Carbohydrate Polymer Technologies and Applications 12 (2025) 101005

Contents lists available at ScienceDirect

Carbohydrate Polymer Technologies and Applications

journal homepage: www.sciencedirect.com/journal/carbohydrate-polymer-technologies-and-applications

# Insights into the production and versatile agricultural applications of nanochitin for sustainable circularity: a review

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ARTICLE INFO

*Keywords:*
Chitin
Nanocrystal
Nanofiber
Plant elicitor
Biocide
Biostimulant

ABSTRACT

Chitin exhibits remarkable biocompatibility and biodegradability; nonetheless, its potential remains largely unexplored due to its hydrophobic nature, which makes it insoluble in water. Industrial marine food wastes, including crustacean shells, are the most plentiful and the potential source of chitin. High-performance chitin nanoparticles have been developed by understanding their biological characteristics and advanced preparation methods. The creation of nano-chitin materials is a significant topic due to its distinct dimensional, optical, mechanical, and other properties i.e. high surface area, low density and high dispersibility. Chitin nanocrystals and nanofibers could be fabricated by depolymerizing and demineralizing crustacean shell waste following various top-down and bottom-up methods, viz. acid hydrolysis, deep eutectic hydrolysis, TEMPO-mediated oxidation, self-assembly, etc. Morphology of the nanochitin and applications pertaining to respective nanofibrillation have been tabulated using the aforementioned methods. The present review summarizes the significant current developments in the synthesis of chitin nanoforms, i.e., nanochitin, nanofiber, or nanocrystal, along with their impact on enhancing plant growth and quality. Nanochitin could be utilized as fertilizers, biostimulant, plant elicitor, biocide as well as for seed treatment and appeared as an organic substitute for sustainable agricultural practices.

## 1. Introduction

A primary approach in the execution of a circular economy within the seafood processing sector involves the identification and adoption of sustainable methodologies for the management of residual seafood waste and by-products. The biomass present in the ocean exhibits distinct structures when compared to terrestrial organisms; nonetheless, it remains a relatively minor focus within the realm of natural biopolymers, significantly overshadowed by the prominence of plant-based resources (Bai et al., 2022). Marine food production is expected to rise by 36 to 74 % by 2050, resulting in an estimated annual generation of 6–8 million tons of waste from crab, shrimp, and lobster shells (Costello et al., 2020). Crustacean waste constitutes up to 60 % of total biomass, and its improper disposal presents significant environmental risks. Therefore, the development of sustainable strategies for the valorization of seafood waste is essential to advance the United Nations Sustainable Development Goals (Cooney et al., 2023).

Chitin being one of the most stipulated polysaccharides extracted from the seafood wastes has seized attention for varying industrial applications. It is a linear amino polysaccharide [$(C_8H_{13}O_5N)_n$] composed of N-acetyl-2-amido-2-deoxy-D-glucose (GlcNAc) units connected by β (1→4) bonds. Commercial chitin is mainly derived from byproducts of crustacean shellfish, especially the exoskeletons of shrimp and crab, which generally comprise 8–40 % chitin Vadivel et al. (2024). Nevertheless, the extraction of chitin from crustacean shells presents significant challenges owing to their intricate hierarchical structure, which consists of crystalline α-chitin nanofibers integrated within a protein-mineral matrix, bonded by robust intermolecular and intramolecular hydrogen bonding (Fittolani et al., 2020) (Fig. 1). Chitin molecules organize into microfibrils (2–5 nm), which are integrated within a protein matrix to create a fibrous network (50–300 nm). The network is organized into a helicoidal Bouligand structure, characterized by the interweaving of chitin with proteins and calcium carbonate (Zhang et al., 2024; Jia et al., 2024). The supramolecular assembly

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https://doi.org/10.1016/j.carpta.2025.101005

Available online 10 September 2025

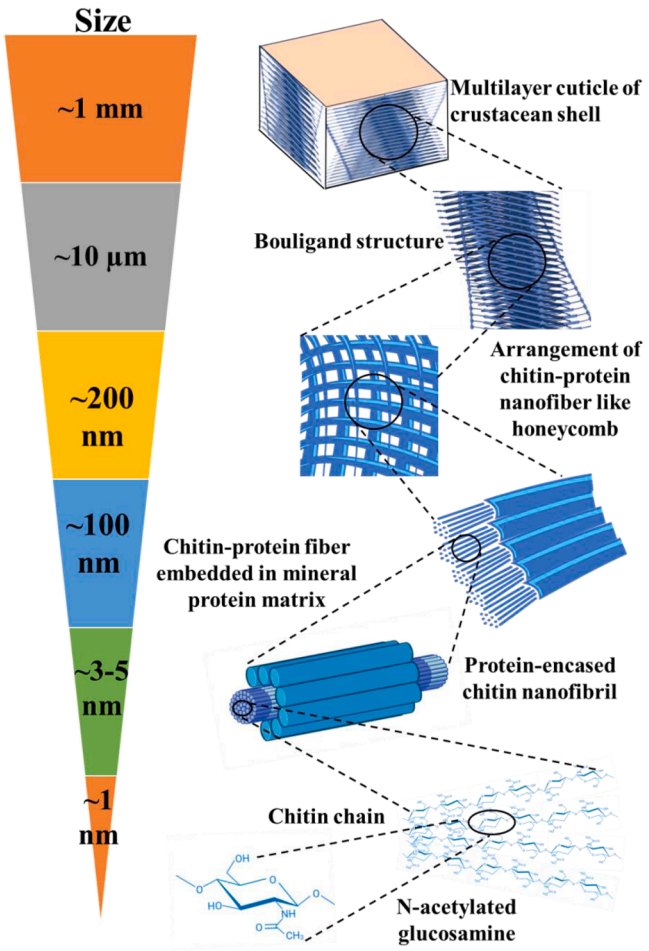


**Fig. 1.** Multiscale hierarchal chitin organisation in crustacean shell exoskeleton.

derived from the Bouligand structure exhibits a lyotropic crystal phase characterized by supramolecular chirality, an essential element of the natural world, ubiquitously found in the exoskeletons of Arthropoda phylum (Luo et al., 2023). Chitin exists in three allomorphs: α, β, and γ. α-chitin has antiparallel chains with strong inter- and intra-sheet hydrogen bonds, while β-chitin has parallel chains with weaker bonding. γ-chitin combines both configurations and is rare in nature (Rudall, 1963). The recalcitrant crystalline structure of chitin renders it insoluble in most solvents, which poses challenges for its processing, even though it possesses biocompatibility and antimicrobial properties (Yang et al., 2020). Nonetheless, nanochitin forms like nanocrystals and nanofibers obtained from α- and β-chitin provide enhanced dispersibility and functionality, attributed to their elevated surface area, aspect ratio, and charge density (Jung et al., 2018; Ngasotter et al., 2022). Nanostructures are generally produced through top-down or bottom-up approaches following the purification of chitin (demineralization, deproteinization and decoloration). It is acknowledged that top-down approaches are the most utilized methods in the present scenario for the extraction of chitin micro- and nanofibrils, largely owing to the practicality and scalability of the process along with the availability of chitin-rich biomass. The methods generally encompass mechanical treatments, that frequently supported by chemical or enzymatic pre-treatments aimed at reducing inter-fibrillar hydrogen bonding and enhanced fibrillation. Bottom-up approaches, while not as frequently employed, typically entail the regulated self-assembly of chitin molecules via solvent dissolution . These techniques are especially significant when exact control over particle size, morphology, or surface functionality is necessary.

While several recent reviews have broadly explored various aspects of nanochitin and its applications across multiple sectors, including materials science, nanotechnology, and food packaging (e.g., Wang, Zhu, Qiao, & Luo, 2024); limited literature is available on in-depth analysis of nanochitin synthesis pathways with a dedicated focus on sustainable agriculture. The present review addresses the gap by offering a comprehensive and perceptive insight regarding nanochitin production technologies, with particular emphasis on the agricultural applications within the framework of circular bioeconomy and life cycle assessment (LCA). Present article has systematically classified and compared both the conventional (e.g., acid hydrolysis, ultrasonication, high-pressure homogenization) and emerging green fabrication techniques-including underexplored methods i.e. electron beam irradiation, microwave treatment and deep eutectic solvent-based hydrolysis with appraisal of relative efficiencies, scalability, and environmental implications. Special attention has been given to the top-down and bottom-up approaches and coupling the synthesis techniques to the resulting physicochemical properties of nanochitin (e.g., surface area, charge density, colloidal stability) along with the relevance to biofunctional roles in agriculture. Nanochitin has been recognized as a promising biostimulant, nanofertilizer, elicitor of plant defense mechanisms, and environmentally friendly biocide. Nanochitin's potential applications to improve seed germination, promote plant growth, and increase resistance to pathogens with decreasing reliance on synthetic agrochemicals have also been contemplated. Tha review has also emphasized on the transformation of crustacean waste into high-value nanomaterials, presenting innovative perspectives on the role of nanochitin as a sustainable alternative to customary inputs. Biopolymers enhanced payoff for resource-efficient farming, soil health restoration, and minimizing environmental impact has alos been underlined.

## 2. Chitin: sources and extraction

Chitin is extensively present in algae, fungi, arthropods, and mollusks. This material functions as a crucial structural element in the exoskeletons of crustacean shells (barnacles, krill, shrimp, crabs, and lobsters), generally comprise 20–30 % chitin, 30–40 % proteins, 30–50 % calcium carbonate, along with pigments such as astaxanthin (Dayakar et al., 2021). Chitin establishes a robust composite network in conjunction with proteins, lipids, and pigments, which is further enhanced by the deposition of calcium carbonate (Dun et al., 2019). Recovery of chitin with improved solubility and higher purity has been reported from fungal sources including *Aspergillus niger* (Cano-Gonzalez et al., 2025), *Flammulina velutipes* (Liang et al., 2024), *Agaricus bisporus* (Hassainia et al., 2018), etc. Chitin sourced from fungal species being devoid of proteins and calcium carbonate is well-suited for applications in biomedical and cosmetic fields. Nonetheless, the yield of chitin might be diminished due to the presence of glucans (El-Feki et al., 2025). The highest chitin yield is reported from *Bombyx eri* (insect); 45 % (Huet et al., 2020), followed by *Homarus americanus* (crustacean); 40.31 % (Kozma et al., 2024), and *Flammulina velutipes* (fungi); 16.8 % (Liang et al., 2024). Industries typically entail harsh chemical treatment steps for chitin extraction including strong acids and alkalis, at elevated temperatures. This method, despite its widespread commercialization, produces toxic effluents, results in products with variable properties, and requires significant energy and financial investment, thereby constraining its sustainability (Kumari et al., 2023). Recent findings indicated that deep eutectic solvents (DESs) serve as an efficient one-pot solution for extracting high-purity chitin, eliminating the need for demineralization and deproteinization steps (Zhu et al., 2017). Unlike toxic and nonbiodegradable ionic liquids, particularly natural DESs (NADESs), offering advantages viz. biodegradability, cost-effectiveness, and straightforward synthesis (Sulthan et al., 2023). These green solvents, derived from eco-friendly precursors, effectively extract biopolymers and provide sustainable alternatives to conventional methods. Biological methods utilizing proteolytic enzymes and microbial

fermentation provide sustainable and cost-effective approaches for chitin extraction, effectively digesting proteins and minerals while maintaining chitin quality. Fermentation may occur independently or in conjunction with enzymatic hydrolysis, utilizing a range of lactic and non-lactic acid bacteria, including *Serratia marcescens, Bacillus* spp., *Pseudomonas aeruginosa*, and *Lactobacillus* spp. (Kumari et al., 2023; Xie et al., 2021). The integration of microbial techniques with reduced chemical intervention can enhance extraction efficiency (Hamed et al., 2016). Extremophilic microorganisms and their enzymes, including *Thermomyces lanuginosus* and *Thermomyces dupontii*, provide effective action on chitin owing to their resilience to elevated temperatures, extreme pH levels, salts, and solvents, thereby they can utilize for feasible chitin's extraction process (Akanbi et al., 2020; Kumar et al., 2021; Kumari et al., 2024). *Halobacterium salinarum* and *Halococcus dombrowskii* accomplished deproteinization rates of up to 98 % from prawn shell waste using minimal chemicals (Dayakar et al., 2021). In a similar manner, enzymatic extraction from *Acetes* sp. resulted in a deproteinization rate of 93.68 %, accompanied by improved hydrolysis (Dhanabalan et al., 2021). The properties of chitin are significantly affected by various process parameters, including pH, temperature, incubation time, and inoculum size. Chitin extracted through biological methods provides superior purity, consistency, and acetylation levels in comparison to chemically derived alternatives. Furthermore, the presence of amino acids in the fermentation broth enhances its value as feed ingredients (Dhanabalan et al., 2021; Liu et al., 2020). Nonetheless, microbial and enzymatic extraction methods are still in the early stages of development and necessitate optimization, particularly for effective protein and mineral removal at commercial scales.

## 3. Nanochitin

Nanochitin (NCh) refers to the biopolymeric nanofibrils isolated from chitin in the form of nanocrystals, nanowhiskers and nanofibers. Chitin nanocrystals (ChNCs) or chitin nanowhiskers (ChNWs) have a thin shape typically ranging from 100–800 nm in length and 6–60 nm in width; while chitin nanofibers (ChNFs) are elongated structures having length in the micrometer range with widths between 10–100 nm (Fernández-Marín et al., 2021). NCh displays demonstrate distinctive physicochemical properties, encompassing such as quantum effects, hydrophilicity and adjustable surface characteristics (Wang et al., 2023). The unique form and their dimensions chiefly rely on the source of native chitin and different extraction methods. The conversion of crystalline regions of native chitin results in the formation of ChNCs and ChNFs with remarkable aspect ratio. A higher aspect ratio of chitin nanomaterials is related to a distinct role by enhancing their unique properties (Yihun, 2022). For instance, enhancement of mechanical strength and thermal stability has been found of resulting biocomposite (NR/ChNCs) by incorporation of ChNCs with higher aspect ratio into natural rubber (NR) latex (Liu et al., 2018). Chiral nematic (cholesteric) ordering in NCh is a resulted from self-assembly of ChNCs/ChNWs into helicoidal structures, characterized by the progressive twisting of nanocrystal orientation along the axis (Luo et al., 2023; Narkevicius et al., 2022). This mesophase occurs naturally in aqueous environments and can be maintained during the transition to solid states. Methods including biomimetic mineralization, self-assembly, and evaporation-induced self-assembly (EISA) have been utilized to facilitate this organization (Chen et al., 2023; Liu et al., 2022). The chiral nematic pattern arises from the intrinsic chirality and aspect ratio of NCh, which are affected by various factors including concentration, morphology, surface charge, pH, ionic strength, temperature, hydrolysis duration, self-assembly time, and sonication (Ramanauskaitė et al., 2025). This structure selectively reflects light, generating iridescent coloration and has influenced the development of bionanomaterials, photonic devices, sensors, and biodegradable optical films (Luo et al., 2023; Narkevicius et al., 2022). NCh, characterized by high surface area, low density, and bioactive properties i.e. biodegradability, biocompatibility, antioxidant, and antimicrobial activity, presents considerable potential for sustainable applications across health, agriculture, and food sectors (Jahed et al., 2017). The chemical reactivity, mechanical strength, nontoxicity, and self-assembly significantly contribute to its overall utility. Fig. 2a presented essential physicochemical properties of NCh, encompassing particle size, crystallinity, surface charge, and degree of acetylation with various applications. The characterization of these attributes is typically conducted using techniques viz. DLS, TEM, zeta potential analysis, and XRD. Nevertheless, differences in methodologies among studies impede direct comparisons of data. Standardized protocols are essential for ensuring reproducibility, aiding regulation, and establishing benchmarks for product performance. Harmonized characterization would facilitate scalable development for both industrial and agricultural applications.

Nanostructured biopolymers, including cellulose nanocrystals (CNCs), cellulose nanofibers (CNFs), nanochitosan, and starch nanocrystals, have been extensively studied for their potential applications in packaging, biomedical scaffolds, and functional nanocomposites (Habibi et al., 2010). NCh, sourced from α or β chitin, presents unique benefits over cellulose or starch viz. inherent antimicrobial properties, an enhanced modulus-to-weight ratio, biodegradability and biocompatibility that are on par with nanochitosan, while exhibiting superior stability at neutral pH (Bai et al., 2022; Ifuku & Saimoto, 2012). Nonetheless, in contrast to CNCs and CNFs, the research and commercialization of NCh are constrained by inconsistent supply chains of chitin feedstock, intricate pretreatment processes i.e. demineralization and deproteinization, and a lower level of process maturity for large-scale continuous production (Kaya et al., 2014). The presence of acetamido groups in chitin enhances dipole alignment and improves piezoelectric performance relative to cellulose, rendering ChNFs/ChNCs more effective for energy harvesting applications. Conversely, CNFs are more abundant, flexible, and cost-effective for large-scale devices (Rajala et al., 2016). Transparent ChNF films derived from squid pens exhibited piezoelectric performance comparable to fluorine-based polymers (Kim et al., 2018). Additionally, nanogenerators constructed from crab shell ChNF and polyvinylidene fluoride/ChNF composites efficiently harvested energy from minor mechanical activities, including walking, speaking, and finger tapping (Hoque et al., 2018). Recent studies emphasized that hybrid systems viz. NCh & nanocellulose composites, which integrate the mechanical and barrier properties of cellulose with the antimicrobial characteristics of chitin, illustrate the potential of cross-biopolymer engineering in developing high-performance sustainable materials. ChNF exhibit reduced mechanical reinforcement compared to CNFs, yet they present additional biofunctional benefits, including antimicrobial properties and biocompatibility. Despite diminishing transparency, the multifunctionality of ChNF amplifies its significance for biomedical and sustainability applications (Lim et al., 2020). The complexation of CNF with NCh improves CNF adsorption on polylactide droplets, resulting in uniform Pickering emulsions. NCh enables the creation of lightweight, highly porous cryogels featuring well-defined internal structures. NCh has also been reported to enhance the water resistance, underscoring its significance in the reinforcement and functionalization of CNF/NCh-based cryogels (Guo et al., 2024). Alterations of emulsion properties by varying the CNC/NCh ratio have demonstrated that combinations of polysaccharide nanoparticles could serve as an effective stabilization material (Guo et al., 2023). Chitin nanomaterials serve as reinforcing agents in polymers, nanopaper membranes, and bioplastic films (Xuan et al., 2025). They are also utilized in biomedical applications, including wound dressings (Ma et al., 2025), emulsion stabilizers (Zhou et al., 2025), antifungal agents (Liang et al., 2018), and scaffolds for bone tissue engineering (Olza et al., 2025). Additionally, the NCh hydrogels provided a simplified fabrication process, enhanced filtration efficiency, and cost-effectiveness, underscoring their scalability for practical water purification systems through the removal of microplastics (Yi et al., 2025). The use of NCh, including ChNCs, ChNFs, and chitin nanosphere formulations, in

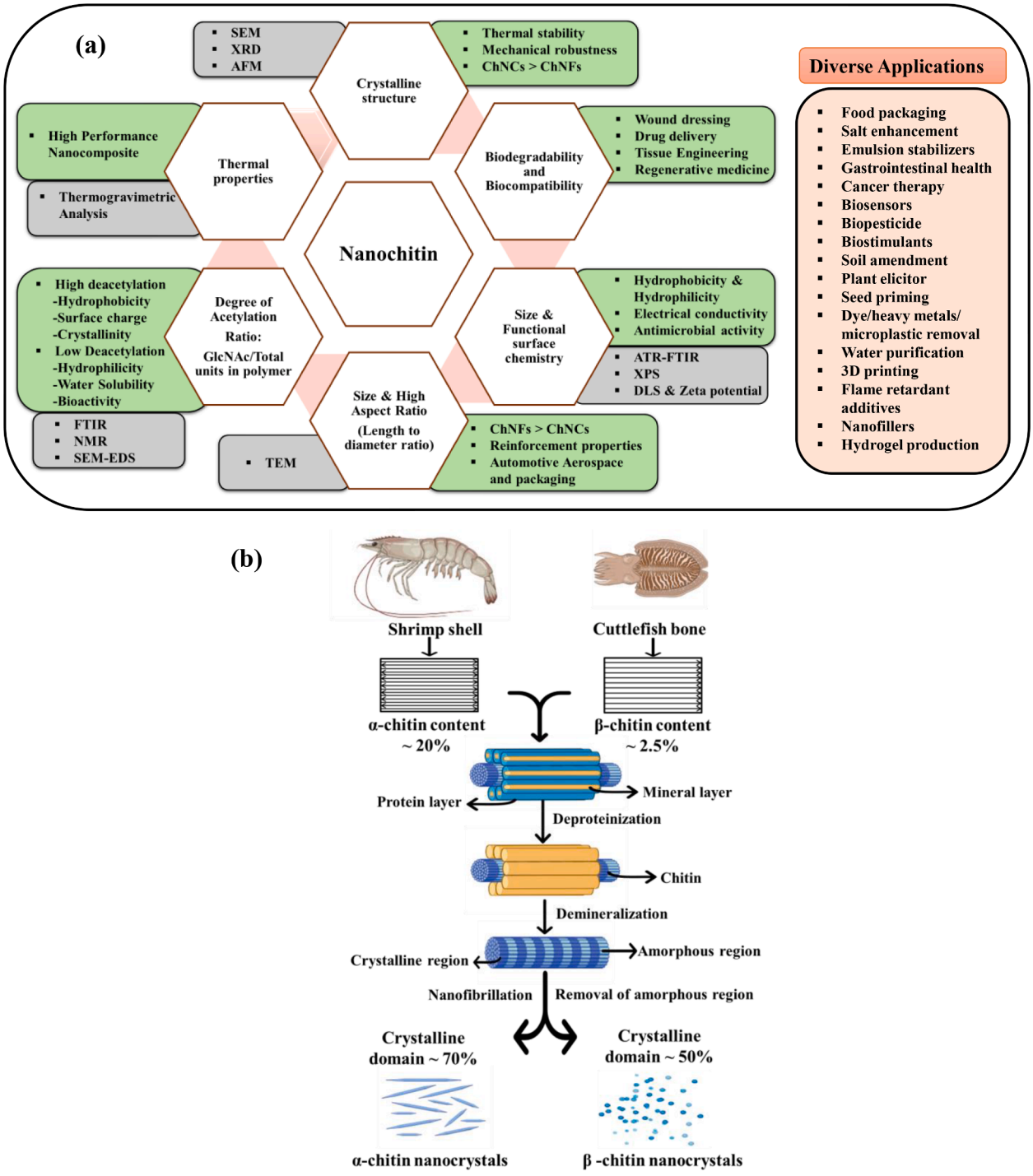


**Fig. 2.** Overview of nanochitin's properties, characterization techniques and applications (a); Nanofibrillation of α-chitin from shrimp shell and β-chitin from cuttlefish bone (b).

complex coacervation for encapsulating *Lactobacillus casei* represents a novel method for developing a compressed probiotic supplement (Luo et al., 2025). This approach contributes to the advancements in design and fabrication of encapsulation vehicles for active ingredients.

### 3.1. Chitin nanocrystals

ChNCs have rod or whiskers like nano structures and are highly crystalline in nature (< 80 %) and its wider aspect ratio (10–50 nm width; 100–400 nm length) varies with the source and extraction process (Salaberria et al., 2015; Qin et al., 2016). The source and synthesis method also affect the nanocrystals' surface chemistry, primarily hydroxyl, amine, acetamide and carboxyl which improves ChNCs dispersibility and aids in surface functionalization. Typical chemical modifications of ChNCs encompass surface thiolation, phosphorylation (Yu et al., 2023), acetylation (Lin et al., 2014), carboxylation (Ma et al., 2019), and the grafting of polymers or functional groups (Wang et al., 2012). The modifications improve characteristics viz. dispersibility, hydrophilicity/hydrophobicity, charge density, and reactivity. Thiolation, accomplished through the acylation of amino groups with N-acetylcysteine or thioglycolic acid, notably modifies colloidal and rheological properties. The thiolated ChNCs have demonstrated

photocatalytic reduction of Cr (VI) without exhibiting acute toxicity in mice (Torlopov et al., 2025). Bio-nanocomposites reinforced with ChNCs have been observed to hold increased tensile strength, Young's modulus (Broers et al., 2018; Yanat & Schröen, 2023), along with the enhanced thermal stability and degradation resistance (Singh et al., 2020). Furthermore, antioxidant and antimicrobial properties of ChNCs rendered them significant for food preservation and biomedical applications (Martínez Salaberria et al., 2017; Yanat et al., 2022). ChNCs extracted from crystalline region of natural chitin by removing amorphous structure from chitin microfibrils. For instance, α-ChNCs was extracted from crab and shrimp shells and β-ChNCs from squid pen and cuttlefish bone (Jung et al., 2019) (Fig. 2b). Diverse preparative techniques have been employed to produce ChNCs, i.e., acid hydrolysis, oxidation mediated via TEMPO and APS, partial deacetylation combined with mechanical or combined treatment. It acts as efficient nanofillers, greatly improving the mechanical strength of hydrogel patches. Their incorporation promotes the systematic regeneration of fibrous tissue, presenting a promising approach for the advancement of clinical biomaterials aimed at postoperative annulus fibrosus repair (Liu et al., 2025). A supramolecular network of wormlike micelles (WLMs) (CTAB/sodium salicylate) reinforced with rigid ChNCs have been reported to create a thermally stable suspension, which shows potential for application in oilfield fracturing fluids where pure WLMs experience a loss of viscoelasticity (Hao et al., 2025).

### 3.2. Chitin nanowhiskers

ChNWs, also known as chitin nanocrystals or chitin nanofibers depending on the context, are nanoscale derivatives of chitin distinguished by their high crystallinity and slender parallelepiped rods or spindle-shaped morphology. ChNWs typically display dimensions measuring in the hundreds of nanometers for length and tens of nanometers for width, leading to a significant aspect ratio (Yang et al., 2020). A range of techniques exists for the preparation of ChNWs, encompassing chemical processes, ionic liquid applications, TEMPO mediated oxidation, deacetylation, and mechanical approaches (Zhang et al., 2024). ChNWs are broadly generated via acid hydrolysis of refined chitin, which effectively eliminates the amorphous regions and resulted in highly crystalline structures (Zhang et al., 2024). The assembly of ChNWs into the Bouligand structure exemplifies the distinctive nature of chiral nematic ordering (Luo et al., 2023). ChNWs exhibit high surface area, low density, biodegradability, and biocompatibility. Their abundant surface -OH and -$NH_3^+$ groups facilitate hydrogen bonding and protonation, which contribute to the formation of stable colloidal suspensions (Etxabide et al., 2022; Kim et al., 2021; Qin et al., 2016). The characteristics of ChNWs render them particularly appealing for use in biomedicine, biomaterials, environmental remediation, and bioactive food packaging (Eze et al., 2024). ChNWs have also been reported to utilize as reinforcing agents in polymer nanocomposites; improving mechanical, thermal, and barrier properties (Mincea et al., 2012). They may also form agglomerated bundles while preserving non-toxic, antibacterial, and low-immunogenic characteristics. ChNWs serve a crucial structural function in polymer assembly and affect the kinetics of drug release (Dubashynskaya et al., 2025).

### 3.3. Chitin nanofibers

ChNFs are slender fibers made from chitin, having diameters $< 100$ nm and length varying from a few to several μm. These ultrafine fibers, extracted from crab shells, exhibit excellent dispersibility in water, making them suitable for various applications. (Endo et al., 2023). ChNFs exhibit a high aspect ratio, outstanding mechanical properties, and an extensive surface area, rendering them particularly appealing for use in biomedicine, materials science, and environmental engineering. These are generally generated through the mechanical disintegration of natural chitin, frequently after undergoing chemical pretreatment (Ma et al., 2021). Several mechanical methods i.e. grinding, ultrasonication, high-pressure homogenization and high-pressure water jet (HPWJ) systems and other bottom-up approaches including elctrospinning and self-assembly, have been commonly implemented either individually or in combination through specific acidic or neutral conditions to obtain ChNFs from natural chitin sources. ChNFs could be isolated from prawn shell chitin, shrimp shell α-chitin, crab shell and squid pen β-chitin . Nanofibrillation has reported to be easier employing prawn shell as source in comparison to the crab shell because finer structure of prawn enables easier synthesis of ChNFs under neutral conditions (Ifuku et al., 2011). Partially deacetylated and TEMPO oxidized CtNFs demonstrate exceptional properties i.e. aggregation-induced emission fluorescence, hydrophobicity, and UV shielding, positioning them as promising candidates for fluorescent nanocomposites and fluorescence-based sensing technologies (Yu et al., 2025). ChNFs were regenerated using a KOH/urea system and used to maintain the stability of selenium nanoparticles via a redox reaction employing ascorbic acid and sodium selenite. Additionally, ChNFs have been illustrated to possess notable in vitro antioxidant activity and cancer cell suppression (Wei et al., 2025).

## 4. Nanochitin preparative methods

The two principal strategies for conversion of chitin into versatile NCh: (i) top down, a hierarchical methodology encompassing the mechanical and chemical disintegration of substantial chitin and (ii) a bottom-up approach that utilizes molecular self-assembly and electrospinning (Fig 3). Contrary to the top-down methods that maintains the nano chitin's inherent crystal structure and rod-like morphology; bottom-up approaches have been reported to alter the crystal structure of nano chitin by using harsh solvents systems. Hence, the top-down is favoured for generation of NCh. The top-down treatment method consistently entails the direct extraction of nano-sized fibers from biomass feedstock, resulting in the disruption of the feedstock's molecular structure. Notwithstanding this, the method upholds the intrinsic crystallinity of the nanofibrils, assuring the retention of inherent crystalline structure throughout the preparation process (Ma et al., 2020). The fabrication of NCh has conventionally relied on the energy-intensive methods viz. acid hydrolysis, grinding, ultrasonication, and high-pressure homogenization. Alternatively, eco-friendly alternatives i.e. electron beam irradiation, microwave treatment, and DES hydrolysis present benefits including milder conditions, shorter processing times, and a lower environmental impact. Apart from chemical synthesis, enzymatic hydrolysis represents an emerging greener approach for the fabrication of NCh. Barandiaran et al. (2022) involved the incubation of α-chitin flakes with lytic polysaccharide monooxygenase (LPMO), resulting in the production of NCh utilized in 2D/3D cell cultures and as bioink for 3D printing. The reaction was terminated on ice, and extracted through ultrasonication, centrifugation, and lyophilization. After 72 h, nanofibers with approximate length (458 nm) and diameter (32.3 nm) were produced, exhibiting cleavage at the β−1,4-glycosidic bond via selective oxidation at C1 and C4.

The increasing emphasis on biomass utilization and circular resource use is anticipated to drive the demand for chitin-rich feedstocks. Mushrooms serve as an alternative to crustaceans; however, their low chitin content and competition with food applications restrict their feasibility (Kitagawa et al., 2025). Therefore, the identification of sustainable, abundant, and non-food-competing raw materials is essential for the future production of NCh. The synthesis of NCh encounters significant challenges related to raw materials, as the selection of feedstock viz. crustacean shells and squid pens, has a direct impact on purity, consistency, and cost at an industrial scale (Bai et al., 2022). Greener alternatives demonstrate potential; however, enzymatic pathways are constrained by elevated costs and limited scalability (Pratiwi et al., 2023). The time efficiency of traditional chemical hydrolysis, requiring 1.5 to 6 h, coupled with expensive purifying processes, significantly diminishes throughput. TEMPO oxidation and partial deacetylation

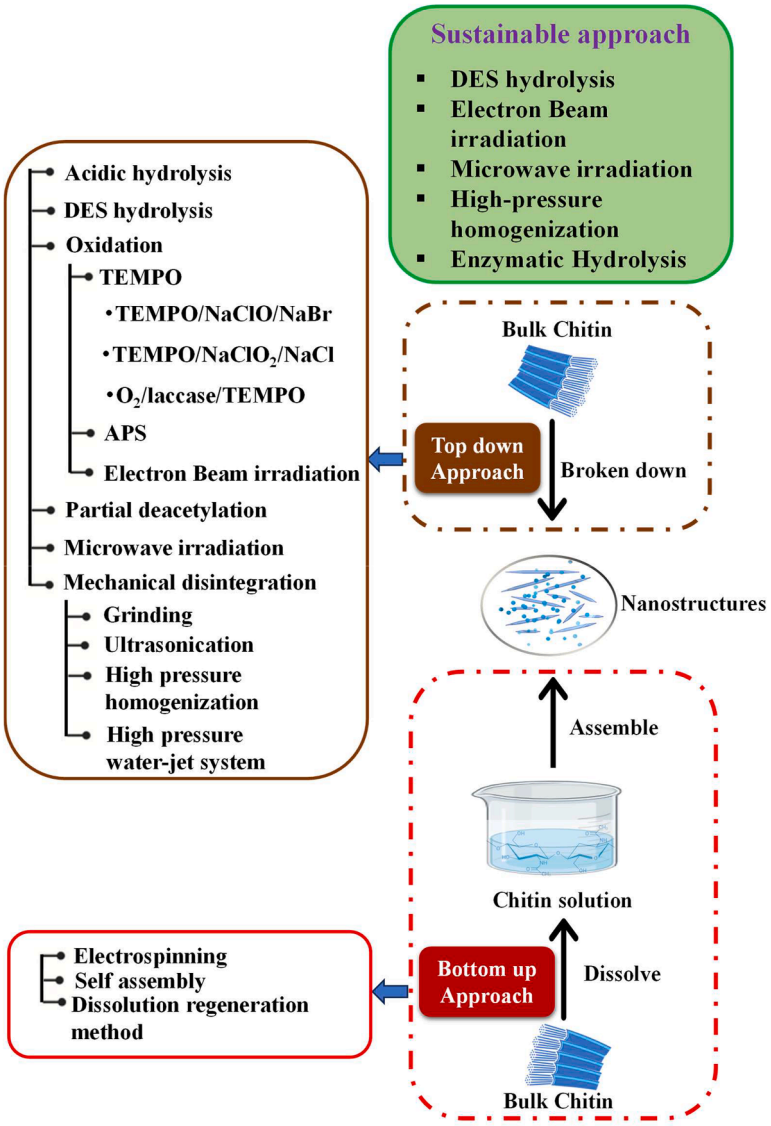


**Fig. 3.** Nanochitin preparation via top down and bottom-up approaches.

require multistep protocols, whereas mechanical methods frequently necessitate pretreatment and result in variable dimensions (Chee et al., 2024). Scalability also presents a significant challenge: chemical methods entail expensive waste management; mechanical approaches require substantial energy and equipment, and bottom-up techniques viz. electrospinning or self-assembly are limited in throughput (Islam et al., 2025). Furthermore, ensuring consistent quality at scale presents challenges, as variability in morphology and crystallinity can hinder industrial adoption (Bai et al., 2022).

### 4.1. Top-down approach

Top-down approaches disintegrate the crystalline region of native chitin into nanoscale structures by various methods, including acid hydrolysis, deep eutectic solvent (DESs) hydrolysis, oxidation mediated through TEMPO, partial deacetylation, microwave irradiation, and other mechanical treatments, including grinding, ultrasonication, high-pressure homogenization, and a high-pressure water jet system.

#### 4.1.1. Acid hydrolysis

Acidolysis is a standard technique to produce NCh, wherein acid selectively degrades the amorphous regions of chitin while preserving the highly crystalline, acid-resistant areas. The disordered regions, characterized by a high concentration of β−1,4-glycosidic bonds, exhibit increased susceptibility to hydrolysis. Hydrochloric and sulfuric acids are frequently utilized, with reaction conditions including acid concentration, time, temperature, and energy input, meticulously optimized to produce high quality ChNCs with an enhanced aspect ratio (Habibi et al., 2010). ChNCs obtained by mixing purified chitin and 3 M HCl in a ratio of 1:30 (w/v) resulted into a colloidal suspension when stirred vigorously at higher temperature (90–100 °C, 1.5–6 h) (Druzian et al., 2019). The suspension is diluted with deionized water, centrifuged and washed multiple times to produce crystallites at neutral pH (Qin et al., 2016). Nanofibrillation has reported to be predominantly relied on usage of HCl and sulphuric acid (Table 1). Nevertheless, acidic hydrolysis typically performed at high temperature; that poses serius environmental concerns. Conversely, Liu et al. (2018) has investigated the hydrolysis of mechanically defibrillated chitin nanofibrils using $H_2O_2$ as a sustainable and green method for producing rod-like chitin nanocrystals from chitin nanofibrils, which exhibited an average length of 350 nm and width of 40 nm. The findings indicated that the α-chitin nanorods, which underwent hydrolysis by $H_2O_2$, retained their crystalline structure. However, there was a notable change in the surface charge of the nanorods, transitioning from positive to negative.

**Table 1**
Production of nanochitin through acid hydrolysis and its applications.

| Nanochitin | Method | Results | Application | References |
|---|---|---|---|---|
| **ChNCs; shrimp shell** | Acid hydrolysis + Ultrasonication | 80–200 nm in length and 30–50 nm | Enhancement of grain yield and quality of winter wheat | Xue et al., 2017 |
| **ChNCs; shrimp shell** | Acid hydrolysis + Ultrasonication | 200 ± 20 nm in length and 8 ± 1 nm in width. | Inhibition of tobacco rot disease | Zhou et al., 2017 |
| **ChNWs; shrimp shell** | Acid hydrolysis + Ultrasonication | 10 – 30 nm in width and 90 – 250 nm in length. | Antifungal activity against wheat crown rot disease | Liang et al., 2018 |
| **ChNWs; shrimp waste** | Acid hydrolysis | | Used as effective adsorbent for crystal violet dye removal from wastewater | Druzian et al., 2018 |
| **ChNWs; shrimp shell** | Acid hydrolysis + Ultrasonication | 15–30 nm in width and 100–250 nm in length | Enhancement of grain yield and crude protein of winter wheat | Cheng et al., 2019 |
| **ChNWs; shrimp shells practical grade** | Acid hydrolysis + Ultrasonication | 50–150 nm in length and 30–50 nm in width, Charge density 63 mmol/kg | Insecticidal activity | Li et al., 2021 |
| **ChNCs; crab shell** | Acid hydrolysis (64 % $H_2SO_4$) | 426 ± 10 nm average length | Strong antibacterial activity against *Escherichia coli* | Oun & Rahim, 2018 |
| **ChNCs; (Crustacean biomass waste)** | p-toluenesulfonic acid-assisted hydro thermal treatment | length and width range of 25–225 nm and 15–30 nm; zeta potential 41 mV | Utilise in fabrication of large-area photonic films, for instance, for applications in vibrant automobile coatings | Zhu et al., 2024 |
| **ChNCs; (*Plesionika martia* shrimp)** | Acid hydrolysis (3 N HCl) | Average length and width 201.49 ± 20.55 nm and 26.55 ± 12.33 nm 12.33 nm | Fabrication of composite (collagen-gelatin) scaffold showing antioxidant activity and non-toxicity | Amal et al., 2025 |
| **ChNCs; (Shrimp shells)** | Acid hydrolysis (Lactic acid) | Average diameter of 20 nm and an average length of 240 nm, Aspect ratio-12; CI- 87.1 % | Used as adhesive for agrowaste cork composite | García-Laynez et al., 2025 |
| **Nanochitin suspension; (Crab shells)** | Acid hydrolysis (acetic acid) | Uniform 276 nm dimension; Degree of deacetylation (DA) 36.86 % | Pickering emulsion stabilizer | Zhou et al., 2025 |
| **Nanochitin suspension; (Cricket legs)** | Acid hydrolysis (0.5 % aq. acetic acid) + 7 Grinding cycle | 338 nm average length and 9–10 nm average width | – | Kitagawa et al., 2025 |
| **ChNCs** | Acid hydrolysis (0.5 % aq. acetic acid) | Average diameter of 21.6 ± 3.0 nm and average length of 228.2 ± 20 nm Crystallinity Index (CI)- 60–62 % | Pickering emulsion template for multimodal sensing | Cao et al., 2025 |
| **αChNC; (Shrimp shells) βChNC; (Squid pen)** | Acid hydrolysis | Rod shape: 115 nm average length and 27 nm width; DA- 96 %; CI- 90 % Sphere shape: 38 nm average diameter; DA- 90 %; CI- 86 % | Helps in bone tissue engineering by improving the osteoinductive properties of chitosan 3D porous biohybrid scaffolds | A.M. Salaberria et al., 2015; Olza et al., 2025 |

Consequently, the colloidal nanocrystals exhibited excellent dispersion in aqueous media with neutral or alkaline pH and demonstrated characteristics of a lyotropic liquid crystal within a specific concentration range.

### 4.1.2. *Deep eutectic solvent hydrolysis*

DESs consist of liquid mixtures of hydrogen bond donors (HBDs) and acceptors (HBAs), creating eutectic systems that exhibit melting points lower than those of the individual components when combined in specific ratios (90–100 °C). The materials serve as environmentally sustainable options for polysaccharide processing, owing to their robust interactions with hydrogen-bond networks in carbohydrates (Zdanowicz et al., 2018). Like ionic liquids (ILs), DESs provide low vapor pressure and high solvent capacity, along with advantages such as low toxicity, biodegradability, recyclability, and cost-effectiveness (Hong et al., 2019). Through the customization of DES formulations, ChNCs can be effectively extracted utilizing acidic DESs, whereas non-acidic DESs promote the formation of ChNFs (Zhao et al., 2025). Sharma et al. (2013) illustrated the dissolution of α-chitin in several DESs, such as choline chloride (ChCl)/thiourea, urea-based mixtures (1:2), and betaine hydrochloride/urea (1:4), employing conventional, microwave, and ultrasound-assisted heating methods. ChCl-thiourea (1:2) was

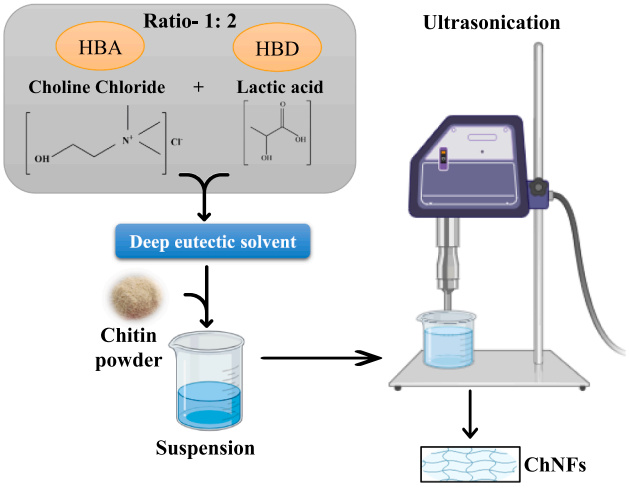


**Fig. 4.** Ultrasonication assisted DES pretreatment for preparation of chitin nanofibers.

utilized to produce ChNFs and calcium alginate bio-nanocomposite gel beads, demonstrating improved elasticity and facilitating the controlled release of 5-fluorouracil (Mukesh et al., 2014).

Various combinations of HBD and HBA have been applied to extract chitin nanocrystals (Fig. 4). For example, p-toluenesulfonic acid and choline chloride fabricated ChNCs as well as combination of different organic acids (oxalic acid dihydrate, lactic acid, malonic acid, citric acid monohydrate, DL-malic acid) mixed with ChCl (2:1) were used as acidic DESs under ultrasonication. The process served dual functions, as along with working as an acylation reagent, it promoted chitin hydrolysis for generation of ChNCs (Cao et al., 2019; Yuan et al., 2020). Moreover, one-pot O-acetylated, esterified and hydrolysed ChNCs produced by using ChCl and Zinc chloride DESs, which act both as hydrolytic medium and catalyst. The study also analyzed effect of several factors viz. reaction time, water and acetic anhydride content and reported 61.6 % yield of acetylated ChNCs with degree of substitution of 0.23 under optimum conditions (2:40 mass ratio of chitin and DES mixed with 4.736 g acetic anhydride, 90 °C, 3 h) (Hong et al., 2019). Zhao et al. (2025) synthesized phosphorylated ChNCs (length, 166.2–185.6 nm; width, 6–13.4 nm) using a urea phosphate-urea based DESs and ultrasonication. An appropriate combination of DESs utilised for production of NCh and its application have been summarized in Table 2.

#### 4.1.3. Oxidation method

The oxidation method, which includes TEMPO, APS (Ammonium persulphate), Electron beam irradiation (EBI) oxidation, is another essential process for creating ChNCs (Table 3). The radical catalyst TEMPO, also known as 2,2,6,6-tetramethylpiperidine 1-oxyl, is a member of the niroxyl radical family. Treatment of chitin with TEMPO and APS resulted into oxidation of the primary hydroxy groups (OH groups) of C6 and its transformation to COOH groups. This makes chitin more dispersible in water by increasing the charge repulsion between molecular chains (Fan et al., 2008). The dispersibility of NCh in water at various pH values is strongly affected by the carboxylate and amine group contents.

*4.1.3.1. APS oxidation method & TEMPO.* APS is a potent oxidant with high water solubility and low toxicity, decomposing at temperatures between 60–75 °C to produce $HSO_4^-$, $H_2O_2$, and $SO_4^{-2}$ radicals. This process facilitates the oxidation of C6 to carboxyl groups and, in some cases, the oxidation of amino groups to nitro groups. APS provides a superior carboxyl yield compared to TEMPO oxidation (Oun & Rahim, 2018). TEMPO-mediated oxidation, commonly applied to chitin and cellulose, facilitates thorough nanofibrillation when subsequently combined with homogenization and ultrasonication (Liu et al., 2021). There are two TEMPO systems: TEMPO/NaClO/NaBr (pH 10) and TEMPO/$NaClO_2$/-NaClO (pH 6.86) (Pang et al., 2017). The proportion of NaClO affects the production of water-soluble polyuronic acid and insoluble NCh (Zeng et al., 2012). Fan et al. (2008) were the pioneers in utilizing the TEMPO/NaClO/NaBr system for the extraction of ChNCs from α-chitin derived from crab shell. The reaction was conducted at room temperature, with the pH carefully maintained at 10 through the addition of 0.5 M NaOH via pH-stat titration and was concluded with the addition of ethanol. Although minor depolymerization may take place under alkaline conditions, this approach has been extensively utilized for the fabrication of NCh (Zhang et al., 2016). Zwitterionic ChNCs containing both $NH_2$ and COOH groups can be synthesized through partial deacetylation, TEMPO/NaClO/NaBr oxidation, and high-pressure waterjet treatment, facilitating their dispersion in both acidic and basic environments. Nevertheless, the alkaline conditions (pH 10) in this system frequently lead to significant depolymerization, resulting in only ~35 % ChNCs. To address this challenge, the TEMPO/$NaClO_2$/NaClO system was established, functioning under mild acidic conditions (pH 6.86), which reduces side reactions and enhances yield (~85 %) (Jiang et al., 2018; Pang et al., 2017). This method utilizes NaClO and $NaClO_2$ as oxidants, thereby removing the necessity for NaBr and enabling effective ChNF preparation at 60 °C.

A novel eco-friendly $O_2$/laccase/TEMPO system has been developed for the fabrication of ChNC through chitin oxidation. Laccase, an oxidase containing copper, facilitates the one-electron oxidation of substrates in the presence of oxygen, whereas TEMPO serves as a mediator, converting C6-OH groups into carboxylates. Chitin suspended in acetate buffer (pH 6.8, 30 °C) underwent treatment with laccase, which adhered to chitin through electrostatic interactions. The oxidized chitin was subsequently subjected to ultrasonication to produce ChNCs. This method effectively maintains the crystallinity of chitin as well as its degree of N-acetylation (Jiang et al., 2018). A gentle, energy-efficient approach has been established to produce hairy ChNCs without the need for mechanical treatment. The sequential TEMPO oxidation of partially deacetylated chitin resulted in the formation of nanorods characterized by protruding molecular chains and a high carboxyl content of 2.72 mmol $g^{-1}$. This structure has been reported to notably

**Table 2**
Nanochitin production following Deep Eutectic Solvent hydrolysis and applications.

| Nanochitin | Method | Composition of DES | Crystal size | Applications | References |
|---|---|---|---|---|---|
| **ChNFs** | Deep eutectic solvent (DESs) | Choline chloride and Thiourea | Diameter 20–30 nm | Used to prepare calcium alginate bio-nanocomposite gel beads, as sustained anticancer drug | Mukesh et al., 2014 |
| **ChNCs (shrimp shell)** | DESs + Ultrasonication treatment | Choline chloride and Zinc chloride | 20 - 80 nm in width and 100 - 700 nm in length | Served both as the non-volatile solvent and as the catalyst for the O-acetylation of chitin. | Hong et al., 2019 |
| **ChNCs (commercial chitin)** | DESs | p-toluenesulfonic acid and Choline chloride | 12–44 nm in width and 206–399 nm in length | Enzyme carriers for the porcine pancreas lipase immobilization, exhibited high catalytic performance and stability (PPL@ChiNC) | Cao et al., 2019 |
| **ChNCs (crab shell)** | DESs + Ultrasonication treatment | Choline chloride with oxalic acid dihydrate/ lactic acid/ malonic acid/citric acid monohydrate/ DL-malic acid | Average diameter and length ranged from 42 - 49 nm and from 257 - 670 nm | Used as an acylation reagent and produce very high mass yield ranging from 78 % to 87.5 % | Yuan et al., 2020 |
| **ChNCs (shrimp shell)** | DESs + Ultrasonication with low energy | Ferric chloride hexahydrate and Betaine chloride (1:1) | Diameter of 10 nm and length of 268 nm, and a crystallinity of 89.2 % | Efficient emulsion stabilizers and yield of approximately 88.5 % | Hong et al., 2020 |
| **ChNWs (*Littorina littorea*/ Periwinkle shells)** | DESs + Microwave irradiation + Ultrasonication | Choline chloride and Oxalic acid (2:1) | 20 nm in diameter and 230 nm in length | Used as a nanocomposite in synthesis of green nanobiofilm | Udayakumar & Anguraj, 2025 |
| **ChNCs (Phosphorylated chitin)** | DESs + Ultrasonication | Urea Phosphate + Urea | Average length and width ranged from 166.2 - 185.6 nm and from 6 - 13.4 nm; CI- 76.8 - 81.8 | Could be used for packaging, insulation, and flame-retardant materials | Zhao et al., 2025 |

**Table 3**
Nanofibrillation employing coalition of oxidation method with top-down approaches.

| Nanochitin | Method | Key Findings | Size | Applications | References |
|---|---|---|---|---|---|
| **Zwitterionic ChNCs** | TEMPO/NaClO/NaBr + Partial deacetylation + high -pressure waterjet | Amino and CC- 1.26 and 0.45 mmol $g^{-1}$ and 38 % yield | 10 nm width and 250 nm length | | |
| **ChNWs** | TEMPO/NaClO/NaBr | – | 33 nm width and 317 nm length | ChNWs-supported proton exchange membranes for fuel cell applications | Zhang et al., 2016 |
| **Zwitterionic ChNFs** | Partial deacetylation + TEMPO oxidation TEMPO/$NaClO_2$/NaClO + Ultrasonication | Degree of deacetylation-15 %; Yield 85 %; Amino and Carboxylate Content (CC)- 0.683 mmol $g^{-1}$ and 0.166 mmol $g^{-1}$ | Widths (5–10 nm) and lengths (200–400 nm | Pickering Emulsifier | Pang et al., 2017 |
| **ChNCs** | TEMPO/NaClO/NaBr and APS oxidation method | Amino and CC - 0.71 and 1.42 mmol $g^{-1}$; Degree of oxidation- 0.12 and 0.24 | Average length (TEMP0) 676 ± 13 nm and (APS) 486 ± 52 nm | Strong antibacterial activity against *E. colil;* reinforcing agent for food packaging films | Oun & Rahim, 2018 |
| **ChNFs** | TEMPO/NaClO/NaBr | – | Needle-shaped CtNFs with a nearly uniform size | Magnetic chitin nanofiber composite, support for enzyme immobilization | Huang et al., 2018 |
| **Amphoteric ChNCs** | TEMPO/$NaClO_2$/NaClO + Partial deacetylation + Ultrasonication | Amio and CC- 1.18 and 0.54−0.58 mmol $g^{-1}$; Degree of deacetylation- 22.8 %; Yield > 94 % and CI > 90 % | Rod like NCs, ~10 nm width and ~544 nm length | – | Jiang, Yu et al., 2018 |
| **ChNCs** | $O_2$/laccase/TEMPO + Ultrasonication | Yield >95 %; CC- 0.43 mmol/g; CI- 93 %, | Rod-like NCs, 24 ± 17 nm width and 480 ± 200 nm length | – | Jiang, Ye et al., 2018 |
| **ChNCs** | Electron Beam Irradiation + high preesure homogenization | Yield 81 %, CC-0.19–0.27 mmol $g^{-1}$, CI – 84 to 94 % | 16–12 nm uniform width and 608–259 nm length | Adsorbents for the removal of heavy metals and dyes during wastewater treatment. | Lee et al., 2023 |
| **Zwitterionic nanochitin** | Phosphorylated partial deacetylation + Carboxylation + TEMPO oxidation | Yield 54.7 %; Charge content- 2.5 mmol $g^{-1}$; Degree of phosphorylation −14; Degree of deacetylation-0.26 and CI- 77.5 %. | 134.09 nm to 885.76 nm length and 18.26 nm to 57.15 nm diameter | Antibacterial activity (*E.coli*) and Pickering Emulsifier | Li et al., 2025 |
| **Hairy (HChNCs)** | Partial deacetylation + TEMPO oxidation | CC-2.72 mmol/g CI- 67 % | 166.04 ± 33.06 nm in length and 13.90 ± 2.77 nm in width | Removal of methylene blue (MB) dye (Absorbing capacity 909.11 ± 17.44 mg $g^{-1}$ of MB) | Ding et al., 2025 |

enhance the efficiency of methylene blue adsorption (Ding et al., 2025).

*4.1.3.2. Electron beam irradiation.* Electron beam irradiation (EBI) is an oxidation technique that cause electrosatic repulsion between the carboxylate ion formed on the C6 position of N-acetylglucosamine unit in α-chitin. It has been employed across various industries to augment the physical and chemical characteristics of materials by chain scissioning of the β (1→4) linkage, as well as mitigating the presence of undesirable contaminants. It has distinctive merits over other techniques, including ease of process (via exposure to an electron beam), mild operating conditions (atmospheric pressure, room temperature), short treatment duration, and minimal environmental impact. Consequently, EBI offers the advantage of reducing the number of processing stages and energy consumption. EBI also facilitates the generation of free radicals via two mechanisms: the removal of hydrogen from a glucose moiety and the cleavage of glycosidic bonds, which is subsequently followed by another degradation process. Following this, the free radicals induce the degradation of sample through either chain scission or oxidation. At doses below 10 kGy, irradiation may induce a cross-linking reaction instead of chain scission. However, at higher doses, chain scission becomes more prevalent, likely attributed to the cleavage of glycosidic bonds (Lee et al., 2018).

A novel, practical and complete eco-friendly approach was reported first time to extract ChNCs and produce dehydrated NCh with the purpose for diverse applications in the commercial sector. The procedure encompassed the separation of solid-state α-chitin through oxidation and chain scissioning utilizing EBI system with different dosages (1000, 2000 and 3000 kGy), followed by fragmentation via high-pressure homogenization (HPH) (25 000 psi for 2 cycles) at pH 11 (Fig 5). The resulting mixture was then neutralized using $CO_2$ bubbling, subjected to a final spray-drying process to obtain an aqueous ChNC suspension and resulted into formation of ChNC-E1000 with anionic carboxylate groups. The process showed high productivity, transparent redispersion comparable aspect ratio, carboxylate ion and crystallinity index with respect to ChNC-TEMPO/NaClO/NaBr and ChNC—HCl hydrolysis. EBI-induced ChNCs were reported as effective adsorbents and potentially be

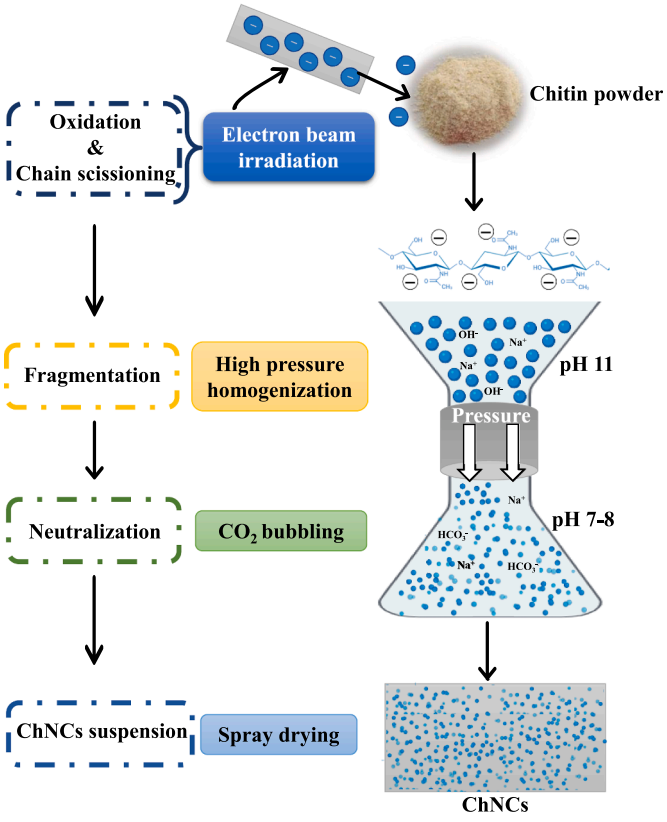


**Fig. 5.** Chitin nanocrystal generation by electron beam irradiation and high-pressure homogenization.

employed in the adsorption of organic cationic dyes and heavy metals viz. iron and copper, by the formation of self-standing hydrogels (Lee et al., 2023).

#### 4.1.4. Partial deacetylation

The partial deacetylation of α-chitin results in an increase in C2 primary amino groups, which enhances cationic charge density and promotes electrostatic repulsion, thereby improving fibril individualization in acidic conditions. To prevent alkali-induced depolymerization, α-chitin underwent treatment with NaOH and $NaBH_4$, followed by heating at 90 °C to facilitate partial deacetylation (Fan et al., 2010). Several studies implement this step following TEMPO oxidation to generate zwitterionic NCh characterized by both cationic and anionic surface charges (Jiang et al., 2018; Pang et al., 2017). For instance, zwitterionic NCh is generated through a process that involves phosphorylation, partial deacetylation, and subsequent carboxylation using TEMPO-mediated oxidation. The resulting zwitterionic NCh exhibiting high crystallinity (77.5 %) and has been observed to possess both the anionic (-$PO_3^{2-}$) and cationic (-$NH^{3+}$) groups, with a phosphorylation and deacetylation degree of 0.14 and 0.26 (Li et al., 2025). Partially deacetylated NCh obtained from waste crab shells demonstrates cationic properties and antibacterial activity under acidic conditions; however, their efficacy has been observed to reduce under neutral to basic pH environments. Methylated NCh is a potential solution to the aforementioned concern; which could be produced by chemical modification through N-trimethylation and possess stable positive charge attributed to the quaternary ammonium groups. Methylated NCh has been illustrated to exhibit consistent dispersibility and robust antibacterial activity against *Staphylococcus aureus* across a wide pH spectrum. The improved stability and functionality facilitate their prospective applications in cosmetics and health food sectors (Kawamoto et al., 2025).

#### 4.1.5. Microwave irradiation

Microwave-assisted extraction (MAE) improves product yield by facilitating synergistic heat and mass transfer, allowing for rapid and uniform heating throughout the sample. Polar solvents absorb electromagnetic energy, leading to dipole alignment, friction, and collisions that increase temperature and enhance extraction efficiency. In contrast to traditional methods, MAE delivers volumetric heating, which presents benefits including reduced processing time, enhanced efficiency, consistent results, minimized solvent usage, and a lower environmental footprint (Rodrigues et al., 2020). The extraction of chitin and/or chitosan using MAE was reported by several researchers. Significant reduction in the duration required for the extraction of chitin (α and β) and chitosan has been reported (from 6–10 h to 10–15 mins). Additionally, an experimental design was presented in which the demineralization process was optimized to extract α-chitin from lobster. This was achieved by subjecting the lobster to microwave irradiation for 23 mins. MAE has been illustrated as an eco-friendly sustainable alternative for the isolation of α-ChNCs from shrimp and yellow lobster and β-ChNFs from squid pens. For lobster, shrimp, and squid, the resulting isolation yields were 85.30, 79.92, and 80.59 % and length of NCh was 314.74, 386.12, and $>$ 900 nm, respectively, by utilizing less concentration of HCl (Fernández-Marín et al., 2021).

#### 4.1.6. Mechanical treatment

Chitin microfibers are linked through intra-molecular hydrogen bonds, and mechanical shear forces are utilized to longitudinally separate them into thin filaments. Mechanical treatments commonly

**Table 4**
Mechanical disintegration for production of nanochitin.

| Nanochitn | Method | Number of cycle/ passes/others | Morphology | Applications | References |
|---|---|---|---|---|---|
| **ChNFs (crab shell)** | Star burst system HPWJS | | 10–20 nm thickness | Active elicitor in *A. thaliana* and rice | Egusa et al., 2015 |
| **N-halamine ChNFs** | Star burst system, HPWJS | 30 cycle | | Antibacterial and antifungal activity | Dutta et al., 2015 |
| **ChNFs (Squid pen, β chitin)** | Grinding and Starburst HPWJ system | | Average diameter 20 nm; Aspect ratio–150–200; Zeta potential −25 mV; CI-80 % | Hydrogel as reinforce agent for Bone regeneration material | Kawata et al., 2016 |
| **Protein/CaCO3 /ChNFs complex (*Chionoecetes opilio*; red snow crab)** | Starburst HPWJ system | 1,5,10.30,50 cycle | | Plant fertilizer tested on tomato (Solanum lycopersicum) in hydroponic condition | Aklog et al., 2017 |
| **ChNFs (red snow crab shell)** | Grinding + Star burst system HPWJS | 5, 50 passes | | Antifungal activity in cabbage and strawberry | Parada et al., 2018 |
| **ChNFs (*Paralithodes camtschaticus*; Red king crab)** | Grinding + HPWJS | 5, 50 passes | 7 to 6 nm thickness | Control fusarium wilt diseases in tomato | Egusa et al., 2019 |
| **ChNWs (shrimp and crab shell)** | High Pressure homogenisation | – | Length 800 nm and width 10–20 nm | Poly hydrogels (vinyl alcohol) for drug delivery | Peng et al., 2019 |
| **ChNFs (commercial chitin)** | ACC HPWJ system | 60 cycle | Average length 2300 ± 1000 nm and width 10 ± 6 nm and | Enhanced propertiesof Pickering emulsifiers | Ishida et al., 2021 |
| **ChNFs (crab shell)** | ACC HPWJ system and TEMPO oxidation | Carboxylate content 0.67–0.79 mmol/g 30 cycle | Average length 492.42 ± 206.44 nm | Improved stodage modulus of ACC CtNFs based hydrogel | Ye et al., 2021 |
| **Nanochitin suspension (Crab shell)** | Partial deacetylation + High Pressure homogenisation | | Diameter of 10 ± 5 nm and lengths ranging from hundreds of nanometers to several micrometers | Preparation for flexible 3D porous air- dried xerogels via Pickering foam | Liu et al., 2022 |
| **Zwitterionic modified nanochitin (Crab shell)** | Partial deacetylation + High Pressure homogenisation (modified by Schiff base reaction) | | 300 and 500 nm length | Tea tree oil-in-water Pickering Emulsifier and antibacterial activity against *E. coli* and *B. subtilis.* | Li et al., 2023 |
| **Nanochitin suspension (Crab shell)** | Partial deacetylation + High Pressure homogenisation | Amino group content 16.5 % | $<$ 400 nm length | Formation of nanochitin aerogels for the remediation of oil spills | Liu et al., 2024 |
| **Nanochitin suspension (Crab shell)** | Partial deacetylation + High Pressure homogenisation + Ultrasonication | | length: ~ 260 nm and width: ~ 16 nm | Fabrication of composite cryogel for pickering emulsifier | Guo et al., 2024 |

employed encompass grinding, ultrasonication, high-pressure homogenization, and water-jet systems, which may be utilized separately or in conjunction, generally under acidic or neutral conditions (Table 4).

*4.1.6.1. Grinding.* Grinding serves as an efficient technique for generating uniform NCh through the mechanical disintegration of crystalline chitin, utilizing counter-rotating grinding stones that exert shear forces. The pulp is processed between static and rotating stones at a speed of 1500 rpm, which effectively reduces structural complexity (Missoum et al., 2013). The procedure may be conducted under either neutral or acidic conditions, contingent upon the source of chitin. Grinding is commonly employed to extract ChNFs by initially eliminating proteins and minerals, succeeded by acid-assisted fibrillation (Ifuku et al., 2010). Uniform α-ChNFs (10–20 nm width, high aspect ratio) have been successfully derived from crab and shrimp shells utilizing this straightforward method (Ifuku et al., 2009). Dried ChNFs exhibited properties comparable to those of never-dried variants and are available in the market as economical fertilizers. Dry chitin, whether treated with or without 1 % acetic acid, provides benefits in terms of preservation, transport, and scalability. ChNFs of comparable dimensions were also obtained from black tiger prawn shells under neutral conditions, necessitating less effort owing to the prawn's finer structure (Ifuku et al., 2011).

ChNFs were obtained through the grinding of crab shell chitin at 1800 rpm for 20 cycles, resulting in fibers with diameters ranging from 20 to 100 nm (Li et al., 2017). Utilizing 1 % acetic acid (pH 3–4) during the grinding process resulted in the production of ChNFs approximately 20–30 nm in width (Chen et al., 2018). In contrast, NaOH-deacetylated chitin, ground at 1500 rpm with acetic acid (pH 4), produced ChNFs measuring around 5.4 ± 1.1 nm (Riehle et al., 2019). While acidic conditions promote fibrillation, their suitability for sensitive applications such as biomedicine and electronics presents a significant challenge.

*4.1.6.2. Ultrasonication.* Ultrasonication serves as a highly effective method for nanofibrillation, facilitating the reduction of particle size and polydispersity through the disruption of aggregates formed by hydrogen bonding. The process operates via cavitation, which involves the formation and subsequent collapse of voids in water. This phenomenon generates shockwaves that disrupt interfibrillar hydrogen bonds and van der Waals forces, effectively transforming microfibers into nanofibers (Lu et al., 2013). The energy released during cavitation, ranging from 10 to 100 kJ/mol, is comparable to the strength of hydrogen bonds (Tischer, Sierakowski, Westfahl, & Tischer, 2010). Ultrasonication, whether used independently or in conjunction with acid hydrolysis, DESs, or TEMPO oxidation, demonstrates efficacy in disrupting interfibrillar hydrogen bonds in chitin. Squid-pen nanofibrils started to individualize within 5 min, achieving complete fragmentation after 80 min (480 nm length). However, extending sonication up to 400 min resulted in no additional changes. In contrast, tubeworm chitin, characterized by a high degree of N-acetylation (0.99) and a low surface charge, demonstrated resistance to dispersion. The application of TEMPO oxidation prior to treatment facilitated dispersion in just 5 min. The source material, in conjunction with ultrasound duration and intensity, significantly impacts NCh properties (Bamba et al., 2017). Zou et al. (2018) synthesized stable ChNFs from chitin gel utilizing low intensity ultrasonication (100 W, 20 kHz, pH 4). The gelation process diminished hydrogen bonding, while ultrasound effectively disrupted van der Waals and hydrogen bonds. In contrast, Lu et al. (2013) generated approximately 20 nm α-ChNFs from dried prawn shells through high-intensity ultrasonication (300 W, 60 kHz, pH 7), noting a rise in crystallinity from 60.1 to 65.8 %.

*4.1.6.3. High-pressure homogenization (HPH).* HPH utilizes a high-pressure reciprocating pump as a means of power transmission and material conveyance to transport materials to the working valve. HPH employs a reciprocating pump to drive material through a valve, generating shear, impact, and cavitation, which leads to the production of fine ChNFs (Yang et al., 2020). Utilizing mildly acidic crab α-chitin, uniform ChNFs (~20 nm diameter) were generated, exhibiting favorable rheological properties; however, there was no significant network formation (Wu, Zhang, Girouard, & Meredith, 2014). In a similar manner, ChNFs (<100 nm width, high aspect ratio) were obtained from yellow lobster waste through the processes of Ultra-Turrax homogenization and ultrasonication, subsequently followed by dynamic HPH (Salaberria et al., 2014). The α-ChNWs having 10–50 nm width and 800 nm length were extracted from the shell flakes of crabs and shrimp. The networks were effectively created through a series of chemical and physical cross-linking processes. The process involves the utilization of salts, which are subsequently subjected to partial deacetylation in a solution containing NaOH (33 % wt). Homogeneity was achieved by applying high pressure in the process (Peng et al., 2019). NCh was produced through the process of mechanical grinding and high-pressure homogenization, yielding ChNF with a width ranging from 80–120 nm and a length exceeding 1 μm (Liu et al., 2018). A novel technique has been proposed for the synthesis of ChNFs derived from the exoskeleton of lobster shells involves the utilization of chitosan at a concentration of 10 wt. % as a sacrificial polymer. The incorporation of chitosan into the initial chitin colloidal suspension, followed by high pressure homogenization at a pH of 3, resulted in a notable decrease in the aggregation of ChNFs (Mushi et al., 2018).

*4.1.6.4. High-pressure water-jet system (HPWJ).* High-pressure waterjet (HPWJ) systems introduce fiber suspensions (10–100 μm) into valve bodies, creating high-speed shear and a significant pressure gradient that triggers fibril breakdown. This treatment results in the partial disintegration of crab shells, exposing nanofibers when subjected to strong mechanical force. Subsequent HPWJ cycles further diminish fiber width through the fibrillation of protein/chitin bundles (Yang et al., 2020).

The Aqueous Counter Collision (ACC) method is a water-jet-based technique that operates without chemicals to defibrillate α-chitin into nanofibers. The process entails the collision of high-speed aqueous jets (50–270 MPa), resulting in the production of nanoscale chitin fibers dispersed in water. ACC has produced 10–20 nm ChNFs characterized by robust network structures (Kose & Kondo, 2011). TEMPO-oxidized α-chitin, subjected to 30 ACC passes, demonstrated improved fibrillation with an approximate length of 492 nm (Ye et al., 2021). In contrast, nano-pulverization at 200 MPa for 60 cycles yielded ChNFs with a width of 10 ± 6 nm and a length of 2300 ± 1000 nm (Ishida et al., 2021).

The Starburst (SB) system utilizes HPWJ technology, employing a hydraulic piston to expel chitin slurry at 245 MPa through a narrow nozzle. This process involves a collision with a ceramic ball, resulting in the formation of nanoscale fibers. Ifuku et al. (2012) successfully produced 16.5 nm ChNFs from dry chitin following 10 passes at pH 3. The optimization of fiber length and thickness was achieved through a maximum of 10 passes, with breakage occurring at 30 passes (Ngasotter et al., 2022). Protein/$CaCO_3$/ChNF complexes were extracted from red snow crab shells (Chionoecetes opilio) utilizing 1–50 cycles at 200 MPa, with a reduction in fiber width observed with increasing passes (Aklog et al., 2016). The implementation of grinder pretreatment significantly improved nanofiber yield, leading to a reduction in SB treatment cycles and associated costs (Aklog et al., 2017). Furthermore, 20 nm ChNFs were generated from β-chitin squid pens through grinding and SB-HPWJ at an acidic pH 3 (Kawata et al., 2016).

### 4.2. Bottom-up approach

The ChNFs are crucial for providing toughness to naturally existing materials. The bottom-up approach is based on electrospinning and self-

**Table 5**
Bottom-up approaches, including electrospinning and self-assembly for nanostructures.

| Nanochitin | Method | Requirement | Size | Applications | References |
|---|---|---|---|---|---|
| **Chitin nanofibrous microspheres** | Self-assembly and thermal induction | | diameter of nanofibers 26 to 55 nm and size of microspheres 3 to 130 mm | Increase attachment efficiency for 3D cell microcarriers. | Duan et al., 2014 |
| **ChNFs** | Multi needle Electrospinning | Use ionic liquid IL [$C_2$mim][OAc] | average diameter of 22 ± 7 nm | | Shamshina et al., 2017 |
| **ChNFs** | Electrospinning of β-chitin from cuttlefish bone | Use Polyethylene oxide (PEO) as a carrier | diameter of about 400 nm | Wound healing | Jung et al., 2018 |
| **CP/PEO nanofiber mats** | Electrospinning using chitin propionate with PEO | Green solvent ethanol and water | diameters 0.6 μm to 1.2 μm | Fluid filtration of fibrous pollutants. | Zhong et al., 2021 |

assembly for the nanofiber fabrication (microscopic to macroscopic) where nanofibers are constructed or assembled by gradually building up to the desired structure at the nanoscale level (Table 5).

### 4.2.1. *Electrospinning*

Electrospinning is a sophisticated electrodynamic technique that generates continuous nanofibers by applying an electric charge to a liquid droplet, transforming it into a jet that stretches and solidifies into fibers. The setup includes a syringe pump, a high-voltage power source, a spinneret, and a collector. The procedure encompasses: (i) the formation of the Taylor cone, (ii) the extension of the jet, (iii) the occurrence of bending/whipping instability, and (iv) the solidification and collection of fibers (Xue et al., 2019). The solvent system is essential in the processes of polymer solvation and jet transport/vaporization. Electrospinning of pure chitosan fibers has been conducted utilizing solvents such as acetic acid, formic acid, and trifluoroacetic acid (Ohkawa et al., 2004).

Fig. 6 depicts the electrospinning of pure β-chitin using polyethylene oxide (PEO) as a carrier polymer instead of formic acid. PEO induced the chain flexibility with improved entanglement by decreasing the charge repulsion between the β-chitin molecular chains. Electrospinning was performed with a 10 cm distance between the needle and the collector. The electrospun nanofibers were obtained using a syringe pump equipped with a 21-G needle, which delivered the polymer solution at a flow rate of 0.8–1.2 mL/h. A high voltage supply of 21 kV was applied, and the collector plate was wrapped with Teflon to facilitate the easy removal of the nanofiber web. Each nanofiber was created at 23 °C and between 50 % and 65 % relative humidity. Lastly, nanofiber web was

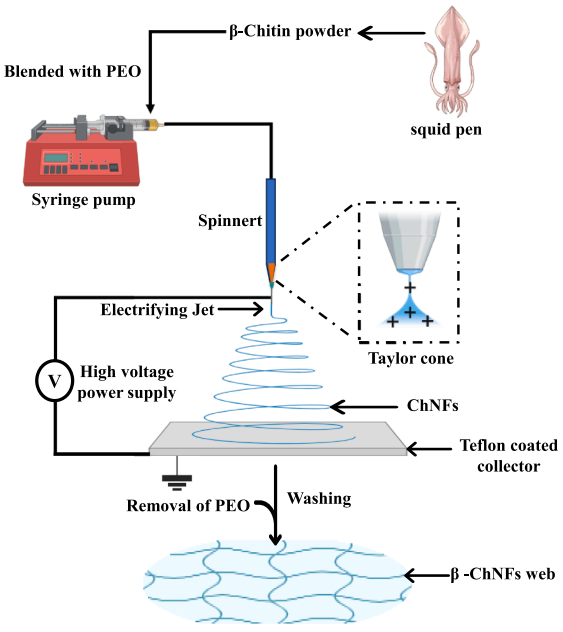


**Fig. 6.** β-ChNFs web preparation by electrospinning.

washed with deionised water till complete removal of PEO. The resulting β-chitin/PEO nanofibers were approximately 400 nm in length, which was compressed after being soaked in water to strip the PEO (Jung et al., 2018). Barber et al. (2013) have reported that ChNFs were electrospun from shrimp shell extract (2 %) dissolved in IL 1-ethyl-3-methylimidazolium acetate [$C_2C_1$Im] [OAc]. The study showed a one-pot method for producing high-surface-area ChNFs from biomass. The first successful electrospinning of chitin from shrimp shell/IL solution without auxiliary solvents showed a viable process via using a renewable feedstock. Another study on multi-needle electrospinning of chitin using IL 1-ethyl-3-methylimidazolium acetate [$C_2$mim] [OAc] has directed to scale up biopolymer electrospinning to practical volumes. The method produced ChNFs with an average diameter of 22 ± 7 nm (Shamshina et al., 2017). Application of green solvents (ethanol and water) in electrospinning with PEO as a co-spinning aid has been reported to in the produce nanofibers and nonwoven mats from chitin propionate (CP). Additionally, electrospun CP/PEO nanofiber mats showed excellent thermal stability, mechanical strength and hydrophobicity having 133-degree contact angles with water (Zhong et al., 2021).

### 4.2.2. *Self assembly*

Self-assembly is a facile bottom-up strategy for the construction of helical and further hierarchical structures by the dispersion of chitin in a specific solvent. Chitin is primarily dissolved in hexafluoro-2-propanol (HFIP) or LiCl/N, N-dimethylacetamide (DMAC) solvents to disrupt hydrogen bonds. Subsequently, either solvent evaporation (HFIP) or the addition of water (LiCl/DMAC) has been reported to initiate the self-assembly, which resulted in the production of superior ultrafine nanofibers. The study has illustrated the generation of complex self-assembling nanofibers from 10 nm (LiCl/DMAC) to 3 nm (HFIP) for individual nanofibrils. The self-assembly of chitin into nanofibers was observed to be impinged the concentration of chitin and water content (Zhong et al., 2010). Recently, the dissolution of β-chitin in an aqueous solution of KOH/urea was found to be more pronounced in contrast to α-chitin. Absence of inter-sheet hydrogen bonding and loose crystal packing structure of β-chitin facilitated the rapid infiltration of solvent molecules that resulted into swelling and dissolution of β-chitin which leads to the formation of β-chitin/KOH/urea complexes. In the presence of a non-solvent, chitin chains formed twisted nanofibers in energetically stable α-chitin crystals. The self-assembly process became more effective with corresponding increase in the chitin concentration, resulting in solution instability and transforming the nanofibrous network into the macroscopic chitin hydrogel (Huang et al., 2021). Chitin dissolution and self-assembly are crucial for sustainable chemistry and biomedical applications due to the exceptional mechanical properties, biocompatibility, biodegradability, and reproducibility.

A novel self-assembly technique utilizing calcium-saturated methanol facilitated the creation of 1D chitin nanowires. Calcium ions partially substituted for interfibrillar hydrogen bonds, facilitating dispersion while preserving the integrity of acetyl groups. The process of solvent exchange utilizing methanol, isopropanol, and water was employed to eliminate $Ca^{2+}$ ions and finalize the disassembly (Oh et al., 2016).

*4.2.3. Dissolution-regeneration method*

Wet spinning is a prevalent technique for the fabrication of ChNFs by the dissolution and subsequent regeneration of chitin by using precipitating agent (Yang et al., 2020). The process produces ChNFs with elevated aspect ratios; however, the spinning efficiency is contingent upon the concentration of the solution and gelling rate (Chee et al., 2024). High concentrations gel rapidly, whereas low concentrations coagulate at a slower rate (Huang et al., 2014). Zhang et al. (2024) formulated an alkaline ternary DESs comprising amino acids, 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU), and urea for the extraction of chitin nanofibers. DBU captures protons from the amino acids, producing ions that interfere with the hydrogen bonds of chitin. Urea facilitates dissolution through hydrogen bonding interactions with chitin. This system inhibits chitin aggregation and facilitates the formation of nanofibers. The broader application is constrained by the limited availability of effective chitin solvents, underscoring the necessity for environmentally friendly alternatives.

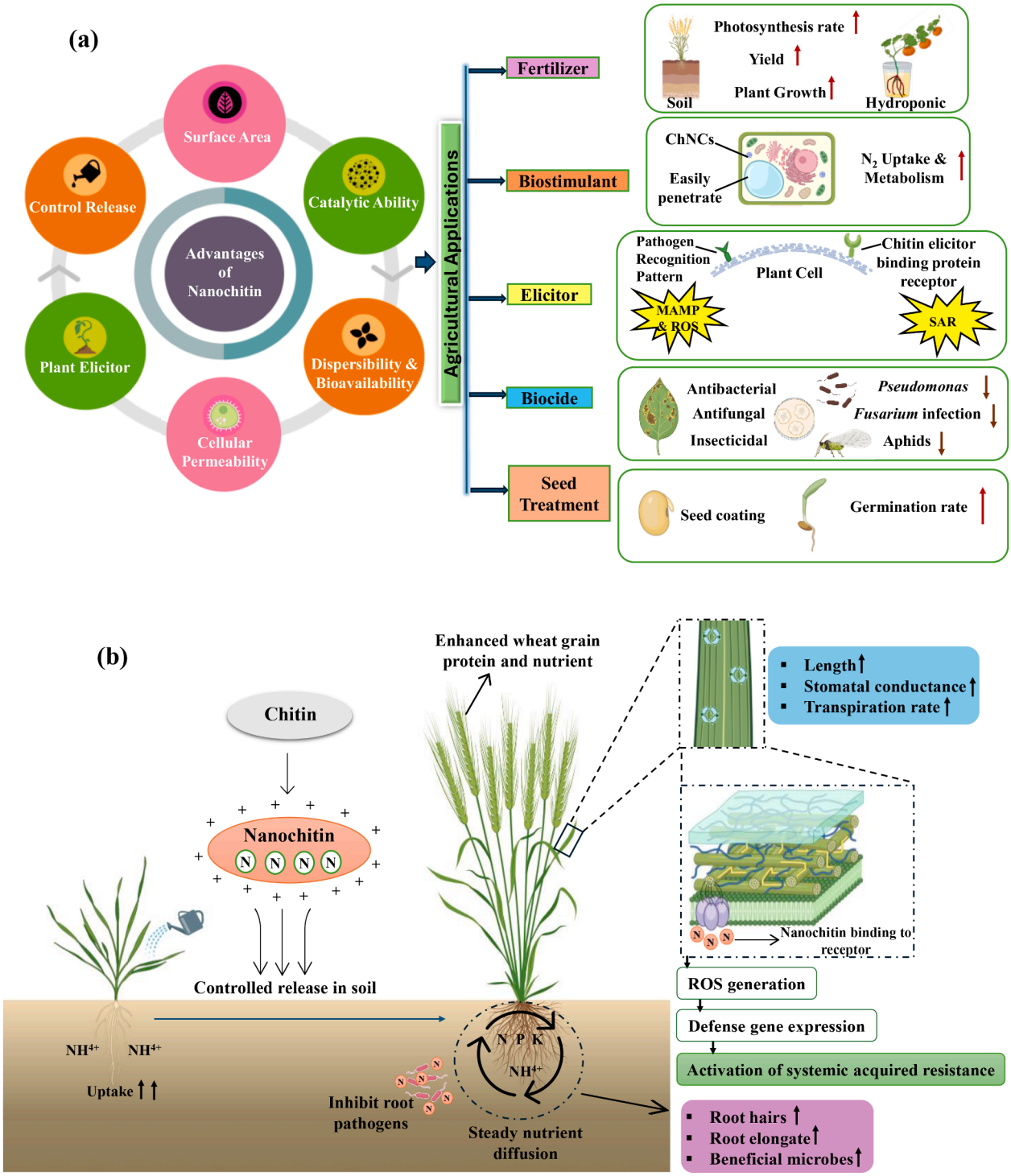


**Fig. 7. a)** Nanochitin primacy towards sustainable agriculture, **b)** Schematic representation of plant growth augmentation by nanochitin application.

## 5. Agricultural applications

The increasing demand for organic alternatives can be attributed to stringent regulations and growing concerns regarding the health and environmental effects of agrochemicals. Sustainable agriculture encourages the utilization of environmentally friendly compounds. Chitin and its derivatives provide effective natural solutions for promoting plant growth (Karamchandani et al., 2024). Heretofore, chitin has exhibited limited applicability in agriculture owing to its high crystallinity and insolubility in water and other conventional solvents. Water-soluble chitin derivatives i.e. NCh, chitosan, or chitin oligosaccharides have appeared as promising substitutes for chitin in agricultural contexts. Despite fewer polycationic charges than chitosan, NCh has demonstrated significant biological activities along with sustaining the core properties of chitin while exhibiting distinctive colloidal behavior as a bionanomaterial (Zhang et al., 2022). Chitin stands out among natural polymers due to its nitrogen content, which varies from 6.1 - 8.3 % and is present in the form of amino groups (Yen & Mau, 2007). NCh functions as a slow-release nitrogen source in soil, where it is degraded by microbes to provide a steady release of nutrients. The biopolymer composition reduces leaching and guarantees a steady supply of nutrients. Furthermore, the presence of calcium in chitin contributes to the enhancement of plant growth (Ravindran et al., 2025; Sharp, 2013). NCh functions as a plant elicitor by activating natural defense mechanisms, regulating hormonal balance, enhancing nutrient absorption, and increasing stress resilience (Shamshina et al., 2020). It possesses enhanced properties as compared to chitin, functioning effectively as a foliar spray or soil additive, thereby promoting growth and seed germination both as a fertilizer and biostimulant (Fig 7a and b). NCh utilized in agriculture is typically sourced from shrimp shells (Xue et al., 2017), crab shells (Egusa et al., 2020), or spent mushroom substrates (Li et al., 2022). Solubility of NCh has significantly augmented its employment in several agricultural applications (Table 6). However, systematic research on NCh as a comprehensive bionanomaterial in agriculture is still in progress.

### 5.1. Fertilizers

Fertilizers provide vital nutrients, including NPK along with micronutrients. Chitin, a biopolymer rich in nitrogen and characterized by a low carbon-to-nitrogen (C/N) ratio (~ 6–7), decomposes swiftly to release these essential nutrients (Shamshina, 2020). The incorporation of this material into soil fosters the growth of chitinolytic microbes, thereby improving nitrogen and calcium levels in the soil (De Tender et al., 2019; Zhan et al., 2021). Tomato plants that received treatment with protein/$CaCO_3$/ChNFs (0.01 %) exhibited enhanced leaf growth, height, and stem diameter (Aklog et al., 2016). A combination of chitin and selenium enhanced phosphorus uptake by 33.3 % and decreased leaching by 29.11 % (Tang et al., 2022).

Researchers treated two winter wheat varieties, multi-spike wheat (MSW) and large spike wheat (LSW), with 0.006 $gkg^{-1}$ of NCh and found significant improvement in their growth by 23.0 % (MSW) and 33.4 % (LSW). NCh treatment also enhanced wheat grain protein, iron, and zinc by 5.0, 10.3, and 22.1 % for MSW and 33.4, 32.0, and 27.0 % for LSW. It augmented the winter wheat grain yield by increasing the flag leaf's transpiration rate during the grain-filling stage, stomatal conductance, intercellular $CO_2$ concentrations, and net photosynthesis rate (Fig 7a) (Xue et al., 2017).

Researchers examined the impact of ChNFs fertilizer and application methods on komatsuna (*Brassica rapa* var. *perviridis* cv. Saori) plant (a Japanese mustard spinach) grown in Entisols (sandy soil) and Andosols. Komatsuna was treated with 0.01, 0.05 and 0.10 % (chitin concentration) solutions of ChNFs, ChNFs/protein, and ChNFs/protein/ $CaCO_3$, respectively. The result indicated that the application of ChNFs to each soil surfaces promote plant growth due to the positive impact of $CaCO_3$ and protein encapsulation as calcium encased ChNFs was easily absorbed by plants (Endo et al., 2023). ChNCs (0.005 % w/v) mixed with fungicides metalaxyl mancozeb and thiophanate methyl has been observed to support tobacco plant growth by increasing stem length and girth as well as leaf area and number (Zhou et al., 2017).

**Table 6**
Application of nanochitin in plant growth promotion and other bioactivities.

| Plant name | Nanochitin treatment | Result | References |
|---|---|---|---|
| ***Arabidopsis thaliana*** | ChNFs (0.1 mg $mL^{-1}$) | Reduction of bacterial infection and necrotic lesion on leaves, caused by *Pseudomonas syringae* pv. tomato DC3000 and *A. brassicicola* pathogen | Egusa et al., 2015 |
| **Tomato** | Protein/CaCO3/ chitin nanofiber treatment with chitin concentration (0.01 %) | Remarkable leaf age growth, plant height and stem diameter | Aklog et al., 2017 |
| **Multi-spike wheat (MSW) and large spike wheat (LSW)** | 0.006 g $kg^{-1}$ of nanochitin | Increase growth by 23.0 % (MSW) and 33.4 % (LSW) and wheat grain protein, iron, and zinc by 5.0 %, 10.3 %, and 22.1 % for MSW and 33.4 %, 32.0 %, and 27.0 % for LSW | Xue et al., 2017 |
| **Tobacco plant** | ChNCs suspension at concentration 0.004 % (w/v) combined with fungicides metalaxyl mancozeb and thiophanate methyl | Remarkable improvement in seedling growth and reduction in mean time to seed germination and inhibit tobacco root rot | Zhou et al., 2017 |
| **Wheat** | ChNCs at concentrations of 300 ppm | Reduction in conidial formation of *Fusarium pseudograminearum* and *F. graminearum* by 89.25 % and 82.28 % | Liang et al., 2018 |
| **Cabbage and Strawberry** | ChNFs (0.1 to 1 mg $ml^{-1}$) | Reduction in spot number caused by *Alternaria brassicicola* and lesion size by *Colletotrichum fructicola* | Parada et al., 2018 |
| **Wheat seed treatment** | ChNCs and tebuconazole, at concentrations of 10 ppm and 30 ppm | Crown Rot disease control efficiencies reach to 79.30 % and 90.02 % | Liang et al., 2018 |
| **Wheat** | ChNWs at concentrations below 6 mg $kg^{-1}$ in the soil | Increment 27.56 % and 13.26 % in winter wheat growth and crude protein concentration | Cheng et al., 2019 |
| **Tomato** | ChNFs (0.1 mg $ml^{-1}$) | Reduction in Fusarium wilt diseases caused by pathogen *Fusarium oxysporum f.* sp. *lycopersici* | Egusa et al., 2019 |
| ***Arabidopsis thaliana, Nicotiana benthamiana* and *P. capsici*** | ChNCs suspension about 0.005 % and 1 μM flg22 | Obstruct *Phytophthora* pathogen invasion | Zhou et al., 2020 |
| **Wheat** | ChNWs suspension (30–50 mg $L^{-1}$) | Pest control and cause mortality rate of wheat aphid around 80 % | Li et al., 2021 |
| ***Lotus japonicus* (Leguminous plants)** | ChNFs suspension - 0.01 % (w/v) | Enhancement of shoot growth and *Mesorhizobium loti* MAFF303099 nitrogen fixation | Gonnami et al., 2024 |

### 5.2. Biostimulant

Bio-stimulants are formulations of compounds applied to plant leaves or soil to enhance plant productivity, including improvement in plant growth, yield, quality, and stress tolerance (Shamshina, 2020). Chitin has long been used as a soil amendment or nitrogen fertilizer to boost the crop growth. Chitin and its deacetylated derivative chitosan have been reported to possess growth-promoting properties by rising NPK and phosphorus contents in *Arabidopsis thaliana*, soybean, sweet pepper, sweet basil, strawberry, and cowpea. The stimulatory effects of ChNWs were investigated on accumulation and translocation of dry matter (DM) and carbon-nitrogen metabolism with further detection of wheat grain yield and crude protein concentration. The application of ChNWs has resulted in enhanced wheat nitrogen uptake at concentrations below 6 mg $kg^{-1}$ in the soil and establishes significant increment 27.56 and 13.26 % in winter wheat growth and crude protein concentration respectively. ChNWs's spindle-like structure, cationic property, nano-scale size, and high surface area promotes its uptake by cell in contrst to the bulk chitin. Nitrogen metabolism has reported to be considerably enhanced by NCh in comparison to the carbon metabolism in wheat. C and N metabolism may be enhanced by ChNWs through the upregulation of metabolic enzymes i.e. Sucrose phosphate synthase and phosphoenolpyruvate carboxylase (Cheng et al., 2019).

The incorporation of ChNFs into the soil has resulted in a modest improvement in tomato growth compared to control and those were treated with crushed crab shells (Egusa et al., 2019). Significant rise in nitrogen uptake efficiency has reportedly driven the growth-promoting effects of chitin treatment. ChNFs has emerged as a valuable compound for managing nutrient uptake and allocation in tomato production even in nutrient-limiting hydroponic conditions. The addition of ChNFs has significantly increased tomato plant growth regarding true leaf count, plant height, and shoot dry weight. Transcriptome analysis revealed that genes related to nitrate transporter and assimilation in root, carbon metabolism and photosynthesis were upregulated by ChNFs treatment. The nitrate transporter, known as Solyc08g078950.2 (LeNRT1.1), which shared similarities with the dual-affinity nitrate transporter AtNRT1.1 (also referred to as AtNPF6.3 and AtCHL1), exhibited increased expression in roots treated with ChNF. Tomato roots treated with ChNFs has apparently upregulated an ortholog of Arabidopsis NLP7, a nodule inception protein (Solyc08g008410.2) with unknown function. Additionally, defence related genes (chitinase and NPR1–1 protein) were also upregulated in ChNF treated plant roots and leaves (Egusa et al., 2020).

### 5.3. Plant elicitor

Synthetic elicitors are a class of small molecules with drug-like properties that protect the crops against diseases. These elicitors stimulate plant defence mechanisms, including activating signalling molecules i.e. flagellin, oligosaccharides, dimethyl sulfide, probenazole and salicylic acid (Malik et al., 2020). Nanobiocides could be a viable and sustainable substitute for plant protection against diseases, owing to their elicitor activity that induces plant disease resistance. The efficacy of ChNCs has been studied in combating *Phytophthora* infections as well as the receptors and mechanisms involved in inducing plant defence response. ChNC suspension (0.005 %) and flg22 (1 μM) were applied to *Arabidopsis thaliana, Nicotiana benthamiana* and *P. capsici* isolates. The results revealed that ChNCs have significantly obstruct the pathogen invasion but unable to manage the hyphal growth of pathogen. It has also been observed to induce plant resistance by promoting reactive oxygen species (ROS) generation, eliciting phenylalanine ammonia-lyase (PAL) activity and pathogenesis-related gene (PR) expression, which suggested that ChNC may induce system-acquired resistance (SAR). ChNCs could present a novel microorganisms associated molecular pattern (MAMP) as it induced defence response against cross kingdom oomycete by using a different mechanism than chitin, involving induction of both BAK1 and CERK1 pathway (Zhou et al., 2020). Similarly, ChNFs can induce systemically disease resistance in cabbage and strawberry plants, making it a promising natural material for controlling diseases in cultivated plants. The spot number caused by *Alternaria brassicicola* and lesion size by *Colletotrichum fructicola* over cabbage and strawberry, were reduced by ChNF (0.1 to 1 mg $ml^{-1}$) mixed in soil. Analysis of gene expression in cabbage plants showed a marked upregulation of defense-related genes before and after pathogen infection (Parada et al., 2018). ChNFs act as an active elicitor that decreased pathogen infection in *Arabidopsis* and rice by inducing ROS production and the expression of defence genes in a CERK1 dependent manner (Egusa et al., 2015).

### 5.4. Biocide

According to the US EPA, biocides are defined as substances that can kill living organisms. Biocides encompass a range of substances that are specifically designed to combat various forms of life i.e. fungi, oomycetes, bacteria, viruses, nematodes, and insects (Beakes et al., 2012). Research reports have indicated that NCh particles surpass other bio-based nanoparticles viz. nanocellulose, alginate, and glucan for agricultural applications. Self-assembled networks of short and long nanoparticles offer superior oil/water interface stability at ultralow concentrations and so has potential for future nanopesticide formulation, which could promote diverse biological activities and diminish the usage of chemical fungicides (Bai et al., 2019). A study aimed to examine the potential insecticidal enhancement of ChNWs in combination with Omethoate (40 % EC), Imidacloprid (10 % WP), and Acetamiprid (40 % WG) for pest control, specifically targeted wheat aphid, an insect with piercing-sucking mouthparts. Following 12 h of treatment with a 30–50 $mgL^{-1}$ NC suspension, wheat aphids experienced a corrected mortality rate of $>$ 80 %. The corrected mortality rate was significantly increased up to 95 % when used in conjunction with conventional pesticide dilutions. The chemical insecticidal efficacy has been observed to strongly boosted by ChNWs. Fluorescein isothiocyanate labelled ChNWs could easily absorbed by insects while sucking plant fluid and transported to whole body through digestive tract. Lower toxicity was found with Sprague-Dawley rats, which indicated that ChNWs could be safe for application in agriculture and food industry. NCs showed great potential for development of water-based nanopesticide formulations for limiting the use of chemical pesticides with future agro-environmental sustainability (Li et al., 2021). N-halamine-based ChNF film (chlorinated conjunction) was modified successfully by employing diluted sodium hypochlorite (0.50 %) and showed effective antibacterial and antifungal activity. The chlorinated ChNF film effectively inhibited Gram-negative and Gram-positive bacteria, including *E. coli* and *Staphylococcus aureus*. Additionally, the films inhibited spore germination by 100 and 80 % against *Alternaria alternata* and *Penicillium digitatum* fungi, respectively (Dutta et al., 2015). Additionally, the application of ChNFs treatment resulted in a decrease in the occurrence of *Fusarium* wilt disease in tomato plants, caused by pathogen *Fusarium oxysporum f.* sp. *lycopersici*. The inhibitory effects of Protein/$CaCO_3$/ChNFs and Protein/ChNFs on disease were found to be more potent than those of crushed crab shells and exhibited a similar level of effectiveness as pure ChNF (Egusa et al., 2019). Similarly, reduction of fungal infection caused by *Alternaria brassicicola* and *Colletotrichum fructicola* in cabbage and strawberry was observed by mixing of ChNFs in soil (Parada et al., 2018). The mixture of ChNC suspension concentration (0.001 % w/v) and fungicides (metalaxyl mancozeb and thiophanate methyl) inhibited the root rot disease in tobacco which indicated superior synergistic effect on fungicide formulation (Zhou et al., 2017). Several researchers have reported the promising antifungal and antibacterial potential of ChNFs (Egusa et al., 2015; Parada et al., 2018). Reduction of bacterial infection and necrotic lesion on leaves, caused by *Pseudomonas syringae* pv. tomato DC3000 and *A. brassicicola* pathogen has been observed in *A. thaliana* (Egusa et al., 2015). ChNCs had shown strong antifungal activity against soil borne pathogen of

wheat, *Fusarium pseudograminearum (Fp)* and *F. graminearum (Fg)*. The application of NCs at 30 and 300 ppm concentrations in the growth medium resulted in notable inhibitory effects on the mycelial growth and conidial production. Following treatment with ChNCs (300 ppm), notable reduction in conidial formation by 89.25 and 82.28 % was observed for *Fp* and *Fg* respectively (Liang et al., 2018).

### 5.5. Seed treatment

Seed treatment involves the application of a substance to coat seeds, which possesses advantageous properties for the plant. Certain seed treatments possess antifungal, antiviral, antibacterial, or insecticidal properties, while others enhance the germination rate and growth of the seeds (Shamshina, 2020). Few seed treatment studies have also conducted using NCh and chemical fungicides. Indoor and field trials of ChNCs suspension (0.004 % w/v) combined with fungicides, metalaxyl mancozeb and thiophanate methyl on tobacco plant have illustrated remarkable improvement in seedling growth and reduction in mean time to seed germination (Zhou et al., 2017). To reduce the incidence of crown rot, seeds are often treated or coated with chemical fungicides such as fludioxonil, tebuconazole, and thiophanate-methyl (Akgül & Erkilic, 2016). ChNWs serve as a biobased fungicide for seed treatment in a pot experiment, resulting in the observation of shorter and greener wheat seedlings. The disease control efficiencies were observed to rose to 79.30 and 90.02 % when seeds were treated with mixture of ChNCs and tebuconazole, at concentrations of 10 and 30 ppm, respectively (Liang et al., 2018).

## 6. Circular economy and environmental sustainability

In recent years, there has been a significant emphasis on economic decarbonization to tackle the pressing global challenges of diminishing natural resources and environmental pollution. To address these limitations, focus is shifting towards sustainable alternatives viz. renewable carbon sources derived from biological origins with an aim of creating biobased materials that exhibit low or even negative carbon footprints (Muiruri et al., 2023). Biogenic carbon derived from biopolymers i.e. cellulose; chitin, starch, and lignin has been progressively integrated into advanced biomaterials; serving as a sustainable substitute for fossil-based inputs. Chitin is notable as a highly promising biopolymer, characterized by its abundance, renewability, and inherent bioactivities (Manoj et al., 2025). The global market for chitosan oligosaccharides (COS), is projected to be valued at USD 3.1 billion in 2024 and is expected to grow to USD 10.9 billion by 2034, reflecting a compound annual growth rate (CAGR) of 13.4 % from 2024 - 2034. India and China are expected to exhibit the highest consumption of COS in the healthcare, beauty, and skincare sectors, with projected CAGR of 11.2 and 12.3 %, respectively, till 2034 (Future Market Insights Inc., 2024). The significance of chitin within the biorefinery framework has been illustrated through an evaluation of a shrimp-based biorefinery conducted by Zuorro et al. (2021). The assessment revealed economic viability, showcasing a net present value of $1.12 million and a cumulative cash flow of $0.26 million per year for the processing of 4110.37 tons of fresh shrimp on an annual basis. The study emphasized important impact metrics from an environmental standpoint, indicating a human health potential of $7.17 \times 10^9$ CTU (comparative toxicity unit)·h $kg^{-1}$ and an ecotoxicity probability of 2.59 CTU·eco $kg^{-1}$. NCh, sourced from chitin-rich waste viz. crustacean shells and fungal biomass, cohered to the principles of circular bioeconomy by transforming waste into valuable, biodegradable nanomaterials. Eco-friendly extraction and nanotechnological methods have minimized the environmental impact with facilitation of regenerative cycles. The diverse applications of NCh in packaging, agriculture, biomedicine, and water purification underscore its sustainable utility, safe decomposition without toxic residues and bridging waste reduction to material innovation. Circular economy methodology involves generation of ChNPs from varied biomass wastes would provide a way for their valorisation with reintroduction into the economy; thereby avoiding the landfilling of biowaste with contribution in circular economy. This will significantly reduce the burden on the environment by mitigating the environmental concerns related to waste disposal and management. NCh has not been associated with human or ecological toxicity; hence, its employment as an alternative to synthetic materials presents a significant prospect to reduce the amount of hazardous chemicals and pollutants released into the ecosystems (Chakravarty & Edwards, 2022; Elieh-Ali-Komi & Hamblin, 2016). The global market for chitin and chitosan derivatives, which was forecast to be 106.9 thousand metric tons in 2020 during the COVID-19 crisis, is expected to grow and reach a size of 281.7 thousand metric tons by 2027 (Analysts, 2020). Approximately 40–55 % of shrimp and over 70 % of crabs have been lost as waste throughout the transformation processing steps (Joseph et al., 2020). The waste of these species often contains chitin ranging from 10–55 % on a dry weight basis, depending on the processing method. Conversion of chitin containing marine waste into valuable products viz. NCh enhances the overall sustainability of the process. Ecologically conscious characteristics of NCh illustrated them as well-suited materials for sustainable product development with eco-friendly solutions.

Although chitin and NCh have environmental sustainability benefits, it is crucial to evaluate the complete life cycle of these substances, encompassing their manufacturing, utilization, and disposal, in order to guarantee their maximum environmental efficacy. LCA is extensively utilized to assess environmental performance, particularly in the context of packaging materials. The evaluation examines the comprehensive environmental impact, pinpoints critical areas, and provides direction for enhancing the product sustainability (Yanant et al., 2025). As the production of NCh advances towards industrial scalability, the implementation of a comprehensive LCA is vital for evaluating its environmental impact. LCA facilitates the assessment of energy usage, material inputs, and emissions throughout each phase of NCh manufacturing, aiding in the identification of environmental hotspots and informing process optimization (Fig. 8). This is especially important for innovative methods that include energy-intensive processes, viz. drying or mechanical disintegration. Additionally, LCA promotes sustainable decision-making by allowing comparison between various chitin sources (e.g. crustaceans, insects, fungi) and supporting the justification for the adoption of more sustainable technologies (Chee et al., 2024). LCA of chitosan production in Guayas-Ecuador revealed significant economic viability and environmental sustainability, showcasing a net present value of $10.38 million, return rate of 67.31 %, and payback period of 3.13 years. The cradle-to-gate LCA conducted on 5000 tons per year of shrimp shell waste indicated minimal environmental impacts with human non-carcinogenic toxicity identified as the primary concern (Riofrio et al., 2021). The production cost ranked among the lowest worldwide, underscoring Ecuador's competitive advantage. The analysis supported the utilization of seafood waste as a viable raw material for biopolymer production in a sustainable manner. Relative to cellulose, chitin and chitosan have demonstrated elevated climate change values of 29.41 and 41.80 kg $CO_2$-eq·$kg^{-1}$, respectively. This is largely attributed to the rigorous chemical demineralization processes (HCl treatment) necessarily required for their extraction from shrimp shells. Carbon footprint for isolation of NCh is 36.65 kg $CO_2$-eq·$kg^{-1}$, which indicated the environmental impact of existing processing methods and the necessity for more sustainable, low-impact extraction technologies (Huang et al., 2025). Incorporating LCA early in the development process will assure that NCh applications are effective in function and responsible in environmental stewardship. A recent study based on LCA considering eighteen impact indicators, has been conducted to investigate the environmental impact of isolating ChNCs and ChNFs from 1 kg biowaste of fungus, shrimp shells, and crab shells. The result reported that the global warming potential ($CO_2$ emissions) of ChNF, ChNC, and CNC production regarding ranged from 18.5–906.8 kg·$CO_2$-eq·$kg^{-1}$ of NCh. The global warming potential of ChNF (fungi) was considerably


**Fig. 8.** Life cycle assessment of nanochitin and its impact evaluation.

lower than ChNC (shrimp) and ChNC (crab), with almost 49 and 29-fold decrease. The utilisation of pure chitin powder yielded a negligible environmental impact of 105.2 kg· $CO_2$-eq·$kg^{-1}$, underscoring that the preliminary demineralisation and deproteinisation processes in the treatment of crab shells substantially influence environmental outcomes. Among the sources, ChNF (fungi) exhibited the minimal environmental impact across all eighteen categories, whereas ChNC (shrimp) demonstrated the maximum impact. Environmental impact levels have been observed to be affected by diverse factors i.e. source (no need of demineralization step for fungi as for crustacean-derived NCh), chemical use (inorganic acids or bases), reaction temperature and duration (affecting energy consumption), purification steps (requiring significant chemical use), and reaction yields (Berroci et al., 2022). In order to reduce the environmental impact, solvents are retrieved through the utilization of a rotary evaporator, enabling their reuse in a future synthesis procedure. Industries engaged in nanoparticle production have set a target solvent-recovery rate of 90 % and have used this metric in the past for sensitivity analyses (Arvidsson et al., 2014). New developments are being made to obtain ChNFs with better environmental performance, which should be taken into account. Recent studies have illustrated that the integration of ChNC into polylactic acid contributes to an increased carbon footprint, primarily due to the current low technical readiness level associated with ChNC production (Yanat et al., 2025). Nonetheless, LCA assists in recognizing the opportunities for enhancement, including reduction of chemical usage, implementation of biological processing methods, and the optimization of drying

techniques. Consequently, LCA acts as an essential tool for directing the sustainable production, application, and disposal of NCh -based materials.

## 7. Conclusion and future perspectives

Chitin, the nitrogen rich polysaccharide could be readily acquired from discarded seafood remnants; is biodegradable and exists abundantly in nature. Remodelling of chitin to nanochitin through a range of top-down and bottom-up approaches has extended its applications. Nanochitin exhibits high mechanical strength, low density, biodegradability, and intrinsic antimicrobial properties, rendering it highly suitable for biomedical, packaging, environmental, and agricultural applications. However, widespread adoption is impeded by several challenges. Variability in feedstock due to reliance on crustacean shell waste leads to variances in purity, mineral composition, and polymer chain length. Processing is complex and time-consuming, necessitating multi-step demineralization, deproteinization, and nanostructuring methods that are frequently chemically intensive and conducted in batches. Scalability is hindered by substantial energy requirements for fibrillation, the expense associated with acids, ionic liquids, or DESs, and ineffective solvent recovery systems. Nanochitin experiences aggregation and stability challenges during drying and storage, which undermine its effectiveness in composites or coatings. Furthermore, although its general properties are well understood, the application-specific customization via surface modification for targeted purposes, viz. drug delivery or selective adsorption, is still inadequately developed, which restricts its wider applicability. Most of the nanochitin fabrication methodologies have depended on the utilization of potent chemicals, entail higher energy consumption, result in adverse environmental impacts. Hence, further investigation is required to advance the development of novel, efficient techniques for extracting nanochitin that minimize product heterogeneity with cost-effectiveness. The potential applications of nanochitin in agriculture are promising, further research is ongoing to optimize formulations, understand long-term effects, and ensure practical feasibility. Additionally, the regulatory aspects of using nanomaterials in agriculture must be considered to ensure their safe and responsible application in the field. The investigation about the preparation, characterization, and agricultural application of these materials is still in its nascent stages. Furthermore, emergence of novel contributions in the field of nanochitin preparation and its subsequent applications in the agriculture will augment the sustainable resource recovery from coastal residual resources. Future research on nanochitin must emphasize sustainable and standardized sourcing of feedstock, particularly through the investigation of fungal and insect-derived chitin to ensure consistency and minimize dependence on crustacean waste. Innovations in processes are essential for the integration of pretreatment, nanostructuring, and purification into continuous, energy-efficient workflows. Membrane-based systems present opportunities to enhance the efficiency of washing and neutralization. Green chemistry methodologies, including the application of recyclable solvents viz. DESs and biobased ionic liquids within closed-loop recovery systems, are crucial for reducing environmental impact. Enhancing surface functionalization and hybridization techniques facilitates the creation of multifunctional composites through the integration of nanochitin with cellulose nanofibers, bioplastics, or nanoparticles to improve performance. Life cycle and techno-economic assessments are essential for guiding scalable production and ensuring environmental sustainability. These research advancements will facilitate the real-world application of nanochitin in the next decade. In biomedicine, nanochitin may be refined for use in antimicrobial wound dressings, tissue engineering scaffolds, drug delivery carriers, and bioresorbable sutures. Nanochitin can be transformed into active biodegradable films with improved moisture and oxygen barriers and inherent microbial resistance for use in the food industry. Functionalized nanochitin filters can selectively eliminate dyes, heavy metals, and organic pollutants from wastewater for environmental remediation purposes. Biodegradable seed coatings and foliar sprays in agriculture can be customized to enhance plant immunity, thereby decreasing reliance on synthetic pesticides. Additionally, customized nanochitin aerogels and films have demonstrated potential in energy storage and electronics with functioning as lightweight, flexible separators for supercapacitors and batteries. They also act as high-performance fillers in bioplastics and sustainable construction materials.

The industrial potential of nanochitin hinges on the alignment of research in feedstock diversification, efficient processing, and material tailoring with application-driven demands. Addressing these challenges may enable nanochitin to not only compete with but also exceed nanobiopolymers viz. cellulose nanocrystals and nanochitosan, positioning it as a crucial material for the forthcoming generation of sustainable, high-performance industries.

## CRediT authorship contribution statement

**Rajni Kumari:** Writing – original draft, Formal analysis, Data curation. **Aakash Chawade:** Writing – review & editing. **V. Vivekanand:** Writing – review & editing. **Nidhi Pareek:** Writing – review & editing, Resources, Project administration, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

RK gratefully acknowledged the senior research fellowship by Council of Scientific and Industrial Research (CSIR, New Delhi) for conducting the research. NP appreciatively expresses gratitude towards ANRF (Anusandhan National Research Foundation, SPG/2021/003648) Government of India for research grant to carry out the work. Necessary facilities provided by Central University of Rajasthan, Rajasthan, India to accomplish the research work are well recognized.

## Data availability

Data will be made available on request.

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