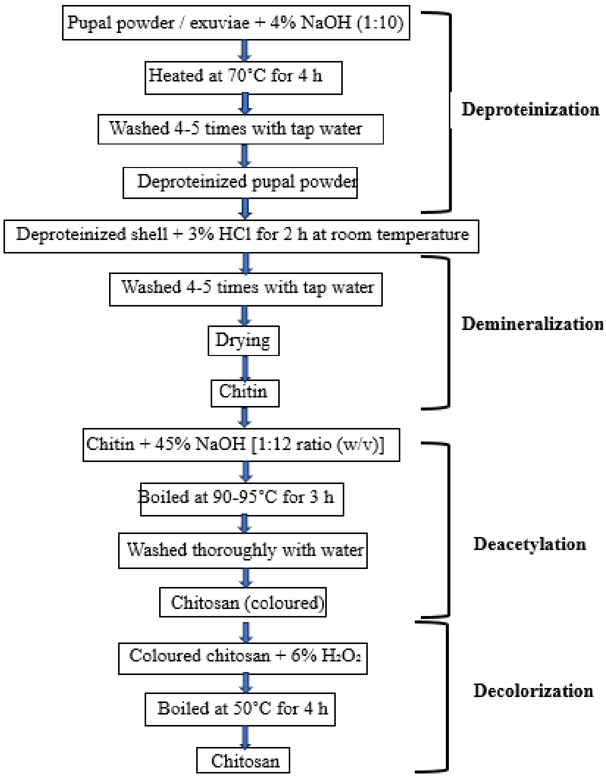

Fig. 3. Flow chart of obtaining chitosan from silkworm pupal powder / exuviae

5. PROPERTIES OF SILKWORM-PUPAL CHITOSAN

5.1 Physicochemical Properties

- White, film-forming powder
- Insoluble in water and most organic solvents
- Linear polyamine with reactive amino and hydroxyl groups
- Capable of chelating metal ions

5.2 Biological and Functional Attributes

- Natural cationic polymer amino groups are protonated at pH < 6.3, enabling solubility and functionality.
- Biocompatible, biodegradable, non-toxic and bio-functional.
- Antimicrobial and antifungal; mucoadhesive; and haemostatic.
- Can be sulfated to anionic forms with anticoagulant activity.
- Film-forming and electrostatic properties make it suitable for wastewater treatment, packaging, controlled drug release and biomedical applications (Kumirska et al., 2011).

A recent study by Pradip (2024) investigated the extraction of chitin and chitosan from different silkworm breeds and exuviae, analyzing their physicochemical properties. The results showed that male pupae had higher chitin (3.242%) and chitosan (2.430%) content compared to females (3.013% and 2.345%, respectively). Among hybrids, crossbreeds had higher chitosan content (2.526%) than bivoltine hybrids (2.308%). Chitin and chitosan content varied across different stages, with bivoltine larval exuviae exhibiting the highest content (26.02% and 19.37%, respectively). The moisture content of larval and pupal chitosan ranged from 7.00-8.00 per cent and 7.20-7.40 per cent, respectively. Viscosity was higher in larval/exuvia chitosan (48-58 cP) compared to pupal chitosan (44-46 cP). The ash content was below 1 per cent, with pupal chitosan ranging from 0.30-0.38 per cent and larval/exuvia chitosan from 0.50-0.80 per cent. The degree of deacetylation and solubility of extracted chitosan ranged from 85-96 per cent and 92-99 per cent, respectively. Notably, silkworm pupae-derived chitosan exhibited superior physicochemical parameters, including high solubility (99%), degree of deacetylation (>85%) and low ash content (<1%), indicating its potential as a high-grade alternative source of chitosan. His study also proved that both the commercially available chitosan and the extracted chitosan from silkworm pupae and exuviae had similar physicochemical properties and could be used as a viable alternative to commercial chitosan.

Luo et al. (2019) compared the properties of chitosan extracted from cicada slough, mealworm, silkworm chrysalis, shrimp shells and grasshopper. They extracted chitosan through sequential chemical treatments *viz*., demineralization, deproteinization and deacetylation, then characterized its solubility, ash, moisture, molecular weight and degree of deacetylation using methods like gravimetric analysis, viscometry, FTIR, elemental analysis and XRD. Rheological behaviour was assessed using a rheometer and morphology was examined by scanning electron microscopy (SEM). The results showed that all chitosan samples had high solubility (91.5-99.3%), with insect-derived chitosans generally outperforming shrimp shell chitosan. The degree of deacetylation (DD%) ranged from 84.1-91.2 per cent, with shrimp shell chitosan having the highest value. The molecular weight (Mw) varied significantly, with silkworm chrysalis having the highest Mw (4.09×10^4 Da) and shrimp shell having the lowest (1.62×10^4 Da). Insect-derived samples had approximately 2-2.5×10^4 Da higher Mw than shrimp shell chitosan. Ash and moisture content were lowest in cicada and silkworm-derived chitosans. Thus, the chitosan extracted from silkworm chrysalis can be used as an alternative for commercial use.

Scanning electron microscopy images revealed distinct morphological differences between the chitosan samples. Cicada slough-derived chitosan had a needle-like structure, while silkworm chrysalis-derived chitosan had a reticular structure. Mealworm-derived chitosan had soft and irregular fibres and grasshopper-derived chitosan had an irregular block and rough structure without porosity. These differences may be attributed to the inherent characteristics of the sources, extraction methods and extents of deacetylation. Overall, the study suggests that insect-derived chitosans, particularly from cicada and silkworm sources, have favourable properties for various applications.

Table 1. Physicochemical properties of chitosan isolated from insects and shrimp shells

Samples	Solubility (%)	Degree of deacetylation (%)	Mw (Molecular weight) (Da)	Ash (%)	Moisture content (%)
Cicada slough	99.3±0.12	84.1%	(3.779±0.068) × 10^4	0.03±0.004	0.18±0.016
Silkworm chrysalis	98.7±0.16	85.5%	(4.090±0.059) × 10^4	0.05±0.003	0.07±0.008
Mealworm	97.4±0.20	85.9%	(3.975±0.072) × 10^4	0.50±0.016	0.19±0.012
Grasshopper	94.3±0.39	89.7%	(3.989±0.021) × 10^4	0.89±0.025	1.8±0.213
Shrimp shell	91.5±1.22	91.2%	(1.620±0.032) × 10^4	0.95±0.040	2.7±0.245

Table 2. Degree of deacetylation at different temperatures calculated by potentiometric titration

Deacetylation time (h)	Degree of deacetylation		
	80˚C	**100˚C**	**110˚C**
4	28±1.4	31±1.1	34±0.7
6	43±1.2	48±1.4	69±0.8
8	63±0.9	71±0.8	82±1.2
10	77±2.0	84±1.6	89±1.9

According to Pal et al. (2016), the deacetylation process of chitin to chitosan is highly temperature-dependent. At various temperatures, the degree of deacetylation (DDA) increases with reaction time: at 80˚C, DDA rises from ~28% at 4 hours to ~77% at 10 hours; at 100˚C, it increases from ~31% to ~84%; and at 110˚C, from ~34% to ~89%. This indicates that higher temperatures and longer reaction times accelerate deacetylation. The potentiometric titration method effectively tracks these changes, confirming its utility for rapid DDA evaluation. However, temperatures above 100˚C may degrade amino groups and reduce efficiency. Notably, the extracted chitosan (at 80˚C after 10 hours) showed higher crystallinity and improved thermal stability compared to chitosan from other marine sources. Furthermore, the study found that the conversion of chitin to chitosan was negligible at temperatures below 80˚C, highlighting the importance of temperature in the deacetylation process.

Chen et al. (2024) studied the degree of substitution (DS) of different carboxymethyl chitosan at the positions C_2-NH_2 and C_6-OH in the chitosan which were substituted by carboxymethyl groups under alkaline conditions to obtain N, O-carboxymethyl chitosan.

Chitosan derivatives have been extensively explored for their potential applications. Quaternary ammonium chitosan salts, carboxymethyl-chitosans and other derivatives have been synthesized, with carboxymethyl-chitosans being the most fully explored (Alves and Mano, 2008). Graft copolymerization of chitosan, particularly using atom transfer radical polymerization, is a promising technique to incorporate desired functionalities for targeted applications.

Low molecular weight chitosans or oligosaccharides (COS) can be obtained from high-molecular weight chitosans through physical, chemical or enzymatic methods (Liang et al., 2018). These COS have low viscosity, are water-soluble and exhibit versatile biological activities, including antibacterial, antitumor and cholesterol-lowering effects (Hamed et al., 2016). They are effective in various applications, such as biomedicine, cosmetics and agriculture.

The production of COS can be achieved through various methods, including gamma-ray irradiation, ultrasonic treatment, acid hydrolysis and enzymatic degradation using specific or non-specific enzymes (Liang et al., 2018). Biotechnological processes have also gained interest, permitting the production of high-value commercial by-products (Philibert et al., 2017).

A study by Fadly et al. (2017) optimized the extraction of carboxymethyl chitosan (CMC) from silkworm pupae using alkalization with varying molarities of NaOH. Another study compared the degree of substitution of reference carboxymethyl chitosan (DS = 2.73) with silkworm pupal carboxymethyl chitosan (DS = 1.63), highlighting the potential of insect-derived chitosan derivatives. Their results revealed that, the best CMC yield (115.94 %) was obtained from 10 M NaOH with the most suitable physio-chemical characteristics like alkalinity (4.22 pH), moisture (10.80 %), ash (0.04 %), nitrogen (4.06 %), solubility (99.28 %) and viscosity (14.22 mPas) for antibacterial activity.

6. GENERAL APPLICATIONS OF SILK-WORM PUPAL CHITOSAN

6.1 Food Industry

Chitosan has been approved by the US Food and Drug Administration as a generally recognized as safe food additive and it has also been approved as a food additive in Japan and Korea since the 1990s. Chitosan and its derivatives are used as food additives, serving as fining agents, texture controlling agents, natural flavor extenders and emulsifying agents. Due to its antibacterial, antifungal and antioxidant properties, chitosan is utilized for its bioactive properties and as a preservative to protect foods from microbial deterioration, including fruits and packaged food. Chitosan's controlled release properties make it suitable for use in edible films and its biodegradability makes it useful for encapsulating nutraceuticals (Perinelli et al., 2018).

Chitosan is considered a suitable alternative to non-biodegradable polymers for food preservation and coating due to its antimicrobial and film-forming properties. Various chitosan-based products, such as ChitoClear, Chitoseen-F and MicroChitosan NutriCology, are marketed as fat reducers, cholesterol-lowering agents and antioxidants. ChitoClear, for example, is promoted as a natural weight loss supplement that can aid in weight management and obesity treatment when combined with a healthy lifestyle. Chitosan's nutraceutical properties include antibacterial, anti-inflammatory, antioxidant, anti-carcinogenic and antiulcer bioactivities, as well as its role as a dietary fiber. Its non-digestibility, high viscosity and water-binding properties enable it to lower cholesterol levels by blocking fat and cholesterol absorption. Studies have shown that chitosan and its derivatives can facilitate weight and body fat loss, reducing systolic and diastolic blood pressure (Philibert et al., 2017).

Chitosan has itself the ability to control some fungal diseases, which deteriorate fruits quality during storage. Romanazzi et al. (2017) studied the efficacy of chitosan in postharvest preservation, where the chitosan is coated over the papaya (*Carica papaya* L.) to know the anti-perishable activity. They reported that about 20-30 per cent loss can be incurred in post-harvest stage. Postharvest deterioration of papaya is a microbiological process; the fruits become a target of several pathogens in the market thus decreasing its acceptability and shelf life. They also concluded that the storage life of papaya was extended up to 33 per cent through the use of calcium chloride (2%) with chitosan coatings on fruits. Chitosan coating on fruits and vegetables has been found to be effective for the reduction of a variety of harmful micro-organisms and extend the shelf-life of these products. By its antioxidative property, it reduces the rate of browning for fruits.

6.2 Beverage Industry

Chitosan is a versatile agent in beverage production, serving multiple purposes in wine, such as refining, acidity adjustment, stabilization and removal of impurities like ochratoxin A, enzymes, heavy metals and pesticides. Its eco-friendly properties make it an effective coagulant for clarifying passion fruit juice, as demonstrated by Domingues et al. (2012) and a natural flocculant for beer clarification. Chitosan's applications also include turbidity reduction, removal of suspended solids and colloids like polyphenols, proteins, polysaccharides and minerals and acidity adjustment in fruit juices and beverages. Furthermore, its antimicrobial properties enable it to preserve drinks from microbial spoilage, including bacteria, yeast and mould, making it a natural preservative, flavour extender and active packaging material.

6.3 Medicine, biomedicine and pharmacy

The pharmaceutical industry has seen significant interest in utilizing chitosan and its derivatives as key components in medication formulations and delivery systems. By leveraging natural products, researchers aim to replace potentially hazardous substances and this approach has shown considerable promise.

Chitosan's versatility is evident in its ability to inhibit efflux pumps and its adaptability in various formulations, including solutions, tablets, gels, fibres, capsules, films and sponges (Ali and Ahmed, 2018). These diverse forms enable chitosan to be administered through multiple routes, such as oral, buccal, nasal, vaginal, ocular, intravesical, parenteral and transdermal delivery, as well as implantable systems. Notably, chitosan tablets have demonstrated sustained drug release, surpassing commercial products and are favoured due to their precision, ease of production and patient preference (Bernkop-Schnurch and Dunnhaupt, 2012). Furthermore, chitosan's mucoadhesive properties make it an ideal candidate for buccal delivery, enhancing absorption and bioactivity by prolonging contact with mucosal surfaces.

Chitosan has diverse medical and biomedical applications, including pharmaceutical formulation and drug delivery, antimicrobial applications, gene delivery, wound healing, regenerative medicine, tissue engineering and cancer treatment. It can be processed into various forms, such as solutions, gels/hydrogels, sponges, microparticles/nanoparticles, membranes and films and fibres/nanofibres, making it a versatile biomaterial for medical applications. Several chitosan-based medical products are commercially available, including HemCon Bandage, a high-performance haemostatic dressing, Reaxon, a nerve conduit that supports nerve regeneration and ChitoSeat, a haemostatic sealant for surgical use. These products demonstrate chitosan's potential in wound healing and tissue regeneration (Hamedi et al., 2018).

According to Dash et al. (2011), chitosan is an ideal dressing for wound-healing applications due to its ability to protect wounds from bacterial infection, promote healing and reduce scarring. Additionally, chitosan can deliver therapeutic payloads, such as fibroblast growth factor, which stimulates angiogenesis and enhances wound healing.

The commercial availability of chitosan-based products demonstrates its potential for clinical applications. With its ability to promote healing, reduce scarring and deliver therapeutic payloads, chitosan is poised to play a significant role in advancing medical research and treatment options.

6.4 Textile and Paper Industry as Dye-Binder for Textiles

Chitosan, a renewable and biodegradable biomaterial, has demonstrated potential in wastewater treatment, particularly for removing dyes from textile effluents. A study by Simionato et al. (2014) investigated chitosan's adsorption capacity in both column and suspension forms using two dyes, Blue Remazol (RN) and Black Remazol 5 (RB). The results indicated that chitosan-packed columns outperformed chitin-packed ones, while suspensions showed better adsorption than columns. When applied to real textile effluents, chitosan effectively removed dyes at acidic pH, completely eliminating coloration. This research suggests that chitosan derived from silkworm chrysalides is a feasible option for immobilizing dyes in textile industry effluents, offering a promising solution for wastewater treatment.

6.5 Scaffolds for Tissue Engineering

The development of biomaterials with tailored microarchitectures is crucial for advancing tissue engineering and regenerative medicine. Chitosan, a biocompatible derivative of chitin, has been widely used in clinical practice. However, creating chitosan scaffolds with controllable microchannels remains a challenge. Researchers have generated chitosan scaffolds with adjustable microchannels using a 3D printing and freeze-drying method, allowing for precise control over microchannel arrangement, diameter and density. These scaffolds promoted cell survival, proliferation and tissue in-growth and improved vascular formation. Additionally, silk nanofibers can be mixed with chitosan to form nano-composite films and porous scaffolds, which can promote cell migration and vascularization in wound healing. Increasing pore size and connectivity can enhance cell adhesion, growth and transport of gases, nutrients and metabolites. Tissue engineering seeks to repair or regenerate damaged tissues or organs by combining supportive scaffolds with specific cells and biomolecules, ultimately leading to the growth of new tissue (Dash et al., 2011).

Chitosan-based scaffolds offer a promising alternative to synthetic scaffolds for cartilage tissue engineering. According to Anitha et al. (2014), chitosan-based membranes and scaffolds have diverse applications, including tissue engineering, wound healing and targeted delivery of anticancer drugs, osteogenic agents and growth factors. These biomaterials possess desirable properties such as biodegradability, cytocompatibility, multifunctionality and tailored mechanical characteristics. Additionally, n-Lauryl-carboxymethylcellulose has been explored as a carrier for hydrophobic cancer therapeutics, demonstrating safety and minimal membrane toxicity due to its amphiphilic nature.

6.6 Chitosan as Antimicrobial Agent in Sericulture industry

Silkworms are susceptible to various bacterial diseases, which can lead to significant crop loss. Chitosan, a biodegradable and non-toxic biomaterial, has been shown to exhibit antibacterial activity against the bacterial pathogens of silkworms.

Research by Li et al. (2010) investigated the antibacterial efficacy of chitosan solution against two strains of Serratia marcescens, a prevalent silkworm pathogen. The findings revealed that chitosan solution, at concentrations ranging from 0.01 to 0.10 mg/ml, demonstrated potent antibacterial activity against both strains. Moreover, the antibacterial effect intensified over time, consistently observed across both bacterial strains.

Priyadharshini et al. (2018) evaluated the *in-vivo* antibacterial effect of chitosan against Staphylococcus aureus and Bacillus thuringiensis in silkworms. Chitosan was extracted from silkworm pupae and used at different concentrations (0.5-4.5%). The results showed that chitosan at 2.5 per cent concentration exhibited maximum antibacterial activity against both pathogens, reducing larval mortality and increasing economic parameters.

Madhusudhan et al. (2023) synthesized chitosan-silver nanocomposites and evaluated their antibacterial activity against bacterial pathogens of tropical tasar silkworm. The results showed that chitosan-silver nanocomposites exhibited stronger antibacterial activity than chitosan alone, with a minimum inhibitory concentration of 0.2 per cent against all tested pathogens.

Vokhidova et al. (2014) studied the fungicidal activity of silkworm pupal chitosan and its nanostructured systems with copper against phytopathogenic fungi. The results showed that chitosan and its nanostructured systems effectively inhibited the growth and development of fungi, with chitosan-copper nanostructured derivatives exhibiting stronger antifungal activity.

Chitosan has been shown to exhibit antibacterial and antifungal activity against various pathogens affecting silkworms. Its application in sericulture can help reduce larval mortality, increase economic parameters and promote disease resistance. Further research is needed to explore the potential of chitosan and its derivatives in silkworm disease management.

7. CONCLUSION

The comprehensive evaluation of chitosan derived from silkworm pupae underscores its vast potential as a sustainable, versatile and high-quality alternative to traditional crustacean-based sources. While chitosan from crustacean shells has dominated the market for decades, its limitations-including allergenicity, seasonal availability, environmental concerns and quality inconsistencies-demand exploration of novel sources. Silkworm pupae, an abundant by-product of India's silk industry, emerge as an underutilized yet highly promising resource. With India generating approximately 40,000 metric tons of dry pupae annually, tapping into this biomass for chitosan extraction offers an eco-conscious solution with added economic and industrial benefits.

Compared to aquatic-derived chitosans, silkworm pupal chitosan demonstrates exceptional physicochemical attributes. It exhibits high solubility (>98%), a superior degree of deacetylation (>85%), minimal ash content (<1%) and favourable molecular weight (4.09×10^4 Da). These features not only meet, but in some cases exceed the benchmarks set by crustacean-derived chitosans, especially in terms of biocompatibility, film-forming ability and antimicrobial performance. Notably, insect-derived chitosans such as those from cicada slough and silkworm chrysalis consistently outperform shrimp shell chitosan in molecular weight and purity, which is critical for biomedical and food-grade applications.

The wide spectrum of chitosan forms-flakes, powders, gels, resins, membranes, fibers, nanoparticles and modified derivatives-further reflects its adaptability. Silkworm pupal chitosan has been successfully converted into carboxymethyl chitosan with a high degree of substitution (DS = 1.63), strong antibacterial activity and optimal solubility and viscosity for pharmaceutical and industrial use. Compared to reference materials, it offers an effective platform for future green material innovation.

Applications span across multiple domains: in the food industry, it acts as a natural preservative and dietary fiber with cholesterol-lowering properties; in beverages, it serves as a natural coagulant, flavor enhancer and preservative; in biomedicine, it is a proven drug delivery vehicle, wound healer and antimicrobial agent; in textiles, it effectively adsorbs dyes; in tissue engineering, it enables scaffold formation with enhanced vascularization and cellular growth; and in sericulture, it acts as a potent antimicrobial agent to prevent disease outbreaks in silkworms.

In conclusion, the integration of silkworm pupal chitosan into mainstream applications is not merely a technical alternative- it is a sustainable and strategic imperative. With superior functional properties, broad-spectrum antimicrobial activity and reduced environmental footprint, pupal chitosan is well-positioned to complement or even replace crustacean-based sources in allergy-sensitive sectors such as healthcare, food packaging, pharmaceuticals and agriculture. Ongoing advancements in extraction technologies, molecular customization and commercialization pathways- fueled by public-private partnerships- can accelerate its adoption, ensuring a circular bioeconomy that valorizes waste while advancing green chemistry and industrial biotechnology.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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