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Extraction and characterization of a chitosan-based biopolymer extracted from shrimp shells

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List of abbreviations

- **°C :** Degree Celsius
- **AA** : Area of the Amorphous Phase
- **AC** : Area of the Crystalline Phase
- **AOAC** : Association of Official Analytical Chemists
- **Aq** : Aqueous
- **ATG/DTG** : Thermogravimetric Analysis/Derived Thermogravimetry
- **bFGF** : Basic Fibroblast Growth Factor
- **$CaCl_2$** : Calcium Chloride
- **$CaCO_3$** : Calcium Carbonate
- **$CuSO_4$** : Copper Sulfate
- **DD** : Degree of Deacetylation
- **DM** : Dry Matter
- **ECM** : Extracellular Matrix
- **FTIR** : Fourier Transform Infrared Spectroscopy
- **2θ** : Diffraction Angle (2 Theta)
- **H_2O_2** : Hydrogen Peroxide
- **HCl** : Hydrochloric Acid
- **IR** : Infrared
- **ISO** : International Organization for Standardization
- **L** : Liquid
- **N** : Nitrogen
- **N** : Total Nitrogen
- **NaOH** : Sodium Hydroxide
- **NF** : French Standard
- **pKa** : Acid dissociation constant
- **s** : Solid
- **VEGF** : Vascular Endothelial Growth Factor
- **XC** : Crystallinity Percentage
- **XRD** : X-ray Diffraction
- **XRF** : X-ray Fluorescence

General Introduction

General introduction

The growing urgency of environmental problems and the need for sustainable solutions have propelled research into environmentally-friendly materials and waste recovery processes to the forefront of scientific and industrial concerns. Among these solutions, biopolymers, and more specifically chitosan, derived from marine crustaceans, have attracted considerable interest due to their exceptional properties and potential for application in a wide range of fields.

Chitosan is a natural polysaccharide obtained by the deacetylation of chitin, a major structural component of crustacean exoskeletons. This biopolymer is recognized for its biodegradability, biocompatibility, and antibacterial and antifungal properties, making it a material of choice for a variety of industrial, biomedical and environmental applications.

This thesis will explore in depth the methods for extracting and purifying chitosan from marine crustacean waste, as well as its physicochemical and biological characteristics. In addition, it will highlight the multiple potential applications of this biopolymer, offering a global vision of its importance in the valorization of marine waste and the promotion of a circular economy. The main objective is to demonstrate how chitosan can not only reduce marine waste, but also contribute to the creation of high value-added products, while respecting the principles of sustainable development. This work is mainly composed of two parts:

Part A: A detailed bibliographical study, including:

- **Chapter I :** A review of polysaccharide generalities.
- **Chapter II :** A review of the various applications of chitosan.

Part B: An experimental study, including:

- **Chapter III :** A presentation of the raw material, extraction procedure, analytical methods and experimental protocols.
- **Chapter IV :** An interpretation of the experimental results obtained.

It will conclude with a summary of the results and future prospects for the study.

Chaptre I

I.1. Introduction

Polysaccharides, regularly alluded to as complex carbohydrates, are macromolecules comprising of long chains of monosaccharide units connected together by glycosidic bonds [1]. The term "poly" means the huge number of sugar units included, recognizing polysaccharides from easier carbohydrates like monosaccharaides (glucose and fructose) and disaccharides (sucrose and lactose).

These organically critical atoms play significant parts in cellular structure, vitality capacity, and communication. Starch, a unmistakable capacity polysaccharide in plants, serves as a essential vitality save. Glycogen, the creature identical put away within the liver and muscles, acts as a promptly accessible vitality source. In differentiate, cellulose, a basic polysaccharide shaping plant cell dividers, gives unbending nature and bolster.

Basically, polysaccharides show exceptional differences. They can be straight or branched, with varieties within the sorts of glycosidic linkages between the sugar units [2]. For occurrence, cellulose comprises of straight chains of β-glucose units connected by β-1,4-glycosidic bonds, shaping solid, sinewy structures [1]. On the other hand, glycogen, a profoundly branched polysaccharide, highlights α-1,4-glycosidic linkages within the fundamental chain with intermittent α-1,6-branch focuses [3].

Past their basic parts, polysaccharides are vital in cellular acknowledgment and signaling. Glycoproteins, which have sugar moieties joined to proteins, play key parts in safe reactions, cell grip, and hormone direction [4]. Glycosaminoglycan's, another course of polysaccharides, are fundamental components of connective tissues and oils in joints [5].

In rundown, polysaccharides stand as complex and multifaceted atoms, contributing essentially to the engineering and work of living living beings. Their basic differing qualities and organic flexibility emphasize their significant parts in keeping up cellular judgment, directing vitality capacity, and organizing perplexing cellular forms over the range of life.

I.2. Different type of polysaccharides

I.2.1. Definition of polysaccharides

Polysaccharides are expansive, complex carbohydrates composed of rehashing units of monosaccharides connected together by glycosidic bonds. Not at all like straightforward sugars, these macromolecules display a tall degree of basic differing qualities, usefulness, and play essential parts in different organic forms.

I.2.2. Different type of polysaccharides

I.2.2.1. Storage Polysaccharides

- **Starch**

Found in plants, starch serves as a essential vitality capacity particle. It exists in two shapes: amylose, a direct polymer of glucose, and amylopectin, a branched polymer with α-1,6-glycosidic linkages [6], permitting for fast enzymatic debasement and glucose discharge.

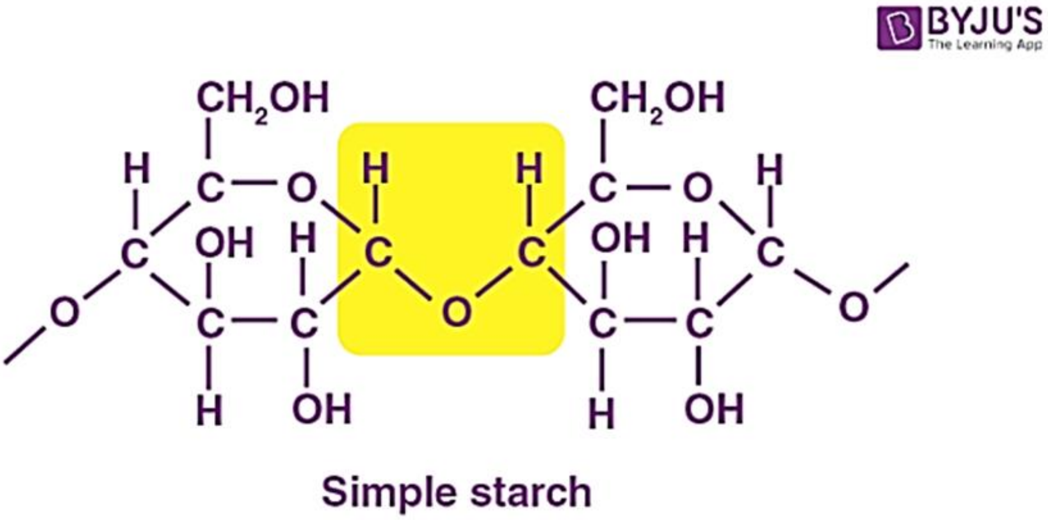


Figure I.1 : Chemical structure of starch [7]

- **Glycogen**

Transcendently put away within the liver and muscles of animals, glycogen could be a exceedingly branched polysaccharide comparative to amylopectin but with more broad branching, empowering fast vitality mobilization [8].

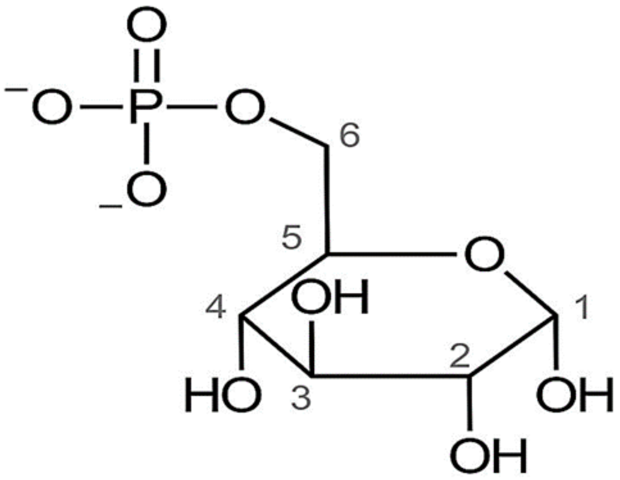


Figure I.2 : Glycogen metabolism [9]

I.2.3. Functional Polysaccharides

- **Glycosaminoglycans (Chokes)**

Found in connective tissues, Chokes are long, direct polysaccharides with rehashing disaccharide units. Illustrations incorporate hyaluronic corrosive, chondroitin sulfate, and heparin, contributing to the oil and basic astuteness of tissues.

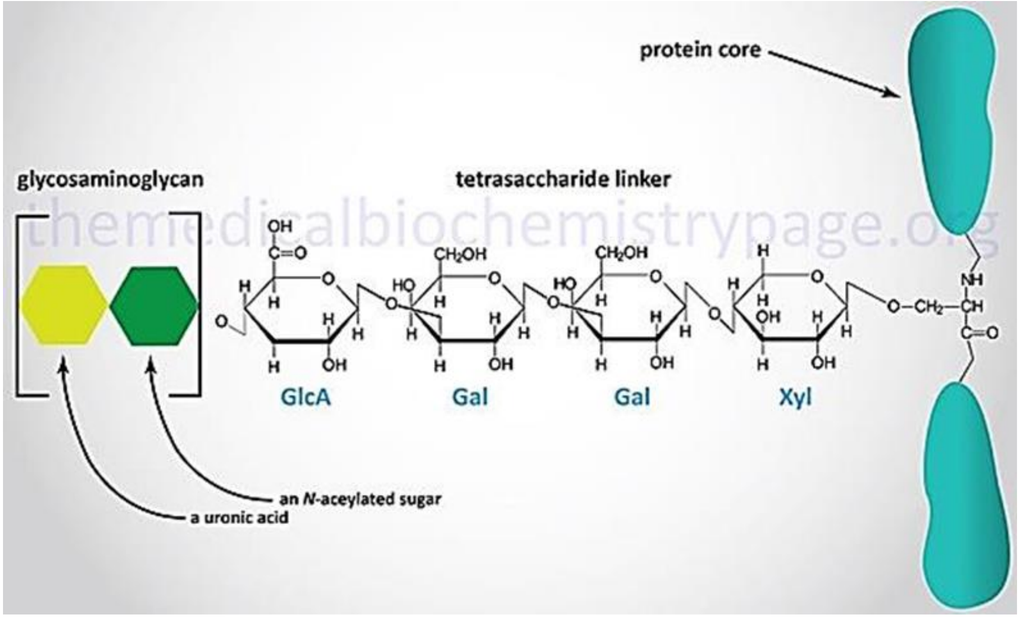


Figure I.3 : Glycosaminoglycan’s metabolism [10]

- **Peptidoglycan**

Display in bacterial cell dividers, peptidoglycan may be a complex structure containing substituting units of N-acetylglucosamine and N-acetylmuramic corrosive. Cross-linked peptides give quality to the bacterial cell divider [11].

I.2.4. Enzymatic Polysaccharides

- **Cellulose**

Mainly composed of β-glucose units connected by β-1,4-glycosidic linkages, cellulose is a constituent of plant cell walls [12]. Hydrogen bonds hold the bundles of linear chains together, giving them stiffness and strength.

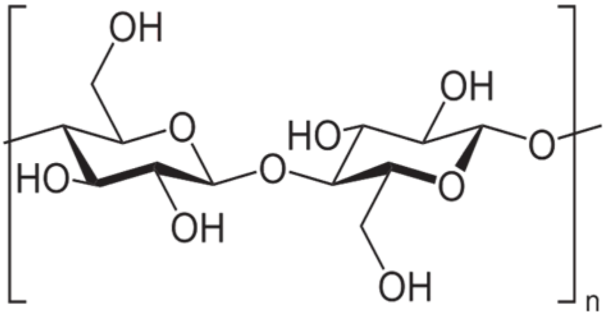


Figure I.4 : Chemical structure of cellulose [13]

- **Chitin**

Made up of N-acetylglucosamine units connected by β-1,4-glycosidic linkages, chitin is found in the cell walls of fungi and the exoskeletons of arthropods [14]. Its sturdy construction provides stability and longevity.

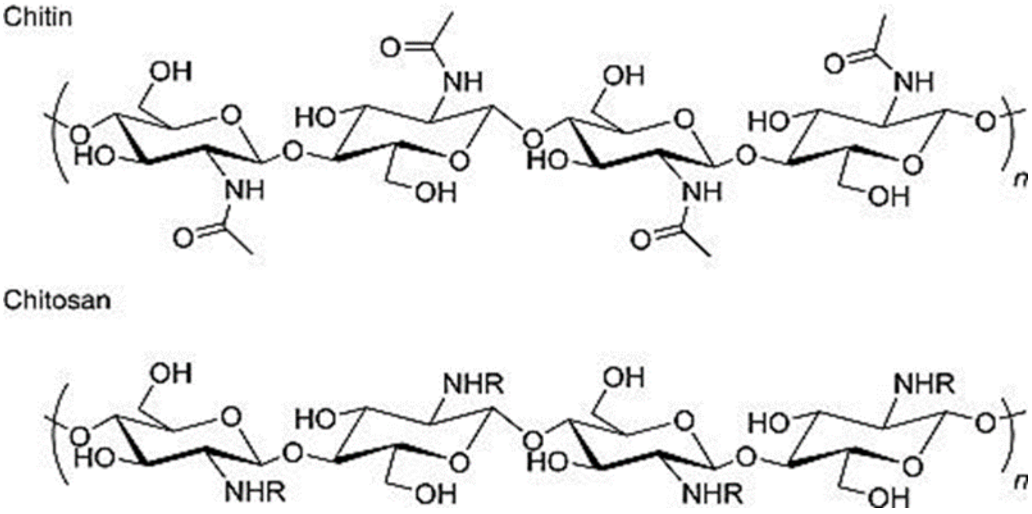
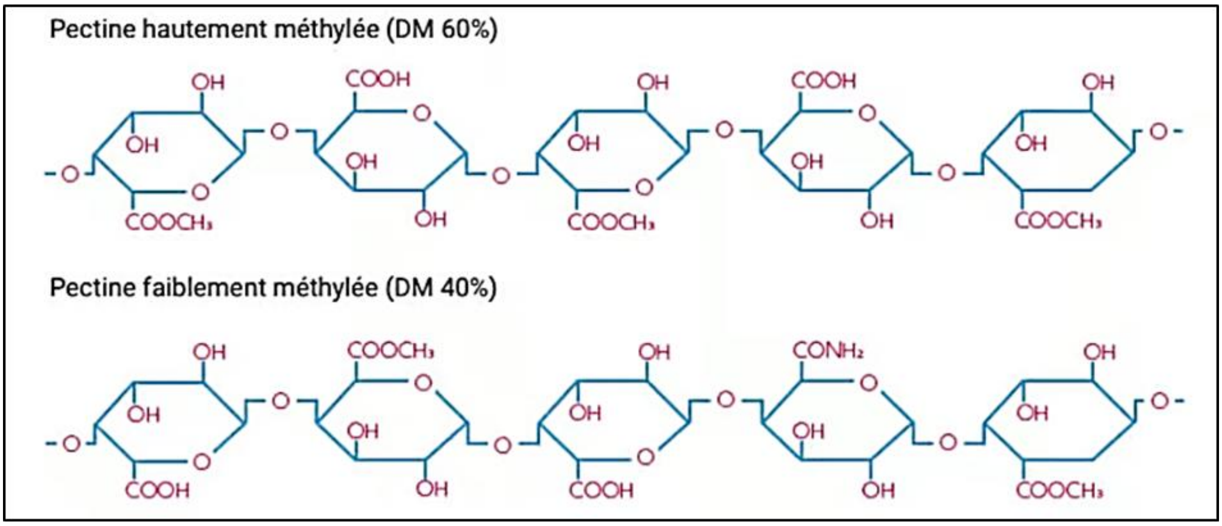


Figure I.5 : Chemical structure of chitin [15]

- **Pectin**

Pectin is a complex structural polysaccharide made up of galacturonic acid units that is present in the cell walls of plants and fruits. It strengthens and increases the pliability of plant tissues [16].


Pectine hautement méthylée (DM 60%)

Pectine faiblement méthylée (DM 40%)

Figure I.6 : Structure of pectin [17]

I.3. Chitin

Chitin is a complex polysaccharide renowned for its prevalence in the biological world, particularly as a principal structural component in the exoskeletons of arthropods and the cell walls of fungi. Structurally, chitin is composed of repeating units of N-acetylglucosamine, creating a linear chain linked by β-1,4-glycosidic bonds [14]. Its fibrous and tough nature grants strength to the exoskeletons of insects, crustaceans, and other arthropods, providing a protective shield against environmental stressors and predators [18]. Additionally, chitin contributes to the rigidity of fungal cell walls, offering support and protection.

Chitin's importance extends beyond its structural role; it plays a crucial part in ecological processes. As a biodegradable polymer, chitin facilitates the decomposition of organic matter, contributing to nutrient cycling in ecosystems. This characteristic underscores its significance in maintaining environmental balance.

Following our investigation through the intriguing terrain of chitin's many uses, we arrive at a crucial change: the conversion of chitin into chitosan. Chitin changes during this transition, losing some of its acetyl groups in a process called deacetylation [19]. Chitosan is created when this process changes the properties of the biopolymer. The resulting chitosan has a distinct cationic character, improved solubility, and higher bioactivity. Beyond its structural roots in fungal cell walls and arthropod exoskeletons, chitosan is a multipurpose biopolymer with uses in environmental research, agriculture, biomedicine, and other fields [20].

I.4. Chitosan

I.4.1. Definition of chitosan

Chitosan, a biopolymer determined from chitin through the method of deacetylation, is characterized by its upgraded dissolvability and special chemical properties. Amid deacetylation [19], a noteworthy parcel of the acetyl bunches on chitin's glucosamine units is expelled, coming about in a polymer with a unmistakable cationic charge. This adjustment confers water-solubility in acidic conditions, setting chitosan separated from chitin, its forerunner.

Beyond its auxiliary beginnings within the exoskeletons of arthropods and parasitic cell dividers, chitosan has earned consideration for its different applications. Its biocompatibility makes it an alluring fabric within the biomedical field, contributing to wound mending and serving as a component in medicate conveyance frameworks [21]. The antimicrobial properties of chitosan have driven to its utilization in nourishment conservation, water treatment, and the advancement of antibacterial coatings.

Chitosan's natural commitments are critical as well. Its capacity to adsorb overwhelming metals and colors in water treatment forms makes it profitable for natural remediation. Moreover, chitosan's biodegradable nature adjusts with supportability endeavors, tending to concerns related to natural affect.

In quintessence, chitosan stands as a flexible biopolymer, molded through a handle of alteration that upgrades its utility over a range of businesses. Its particular properties proceed to fuel investigation and advancement, situating chitosan as a profitable asset within the crossing point of nature and innovation.

Chitin stands out among the various structural polysaccharides as an exceptional biopolymer that supports the strength of fungal cell walls and exoskeletons on arthropods. Because of its distinct characteristics, chitin—which is made up of N-acetylglucosamine units connected by β-1,4-glycosidic bonds—is an intriguing material to study [16]. We explore the world of chitin, its structural complexities, and how it is converted into chitosan, which opens up a wide range of applications in diverse sectors.

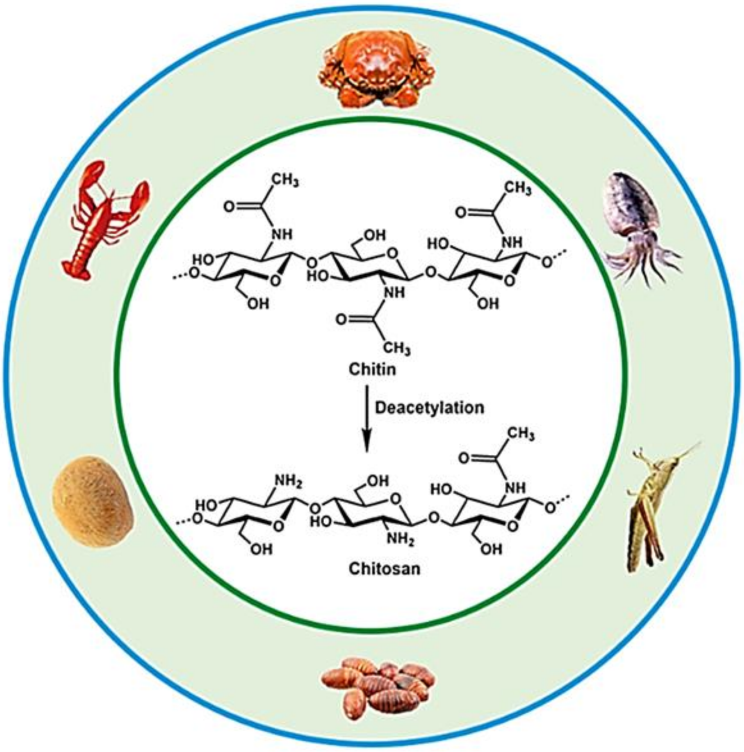


Figure I.7 : Transformation of initial product to chitosan [22]

I.4.2. Historical of chitosan

I.4.2.1. Early Revelations and Roots

- 1811: The compound chitin, the antecedent to chitosan, was to begin with disconnected by Henri Braconnot, a French chemist, from mushrooms.

- 1859: The term "chitin" was coined by Albert Ladenburg to depict the compound.

I.4.2.2. Chitosan's Rise

- 1859-1894: Over the another few decades, headways in understanding the structure and properties of chitin driven to encourage ponders.

- 1894: Chitosan was to begin with created by Raoul Gabriel Henri Verdier by treating chitin with potassium hydroxide.

I.4.2.3. 20th Century Advancement

- 1930s-1940s: Advance inquire about and advancement extended the understanding of chitosan's properties, driving to its extraction from different sources.

- 1970s: Dr. Muzzarelli and his group in Italy were urgent in popularizing chitosan due to its interesting properties and potential applications.

- 1980s: Chitosan picked up consideration due to its biocompatibility, non-toxic nature, and biodegradability, driving to broad inquire about in different areas, particularly in pharmaceutical, horticulture, and industry.

I.4.2.4. Advanced Applications and Progressions

- 1990s-2000s: Chitosan found far reaching utilize in different businesses. Its biodegradability, non-allergenic nature, and antimicrobial properties got to be critical drivers for its application in medication, pharmaceuticals, agribusiness, wastewater treatment, and more.

- 2010s-2020s: Continuous investigate centered on optimizing chitosan-based items and their applications, investigating unused strategies for extraction and amalgamation, and making strides its properties for assorted employments.

I.4.2.5. Later and Future Patterns

Chitosan proceeds to advance as a fabric with promising applications in areas like medicate conveyance frameworks, wound mending, tissue building, farming (as a biopesticide or biostimulant), and natural remediation. He chronicled travel of chitosan reflects an advancing understanding of its properties, driving to the revelation of its different applications over diverse businesses. Continuous investigate and headways proceed to improve its potential and open unused roads for its utilization in a wide run of areas.

I.4.3. Proprieties and chemical structure

I.4.3.1. Physico-chemical properties

- **Solubility**

Chitosan is soluble in acidic solutions and dissolves in conditions where the pH is lower than its pKa [23]. This characteristic is crucial for medication delivery applications because it makes it possible to create controlled-release systems with customized release kinetics.

- **Molecular Weight**

Chitosan's viscosity and mechanical qualities are influenced by its molecular weight, which varies from low to high. Increased viscosity, which is linked to higher molecular weights [24] , affects how well a material performs in formulations and in biomedical applications like scaffolds and matrices.

- **Degree of Deacetylation (DD)**

The cationic charge and reactivity of chitosan are determined by the degree of deacetylation, which is measured as the proportion of acetyl groups extracted from chitin. Its solubility, antibacterial activity, and interactions with other molecules are all improved by a higher DD [25].

- **Crystallinity**

The structure of chitosan is semi-crystalline. Its mechanical strength and rigidity are dependent on its degree of crystallinity[23]. This characteristic is taken into account in applications including films, membranes, and tissue engineering where structural integrity is essential.

- **Viscoelasticity**

Chitosan exhibits viscoelastic behavior, which affects its capacity to form films and its possible use in wound dressings, where adherence and flexibility are necessary [26].

- **Charge Density**

The amino groups in chitosan contribute to its positive charge density. Its interaction with negatively charged substances is influenced by this feature[27], which makes it useful for flocculation and metal ion adsorption.

- **pH Sensitivity**

The solubility and charge of chitosan are greatly sensitive on pH [28]. This sensitivity can be used in medication delivery systems where it's necessary to have regulated release at particular pH levels.

- **Film-Forming Properties**

The molecular weight, concentration, and degree of deacetylation of chitosan all affect its capacity to form films [29]. These films may find use in controlled drug release systems, packaging, and wound dressings.

- **Mechanical Strength**

Chitosan films or constructs are appropriate for a variety of structural applications since their mechanical strength can vary according on their molecular weight [29], degree of deacetylation, and other processing parameters.

- **Water Binding Capacity**

Chitosan's ability to absorb and bind water affects how it swells and retains moisture, which has an impact on how it is used as a biopolymer in food applications and how it heals wounds [30].

I.4.3.2. Chemical properties

- **Amino Groups**

As a byproduct of deacetylation, the presence of amino groups along the polymer chain of chitosan defines its chemical identity. Chitosan's distinctive cationic properties are a result of these amino groups. The degree of deacetylation affects the density of these groups, which affects how chitosan interacts with other molecules [31]. Chitosan's solubility and reactivity in various environments are influenced by chemical reactions involving protonation and deprotonation, which are made possible by the amino groups.

- **Reactivity**

Chitosan is a diverse platform for chemical alterations because of its reactivity, which is a result of the amino groups. A variety of reactions, including acylation, Schiff base production, and crosslinking, can occur with the amino groups. Researchers can modify chitosan chemically to make it suitable for a certain use. For instance, adding functional groups to chitosan can increase its solubility or give it new characteristics [23], increasing its use in a variety of applications such as environmental cleanup and medication delivery.

- **Chemical Modifications**

Using chemicals to alter chitosan's characteristics is a potent strategy. Chitosan's stability, behavior, and interactions with other compounds can all be changed by grafting different moieties onto its molecules. To increase its compatibility with non-polar compounds, for example, adding hydrophobic groups can increase its range of applications [32]. Because chitosan can be chemically modified exactly, it can be carefully engineered to work in a variety of situations.

- **Hydrolysis and destruction**

Because chitosan is a polysaccharide, it is prone to enzymatic and hydrolytic destruction. Predicting the stability and long-term behavior of these processes requires an understanding of the chemical pathways involved. In order to ensure regulated release and minimal environmental impact, researchers frequently take the kinetics of chitosan degradation into account when building biodegradable materials or drug delivery systems[33].

- **Metal Ion Complexation**

Complexation processes arise because of the strong attraction of chitosan's amino groups for metal ions [34]. This ability of chitosan to selectively bind and remove heavy metal contaminants is used in applications like water treatment. Coordination complexes have a role in the chemical reaction between chitosan and metal ions, providing a sustainable approach to environmental cleanup.

I.4.3.3. Biological properties

- **Biocompatibility**

One of chitosan's most distinctive biological qualities is its remarkable biocompatibility. It is a material that is essential in a variety of biological applications since it blends in smoothly with live tissues. Chitosan promotes a favorable environment for cellular interactions in wound dressings, tissue engineering, and drug delivery systems, reducing adverse reactions and fostering tissue regeneration [35].

- **Antimicrobial Activity**

The amino groups in chitosan provide a cationic charge that has strong antibacterial effects. Chitosan has the ability to break down microbial cell membranes, which stops bacteria, fungus, and other diseases from growing . Chitosan serves as a natural and efficient barrier against infections in wound care products, medical equipment, and food preservation due to its inherent antibacterial activity [36].

- **Bioadhesion**

Chitosan is a great option for applications needing continuous interaction with biological surfaces because of its bioadhesive qualities [37]. For instance, chitosan's capacity to stick to mucosal membranes during drug delivery prolongs drug release and improves therapeutic efficacy. When creating mucoadhesive formulations for regulated drug delivery to particular anatomical locations, bioadhesion is also helpful.

- **Hemostatic Effect**

Chitosan's positive charge facilitates its interaction with blood components, which is one of its hemostatic qualities. Materials based on chitosan have the potential to enhance blood clotting and expedite the coagulation process in wound treatment. This hemostatic action is very helpful in controlling bleeding and promoting quicker healing of wounds.

- **Immunomodulation**

There is increasing interest in chitosan's impact on the immune system. Research indicates that chitosan has the ability to regulate immunological reactions, which could improve the host's defense mechanisms [38]. This characteristic is investigated in vaccine delivery methods, where chitosan may help boost vaccine efficacy and enhance immunological responses.

- **Biodegradability**

One of the most important biological characteristics of chitosan is its capacity for biodegradation [39]. It can be broken down enzymatically into non-toxic components because it is a polysaccharide. Because of this characteristic, chitosan is a sustainable option for a range of applications, including implanted medical devices and biodegradable packaging. It also conforms to ecologically friendly standards.

- **Tissue Regeneration**

The bioactive properties of chitosan also apply to tissue regeneration. Chitosan scaffolds offer a scaffolding support for cell adhesion, proliferation, and differentiation in tissue engineering. This characteristic is used to create structures that regenerate cartilage, bone, and other tissues [40].

I.5. Conclusion

In conclusion, chitosan is one particularly notable example of how polysaccharides, a varied class of complex carbohydrates, serve vital roles in a variety of biological and industrial applications. Chitosan, which is derived from chitin, has a fascinating past as well as special qualities that make it incredibly adaptable. Its high viscosity, capacity to form films, and solubility in acidic solutions are some of its physico-chemical properties. Amino groups increase the chemical reactivity of the substance, making a variety of changes possible. In terms of biology, chitosan is praised for its antibacterial, biodegradable, and biocompatibility qualities, which make it a useful substance in many fields.

Chitosan has many practical and significant uses. It is used in medicine for tissue engineering, drug delivery, and wound healing. Chitosan provides insect resistance and encourages plant development in agriculture. Its thickening and preserving qualities are advantageous to the food sector, and its ability to eliminate contaminants and heavy metals highlights its significance for environmental preservation. Because of its many uses and distinctive qualities, chitosan is ideally positioned to serve as a foundation for new developments in the field of polysaccharides, given the considerable progress and untapped potential in this field.

Chaptre II

II.1. Introduction

Chitosan, which is made from the exoskeletons of crabs, is now an essential part of materials used in biomedical engineering. Because of its biocompatibility and biodegradability, it is perfect for use in drug delivery systems and scaffolds for tissue engineering, where it promotes targeted drug release and cell proliferation [39].

It is based dressings are essential for wound healing because of their hemostatic and antibacterial properties, which facilitate quicker clotting and reduce the risk of infection [36]. These dressings help both acute and chronic wounds by fostering the ideal environment for tissue regeneration. Chitosan's adaptability goes beyond medicine to include environmental cleanup, cosmetics, and agriculture, demonstrating its broad range of uses and inventive possibilities in a variety of industries [53].

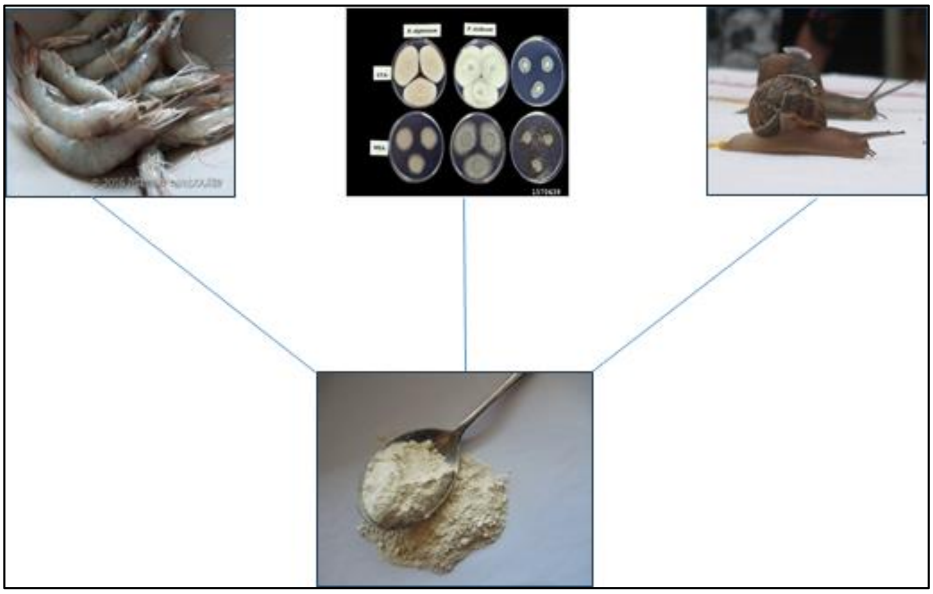


Figure II.1 : Schema de producing chitosan from the raw material

II.2. Application of chitosan

Chitosan is an extremely versatile and promising biopolymer found in the complex structure of crab shells. Because of its special properties and variety of applications, chitosan—which is derived from chitin, a naturally occurring polysaccharide that is abundant in the exoskeletons of crustaceans—has drawn interest from researchers and industry players. Chitosan is fundamentally unique in that it is biocompatible and has a unique chemical composition. Its structure, which is made up of repeating glucosamine and N-acetylglucosamine units [54], gives it a polycationic quality as well as a number of beneficial properties [50]. Its exceptional ability to interact with biological systems has made it invaluable in the field of biomedical applications. Its mucoadhesive and biocompatible properties have revolutionized medicine by enabling applications ranging from drug delivery systems and tissue engineering scaffolds [35]. to wound healing solutions. Beyond the field of medicine, chitosan has applications in food technology, agriculture, water treatment, and environmental remediation [51]. Its use as a biopesticide, soil conditioner, and water purifier highlights the benefits of chitosan in encouraging ecologically friendly behavior [41].

II.2.1. Biomedical Engineering Materials

The biomedical applications of chitosan are not limited to traditional areas such as wound healing, medication transport, and tissue engineering [55]. It exhibits promise in dental materials to improve periodontal therapy's bone regeneration and dental implant procedures. Additionally, chitosan-based bioimaging contrast agents provide enhanced visualization in diagnostic imaging, which has the potential to completely change the way that diseases are detected and tracked. Chitosan-based sealants and adhesives offer a suture-free option for tissue restoration in surgical settings, exhibiting adhesive qualities and biocompatibility on damp surfaces. Chitosan-coated biomedical textiles have antibacterial qualities that make them appropriate for use as surgical gowns and wound dressings [55]. Chitosan scaffolds also have the potential to improve recovery from spinal cord damage or peripheral nerve injury in nerve regeneration studies [35].

The mucoadhesive properties of chitosan are advantageous for ophthalmic applications, allowing for extended drug release and better treatment results for eye diseases [56]. Furthermore, the application of chitosan coatings on cardiovascular devices seeks to improve biocompatibility and decrease thrombogenicity, so offering patients with cardiovascular diseases more resilient and secure implants [57]. These uses highlight how chitosan may be applied to a wide range of biomedical sectors to address complicated challenges.

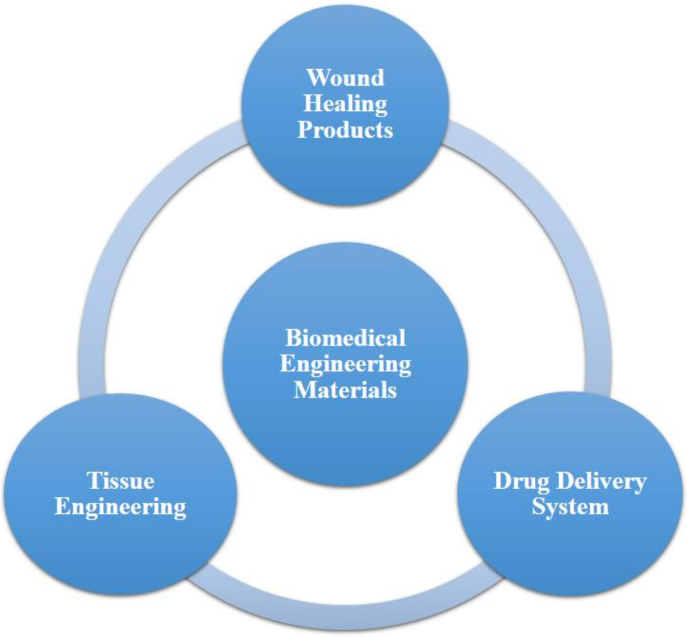


Figure II.2 : Scheme of Biomedical Engineering fields

II.2.1.1. Advantages

- ✓ Chitosan is a substance that is appealing for medical applications like implants and drug delivery systems since biological tissues often tolerate it well [58].
- ✓ Chitosan can be employed in short-term applications where material deterioration is desirable, like absorbable sutures, because it decomposes naturally [59].
- ✓ Chitosan can be chemically modified to alter its properties, making it versatile for a range of biomedical applications from bone implants to drug delivery systems [55].

II.2.1.2. Disadvantages

- ✓ The quality and properties of chitosan can vary depending on the source from which it is extracted, leading to variations in its performance and biological compatibility [32].
- ✓ The process of producing and purifying pure chitosan can be difficult, which might influence the final materials' effectiveness and cause uniformity problems [60].
- ✓ Even while the body normally tolerates chitosan well, there are situations in which it might cause an immunological reaction, particularly when used excessively or not properly purified [60].

II.2.2. Drug delivery system

Chitosan plays a critical role in drug delivery systems by utilizing its special qualities to increase therapeutic efficacy and reduce side effects. Because of its mucoadhesive properties and biocompatibility [56], it is a great option for a number of drug delivery applications Due to chitosan's capacity to stick to mucosal surfaces, including those in the gastrointestinal tract, nasal cavity, and ocular tissues, drug-loaded carriers and target sites can have extended contact, which improves drug absorption and bioavailability. Chitosan nanoparticles, also known as microparticles, shield drugs from enzymatic degradation during oral drug administration, enabling regulated release and targeted delivery [61]. Because chitosan is cationic, it can interact electrostatically with negatively charged cell membranes to promote intracellular medication delivery and cellular uptake, especially for macromolecules like proteins and nucleic acids.

Table II.1 : overview of the diverse applications of chitosan in drug delivery systems.

Application	Description
Oral Drug Delivery	Chitosan enhances the absorption of drugs across intestinal epithelia and prolongs drug release.
Gene Delivery	Chitosan nanoparticles are used to deliver genetic material due to their biocompatibility and biodegradability.
Cancer Therapy	Chitosan nanoparticles can target cancer cells and deliver chemotherapeutic agents more effectively.
Transdermal Drug Delivery	Chitosan forms films and hydrogels that can be used for controlled drug release through the skin.
Ocular Drug Delivery	Chitosan-based systems are used to enhance drug residence time on the eye surface, improving efficacy.

II.2.2.1. Advantages

- ✓ Mucoadhesive qualities in chitosan enable extended interaction with mucosal surfaces, like those in the nasal cavity or gastrointestinal system, improving the absorption and bioavailability of medications [56].
- ✓ Chitosan can be included into a variety of drug delivery forms, including films, hydrogels, nanoparticles, and microparticles. This enables the controlled release of medications over a prolonged period of time, enhancing both patient compliance and therapeutic efficacy [61].

- ✓ Drug delivery systems based on chitosan can be designed to target certain tissues or cells, lowering systemic adverse effects and raising the therapeutic index of medications [61].

II.2.2.2. Disadvantages

- ✓ Drug delivery systems may operate differently depending on the source and production processes used for chitosan, which can affect its molecular weight, degree of deacetylation, and purity [62].
- ✓ Even while the body normally tolerates chitosan well, there are situations when it can cause an immunological reaction, especially if the formulation contains contaminants or if a lot of it is used [63].

Chitosan hydrochloride, a chitosan salt, was first approved in 2002 by the Pharmacopeia. Almost a decade later, chitosan was included as an excipient in both the European Pharmacopeia 6.0 and the 29th edition of the United States Pharmacopeia (USP) 34-NF [50]. These monographs provide detailed assays and set limits for the use of chitosan as a pharmaceutical excipient. Since its approval, there has been a significant and sustained increase in the number of publications on chitosan's application in drug delivery, indicating ongoing interest and research in this field.

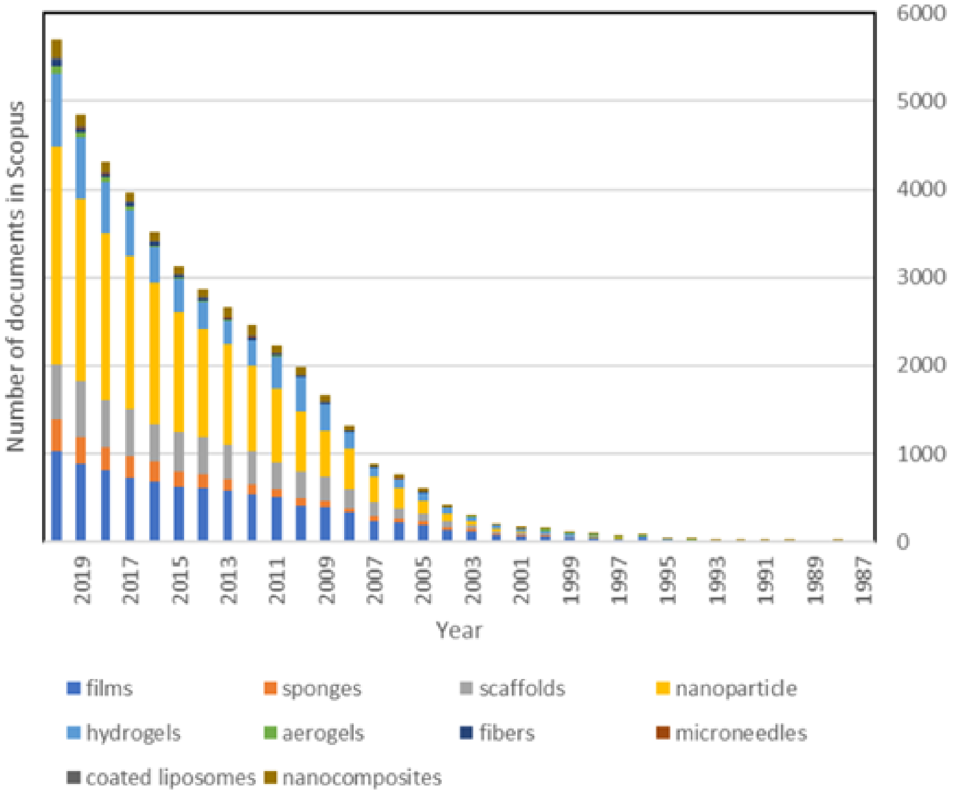


Figure II.3 : Publications about chitosan drug delivery in Scopus (1987–2020) [50].

II.2.3. Wound Healing Products

Chitosan is more effective at healing wounds because of a number of properties, such as non-toxicity, biocompatibility, and biodegradability. Through the promotion of blood vessel creation, cell division, and extracellular matrix (ECM) synthesis, it expedites the healing process [64]. Chitosan speeds up healing by promoting the growth of fibroblasts, keratinocytes, and endothelial cells, which promotes tissue regeneration. Furthermore, it triggers the activation of growth factors that are necessary for the creation of new blood vessels that will supply oxygen and nutrients to the wounded area, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Furthermore, chitosan increases the synthesis of collagen and other extracellular matrix constituents, giving regenerated tissue its structural integrity and tensile strength. It has demonstrated potential in a number of clinical and experimental contexts as a wound-healing agent. Because of its versatility, chitosan can be used to create hydrogels, films, dressings, and other pharmaceutical forms that guarantee the continuous release of medicinal substances. When chitosan films are applied directly to wounds, a moist environment is produced that both promotes cell migration and proliferation and acts as an infection barrier [36].

Moreover , chitosan not only facilitates tissue regeneration but also demonstrates immunomodulatory properties that help control the inflammatory response at the location of the lesion. By reducing excessive inflammation, this modulation aids in the creation of a healing environment. The antibacterial qualities of chitosan also aid in lowering the chance of wound infections, which speeds up the healing process [65]. In addition, chitosan improves patient satisfaction by improving the cosmetic result of wound healing through the orderly deposition of collagen fibers, which reduces the creation of scars.

The versatility of chitosan suggests that it could be useful in combined treatment plans. Healthcare practitioners can combine it with other therapeutic substances or technology, such growth factors or hyperbaric oxygen treatment, to improve its wound-healing capabilities. In addition to maximizing the therapeutic effects of chitosan, this synergistic method takes into account the unique requirements of every patient and their wounds. Furthermore, new developments have looked into how chitosan may be used to transfer stem cells and nanoparticles, which could increase its uses in regenerative medicine. Chitosan, all things considered, offers a plethora of benefits for better healing results and patient well-being, marking a promising new frontier in wound care [65].

Table II.2 : Overview of various applications of chitosan in wound healing.

Application	Description
Antimicrobial Activity	Chitosan exhibits antimicrobial properties, preventing infections in wounds.
Hemostasis	Chitosan promotes blood clotting and is used in dressings to control bleeding in wounds.
Moisture Retention	Chitosan-based dressings maintain a moist environment, which is beneficial for wound healing.
Anti-inflammatory Effects	Chitosan reduces inflammation and promotes faster healing by modulating the inflammatory response.
Scaffold for Tissue Regeneration	Chitosan provides a scaffold for cell proliferation and tissue regeneration, aiding in wound repair.

II.2.3.1. Advantages

- ✓ Hemostatic qualities of chitosan allow it to decrease bleeding and encourage blood clotting, which is advantageous for wound care and hemorrhage control [66].

- ✓ Due to its broad-spectrum antibacterial activity against bacteria, fungus, and certain viruses, chitosan can aid in the healing process and help prevent wound infections [35].
- ✓ By encouraging cell migration, proliferation, and tissue remodeling, moist wound environments are favorable to wound healing and can be maintained with the use of chitosan wound dressings [65].

II.2.3.2. Disadvantages

- ✓ There may be varied results when using chitosan to treat wounds because of factors like the nature and severity of the wound, the patient's unique traits, and the source and purity of the chitosan [65].
- ✓ Even though they are uncommon, allergic reactions to chitosan can happen to certain people, especially if they are known to be allergic to shellfish, which is the common source of chitosan [54].

II.2.4. Environmental Remediation Technologies

Environmental remediation applications have shown a great deal of interest in chitosan [45], a versatile biopolymer produced from chitin. Its capacity to chelate metal ions makes it easier for the metals to become immobile or precipitate [34], which is one common application in the removal of heavy metals from soil and water. Furthermore, chitosan is useful for adsorbing organic pollutants including dyes, insecticides, and medications due to its large surface area and functional groups, especially in wastewater treatment settings [51].

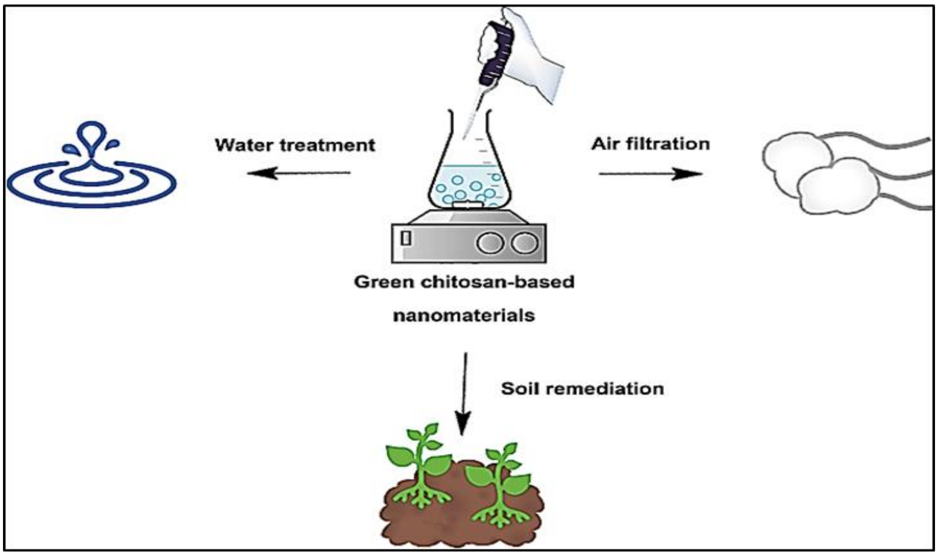


Figure II.4 : Green Nanomaterials for Environmental Remediation [67]

Composites incorporating chitosan, including clay nanocomposites or metal oxide hybrids, have been created to improve cleanup capabilities and adsorption efficiency [52]. By acting as a biostimulant, chitosan helps remove organic pollutants from the environment by promoting the growth of microbial populations participating in biodegradation processes. Chitosan supplements enhance soil structure, lessen erosion, and minimize contaminant leaching in contaminated locations, supporting soil stability and erosion control initiatives. Additionally, when incorporated into different water treatment technologies, such as filtration systems and adsorbent cartridges, chitosan-based compounds offer environmentally beneficial alternatives for the removal of water pollutants. However, putting chitosan-based remediation methods into practice necessitates careful consideration of regulatory frameworks and economic sustainability, which calls for in-depth impact research. Concerns over synthesis methods and long-term environmental effects will have a big influence on how chitosan is used in environmental rehabilitation efforts going forward. We use it also in water treatment, and this table shows some forms of chitosan and its uses.

Table II.3 : Uses of Chitosan in Environmental Remediation Technologies

Application	Chito-Form	Description
Air Purification	**Chitosan-Based Filters**	used to collect airborne contaminants in air filtration systems.
Marine Oil Spill Cleanup	**Chitosan Sponges**	used to remove oil from surfaces of water when spills occur.
Industrial Effluent Treatment	**Chitosan Coagulants**	used to filter impurities out of industrial effluent by coagulating it.
Heavy Metal Removal	**Chitosan Chelating Agents**	used in a variety of settings to bind and extract heavy metals like lead, copper, and mercury.
Pesticide Removal	**Chitosan Film**	used to absorb and break down pesticide residues found in runoff from farms.
Water Treatment	**Chitosan Flocculants**	used to collect pollutants and suspended particles for simpler cleanup.
	Chitosan Beads	used to remove organic contaminants, dyes, and heavy metals from water.
	Chitosan Membranes	used to filter contaminants out of wastewater using filtration devices.

II.2.4.1. Advantages

- ✓ Many remediation technologies can be applied directly at the contaminated site (in situ treatment), minimizing the need for excavation, transportation, and disposal of contaminated materials, which can be costly and disruptive [67].
- ✓ Some remediation technologies, such as phytoremediation and bioremediation, harness natural processes and organisms to degrade or sequester contaminants, offering sustainable and cost-effective solutions over the long term [51].
- ✓ Remediation technologies that are non-invasive, environmentally friendly, and socially acceptable are more likely to gain support from local communities and regulatory agencies, facilitating project implementation and success [15].

II.2.4.2. Disadvantages

- ✓ The cost of environmental remediation initiatives can be high, especially when dealing with complex or large-scale pollution sites. Planning, carrying out, and monitoring these operations all require substantial financial resources [20].
- ✓ There are delays in site cleanup and possible exposure concerns because many remediation solutions take a long time to provide the intended effects, particularly for persistent pollutants or in areas with limited natural attenuation processes [67].

II.2.5. Agricultural Formulations

Renowned for its strong antibacterial properties against a wide range of pathogens, including bacteria, viruses, and fungi, chitosan has become a promising instrument in agricultural methods for managing diseases. Its ability to impede the growth and spread of these bacteria highlights its potential use in crop protection. Beyond its ability to fight infections, chitosan has a complex interaction with plant systems, inducing defense mechanisms that increase plants' resistance to disease. Because of its two uses, chitosan is a useful tool for plant protection tactics [68].

Furthermore, studies have shown that chitosan can encourage plants to produce secondary metabolites. These metabolites are essential for strengthening the defensive systems of the plants and have a wide range of potential uses in the pharmaceutical and cosmetics sectors, among others [63]. Furthermore, chitosan-containing formulations support sustainable agriculture by providing safety and biodegradability as well as other environmental advantages.

Moreover, research has demonstrated that chitosan can stimulate the production of secondary metabolites in plants [68]. These metabolites offer a variety of possible applications in the pharmaceutical and cosmetics industries, among others, and are crucial for bolstering the defense mechanisms of the plants. Additionally, formulations containing chitosan promote sustainable agriculture by offering biodegradability, safety, and other benefits to the environment.

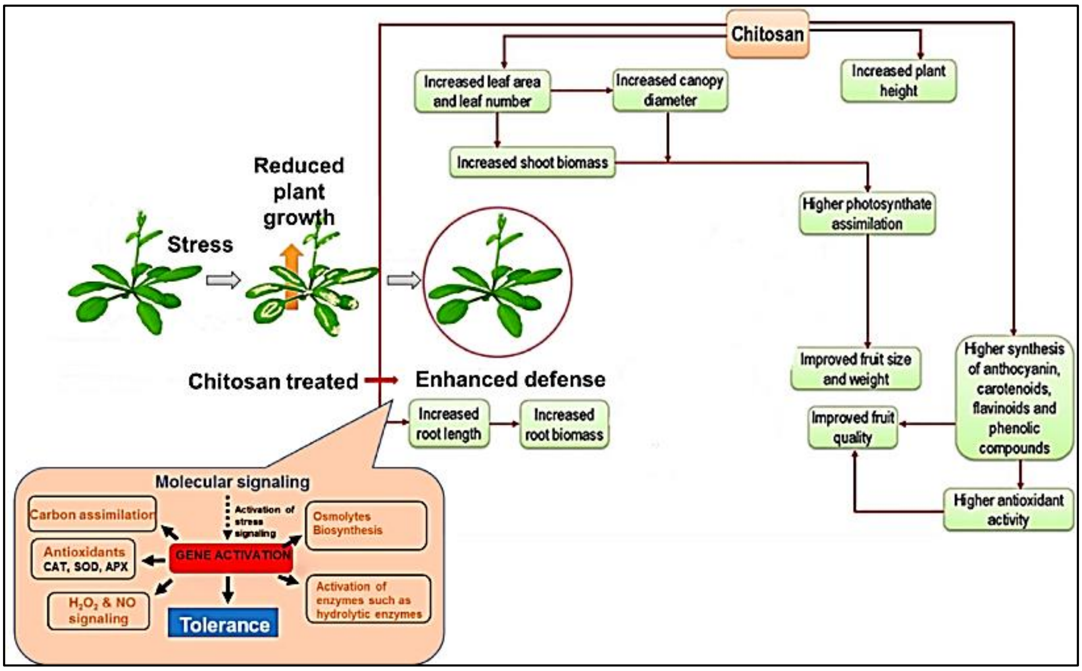


Figure II.5 : General description of chitosan-mediated plant growth regulation under stress conditions

II.2.5.1. Advantages

- ✓ It is possible to apply active substances precisely to target pests, weeds, or nutritional deficits with formulations that minimize off-target effects, minimize environmental damage, and maximize resource utilization [43].
- ✓ Advanced formulations can limit leaching, runoff, and volatilization, protect non-target creatures and ecosystems, and prevent environmental pollution [42]. Examples of these formulations include slow-release fertilizers and microencapsulated insecticides.
- ✓ Agricultural formulations can be adapted to particular crops, pests, or soil types, enabling specialized approaches to handle regional agricultural issues and maximize crop yields with the least amount of negative environmental effects [69].

II.2.5.2. Disadvantages

- ✓ Certain formulations might necessitate specific tools, instruction, or methods of application, which would make farming operations more complicated and logistically demanding and might prevent smallholder or resource-constrained farmers from adopting them [69].

- ✓ In order to guarantee safety, effectiveness, and environmental compliance, formulations must go through stringent testing and regulatory approval procedures. This can cause delays in market access, raise development costs, and restrict the supply of novel products [68].

Table II.4 : Mechanisms of Action of Chitosan in Agricultural Formulations

Application	Mechanism of action
Plant Growth Promoter	increases the permeability of the cell wall, which improves nutrient intake.
	increases the synthesis of phytohormones, including as auxins and gibberellins.
Biostimulant	activates genes involved in stress reactions, growth, and development.
	stimulates the enzymes necessary for photosynthesis and nitrogen metabolism.
Elicitor of Plant Defenses	increases the synthesis of pathogenesis-related proteins and phytoalexins, which induces systemic resistance.
Pesticide Carrier	reduces required dosages and lessens the impact on the environment by allowing for the controlled and continuous release of pesticides.
Seed Coating	Promotes better water retention and pathogen defense to increase seed germination.
	Enables early seedling growth by enhancing nutrient absorption and supplying a barrier of defense.

II.2.6. Tissue engineering

At this moment, cadavers and living donors are the main sources of organs and tissues for transplantation. However, there are drawbacks to this strategy, including concerns about tissue compatibility and the spread of disease. Furthermore, there is a widening gap between the demand and supply for transplants. Through the creation of commercially available tissues and organs, tissue engineering has come to light as a viable remedy for these constraints. This interdisciplinary field seeks to preserve and restore the functionality of damaged or malfunctioning organs and tissues by developing biological replacements [40].

Scaffolds are essential to tissue engineering because they replicate the extracellular matrix and give seeded cells a platform on which to grow. Because of its remarkable biocompatibility and biodegradability, chitosan is currently an essential biomaterial used in the construction of scaffolds. Because of its structural resemblance to extracellular matrix proteoglycans, it is especially well suited for tissue engineering of bone and cartilage. Furthermore, the porous structures of chitosan—achieved through manufacturing processes—mirror the natural porosity of bone, which increases the material's effectiveness in the regeneration of bone tissue [35].

Moreover, the innate antimicrobial and antifungal characteristics of chitosan alleviate tissue compatibility issues related to transplants. Chitosan promotes tissue regeneration in bone and cartilage engineering by facilitating the controlled release of growth factors in conjunction with collagen, hyaluronic acid, and hydroxyapatite. The best method for making chitosan-based scaffolds is freeze-drying, which results in high interpore morphology and the best possible outcomes for bone tissue engineering [5].

Chitosan is used in epidermal, vascular, epithelial, and ligament tissue engineering in addition to bone and cartilage [40]. Recent developments include scaffolds made of a chitosan-silk blend that are intended to mimic the viscoelastic properties of native cartilage tissue in order to improve cartilage engineering.

Chitosan's versatility makes it a promising material for a variety of tissue engineering applications, such as neuron, muscle, salivary gland, and blood vessel regeneration. To fully realize chitosan's potential in tissue engineering on a worldwide scale [59], more research is necessary.

II.2.6.1. Advantages

- ✓ Offering novel therapeutic alternatives for patients with injuries, degenerative diseases, or organ failure, tissue engineering has the potential to regenerate or replace sick or damaged tissues and organs [40].
- ✓ To improve the biological functionality of designed tissues, tissue engineering procedures can include growth factors, cytokines, and other bioactive substances to encourage cellular proliferation, differentiation, and tissue regeneration [40].
- ✓ With the use of tissue engineering techniques, it is possible to create customized tissue constructions that are adapted to each patient's unique requirements, including size,

shape, and biological characteristics [38]. This lowers the possibility of rejection and increases compatibility.

II.2.6.2. Disadvantages

- ✓ The intricacy of cellular interactions, tissue architecture, and physiological cues involved in tissue formation and homeostasis make it difficult to replicate the complex structure and function of native tissues and organs in vitro [70].
- ✓ Insufficient vascularization of engineered tissues can make it difficult for the cells to remain viable and operate over time [70]. This can result in problems including necrosis, poor integration with host tissue, and unfavorable long-term consequences.

II.2.7. Food and Cosmetics Additives

Chitosan It functions in food as a naturally occurring antibacterial and preservative, successfully preventing the growth of bacteria and fungi to increase the shelf life of perishable goods like seafood, meats, fruits, and vegetables[71]. Its capacity to create protective films improves the texture and freshness of food while it is being transported and stored. Furthermore, chitosan helps to clarify and stabilize drinks like wine and fruit juice, which improves their aesthetic appeal to customers.

Furthermore, because of its antioxidant and cholesterol-lowering qualities, chitosan may have health benefits beyond its role in preservation in functional foods and nutritional supplements [72]. Its film-forming and moisturizing properties make it a sought-after ingredient in cosmetics, hydrating and enhancing the texture of skin. Moreover, the intrinsic antimicrobial qualities of the product help preserve it, guaranteeing its durability and safety.

All things considered, chitosan's natural source, biocompatibility, and versatility make it an invaluable tool for the culinary and cosmetics sectors [50]. It helps preserve shelf life, improve product quality, and satisfy consumer needs for natural and sustainable components.

II.2.7.1. Advantages

- ✓ Vitamins, minerals, and dietary supplements are examples of additives that can be added to food and cosmetic items to improve their nutritional content, make up for nutrient shortfalls, or give consumers additional health advantages [72].

- ✓ In food and cosmetic formulations, additives have a variety of functional uses, such as emulsification, stability, thickening, and texturizing, which enhance the performance, usability, and consistency of the final product [71].
- ✓ The sensory qualities of food and cosmetic items are improved by flavor enhancers, colorants, and texture modifiers, which increases consumer acceptance and enjoyment of the product overall [48].

II.2.7.2. Disadvantages

- ✓ Overconsumption of some additives, especially in high amounts or over an extended period of time, can lead to metabolic problems, nutritional imbalances, and unfavorable health effects [73]. Examples of these additives include artificial sweeteners, flavor enhancers, and synthetic colorants.
- ✓ Even though additives are safe and helpful when used as intended, negative publicity or misconceptions about certain chemicals may affect customer perception and purchasing behavior, resulting in decreased consumer trust, demand, or acceptance of products containing additives [73].

II.3. Unlocking the Potential: The Multifaceted Applications of Chitosan

Due to its many industrial applications, chitosan, a naturally occurring biopolymer derived from chitin, has garnered a lot of interest. This polysaccharide, which is mostly found in crustaceans like fish and crabs, has special properties like its ability to break down naturally, its antimicrobial activity, its non-toxicity, and its compatibility with nature, making it an extremely versatile polymer with a wide range of uses [12].

- Chitosan has been investigated in the biomedical area for its potential in drug delivery systems, wound healing, tissue engineering, and as a scaffold for regenerative medicine [66]. Its hemostatic qualities and capacity to encourage cell adhesion and proliferation make it an important component of wound dressings and surgical implants.
- Chitosan has been used in agriculture as a plant growth enhancer and biopesticide. Its antifungal and antibacterial qualities aid in crop disease prevention, and its biostimulant qualities promote plant development and increase yield [43]. Furthermore, chitosan-based formulations for crop protection and sustainable farming methods have demonstrated potential in agricultural biotechnology.

- Applications for chitosan include soil remediation, wastewater treatment, and water purification in environmental remediation [51]. Its capacity to absorb organic contaminants and heavy metals makes it a useful tool for pollution mitigation and environmental detoxification.
- Chitosan is also used in the food and cosmetics industries as a thickening agent, natural preservative, and encapsulating material for active substances. Its biocompatibility makes it perfect for cosmetic formulations, and its film-forming qualities make it appropriate for edible coatings that prolong the shelf life of perishable foods [72].

The many uses of chitosan demonstrate the material's potential as an environmentally friendly and sustainable resource with a broad range of advantages in a number of industries, including agriculture, healthcare, and environmental protection. Chitosan is positioned to become more and more important in solving today's problems and promoting innovation across a range of industries as research and technology developments progress.

II.4. Disassembling Chitosan

Chitosan is an extraordinary natural polymer that is produced when chitin, which is present in crab shells, is deacetylated. It is a unique polysaccharide with a linear structure that is mainly made up of glucosamine and N-acetylglucosamine. It also has biodegradable and biocompatible qualities. This makes it useful in a variety of industries, such as environmental applications, food science, and biomedicine. Chitosan, a substance well-known for its adsorption powers, is used in water treatment to extract impurities and heavy metals. Its antibacterial properties are very useful in medical and food preservation environments. Because of its capacity to form films and gels and its compatibility with biological systems, it finds application in medicine for a variety of reasons, ranging from wound dressings to drug delivery systems.

Furthermore, chitosan is sold as a dietary supplement, despite the paucity of scientific data to back up its claimed advantages in weight loss. Despite this, research and innovation are still motivated by its adaptability and environmental friendliness, which bodes well for future developments across a range of industries.

II.5. Conclusion

To sum up, chitosan is a biopolymer that is exceptionally flexible and has a vast range of possible applications. Its distinct physicochemical qualities, including as antibacterial activity, biodegradability, and biocompatibility, make it a desirable material for a range of creative applications. Because of its increased versatility as a result of chemical changes, chitosan can be used for a variety of industrial and commercial applications. Large-scale manufacturing and standardization are hampered by the heterogeneity in its qualities brought about by variations in the sources and processing techniques.

The fact that chitosan is environmentally friendly is one of its main benefits. Chitosan, a naturally occurring material derived from chitin, provides a sustainable substitute for synthetic polymers. In light of growing environmental consciousness and the need for greener technologies, this sustainability component is very important. However, significant advancements in extraction and processing methods are required to lower the cost of chitosan production, as high production costs continue to be a key obstacle to its broad use. Regulatory barriers also necessitate thorough research to set safety and efficacy requirements, particularly in the food and biomedical industries.

A interdisciplinary strategy including material scientists, biochemists, engineers, and regulatory specialists is required to realize the full potential of chitosan. Through cooperative research and innovation, current barriers can be surmounted and the usefulness of chitosan can be improved. The following chapter will concentrate on examining particular case studies and new developments that demonstrate the continuous development of chitosan applications. Through these case studies, chitosan's transformative potential in improving sustainability and technology will be highlighted, offering greater insights into how it can be further refined and deployed.

Chaptre III

III.1. Introduction

This chapter will cover several important topics, starting with the extraction of chitin from shrimp shells and its chemical transformation into chitosan. We will then examine the physico-chemical characterization of chitosan, focusing on key variables such as its solubility and degree of deacetylation, in order to confirm its suitability for marketing. Characterization techniques will include X-ray diffraction (XRD), thermogravimetric analysis (ATG/DTG), Fourier transform infrared spectroscopy (FTIR) and X-ray fluorescence (XRF). In addition, we will use an optical microscope to explore other characteristics, including melting temperature and morphology.

III.2. Display of initial product

The raw material used in this research is shrimp shells obtained from an aquaculture farm located in Skikda province. More precisely in the commune of Marsa under the name Experimental shrimp breeding farm (CNRDPA)).

Figure III.1 : Shrimp farming

III.3. Preparation of Shrimp Shell Prior to Processing

A mass of shrimp have been carefully peeled, then washed several times with tap water, then with distilled water, then boiled in distilled water - this step removes the fat contained in the shrimp shells. They are then drained and air-dried for 48 hours. Finally, the shrimp exoskeletons are ground to a fine powder using an electric grinder.

III.4. Chitosan extraction process

The fundamental process involves extracting chitin from shrimp shells using sequential treatments with alkaline and mineral acid solutions. Initially, the shrimp shells undergo deproteinization with a concentrated alkaline solution to remove proteins, followed by demineralization with a strong mineral acid to eliminate calcium carbonate and other minerals. After purification, the chitin is subjected to deacetylation under controlled alkaline conditions, transforming it into chitosan. To extract chitin, approximately P_0=90g of ground shrimp exoskeletons are processed through these three essential stages. First, the exoskeletons are treated with sodium hydroxide to achieve deproteinization. Next, they are immersed in hydrochloric acid for demineralization. Finally, the purified chitin is treated with a concentrated alkaline solution to undergo deacetylation, yielding high-quality chitosan.

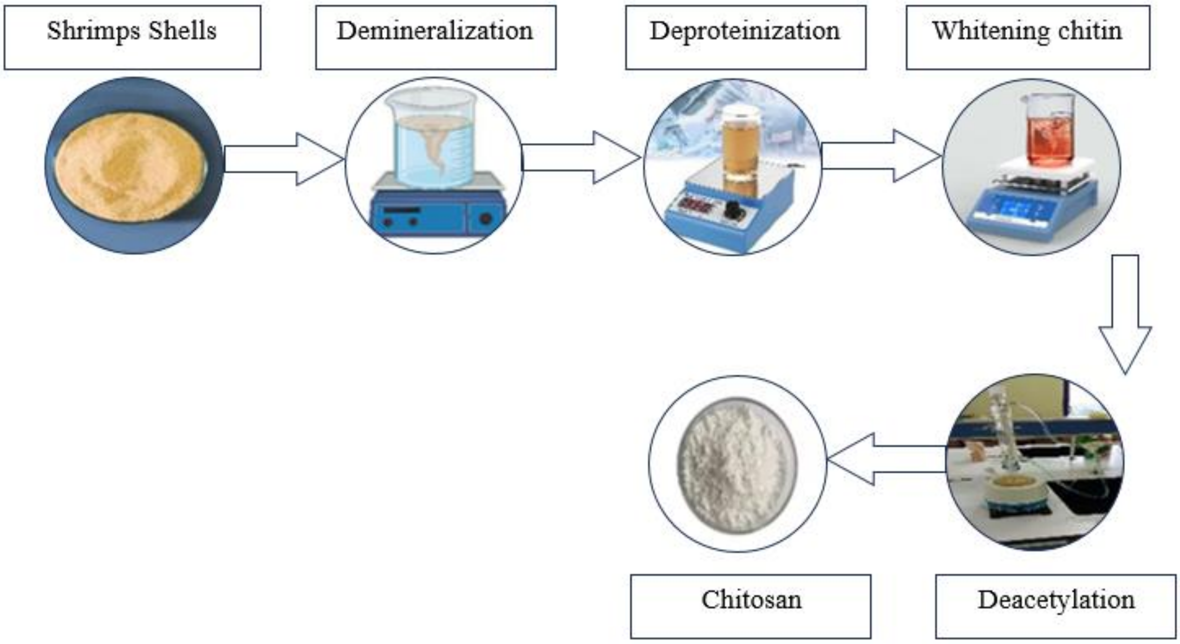


Figure III.2 : Extraction chitosan from shrimps shells.

III.4.1. Demineralization

In a beaker, the pretreated shrimp shells (washed, ground, and dried) were carefully mixed (90g) in an aqueous solution of hydrochloric acid (HCl) [74](1N) with a ratio of 1/15 (m/v). The mixture was magnetically stirred at room temperature overnight to dissolve the minerals accompanying the shells. Calcium carbonate ($CaCO_3$), the main mineral component of the shell (between 30 and 50%), reacts with HCl to form calcium chloride, water, and carbon dioxide as described in reaction (I):

$$2HCl\ (aq) + CaCO_3(s) \rightarrow CaCl_2\ (aq) + H_2O\ (l) + CO_2\ (g) \ldots \text{Eq.1}$$

Finally, the contents of the beaker were filtered through a filter cloth and washed thoroughly with distilled water until neutral. The resulting powder was dried in an oven at 50°C.

III.4.2. Deproteinization

The demineralized shells, after washing with distilled water, were mixed in a 10% sodium hydroxide (NaOH) solution in a proportion of 1/20 (m/v) (dry shell weight/NaOH volume). The mixture was heated to 100°C in a heating flask for 6 hours. This treatment helps eliminate proteins, which are present at a rate of 30 to 40% in shrimp shells. At the end of the treatment, the contents of the beaker were again filtered through a filter cloth and thoroughly rinsed with distilled water until neutral. Distilled water was replaced with acetone for the final rinse to remove residual lipid impurities [75]. The filtrate was then transferred to an oven at 50°C overnight.

III.4.3. Bleaching

This step involves removing pigments in the crustacean shells that form complexes with chitin, such as β-carotene derivatives [76]. Bleaching chitin is often done by treating it with oxidizing agents such as H_2O_2 (2.5N) in a mass ratio of 1/10 (solid/liquid; g/ml) at 30°C for 3 hours. The mixture is then filtered and washed several times with distilled water to remove any remaining components, obtaining a neutral medium. The chitin is then dried in an oven at 35°C for 24 hours.

III.4.4. Deacetylation

The deacetylation of the extracted chitin is performed with a highly concentrated NaOH solution (40-50%) at a temperature of 100°C or higher. In our case, we chose to work with 50% NaOH in a 1/20 (m/v) ratio, where the mixture was refluxed at 120°C for 3 hours. At the end of the treatment, the contents of the beaker were filtered and thoroughly washed with distilled water until neutral. To speed up the dehydration process, the mixture was rinsed with ethanol and placed in an oven overnight at 50°C, then ground to obtain chitosan in powder form [77]. After each drying, the dry material was weighed to determine the yield of each reaction.

III.5. Characterization of chitosan

III.5.1. Yield evaluation

Yield refers to the mass of the extract determined after synthesis and extraction, expressed as a percentage relative to the initial mass of the shrimp shell powder subjected to extraction. The yield (R) is calculated using the following formula:

$$\boldsymbol{R}(\%) = \frac{\boldsymbol{M}}{\boldsymbol{M_0}} * \mathbf{100}$$

Where:

- R: Yield expressed as a percentage
- M: Mass in grams of the resulting dry extract
- M_0: Mass in grams of the plant material to be processed

III.5.2. Determination of Dry Matter

It is determined by drying in a ventilated oven to a constant weight according to the official method (AOAC, 1995). The moisture content corresponds to the difference in weight between one gram of sample powder and its weight after being heated at 105 ± 2 ºC for 2 hours. The results are expressed in g/100 g fresh matter (FM) or g/100 g dry matter (DM).

$$\boldsymbol{Dry\ matter\ content} = \frac{(\boldsymbol{M_2} - \boldsymbol{M_0})}{(\boldsymbol{M_1} - \boldsymbol{M_0})} * \mathbf{100}$$

- M_0: the mass in grams of the crucible;
- M_1: the mass in grams of the crucible with the test sample;
- M_2: the mass in grams of the crucible with the dry matter.

III.5.3. Determination of Ash Content

This analysis is carried out according to the official method (AOAC, 1995), and involves placing 2 grams of previously dried powder into a porcelain crucible, taring it, and then incinerating it in a muffle furnace at 550°C for 4 hours. The weight of the residue corresponds to the mineral content. The results are expressed in g/100 g of dry matter (DM).

$$\boldsymbol{Ash\ content} = \frac{(\boldsymbol{M_2} - \boldsymbol{M_0})}{(\boldsymbol{M_1} - \boldsymbol{M_0})} * \mathbf{100}$$

- M_0: the mass in grams of the incineration crucible;
- M_1: the mass in grams of the crucible with the test sample;
- M_2: the mass in grams of the crucible with the ash.

III.5.4. Determination of Crude Protein Content

Total nitrogen (N) is determined using the Kjeldahl method, following the alternative procedure II (AOAC, 1995). This quantification method consists of three steps: digestion, distillation, and titration.

III.5.4.1. Digestion

Digestion involves converting the organic nitrogen of the dry sample (0.5g) into mineral nitrogen (ammonium sulfate) in the presence of 10 ml of concentrated sulfuric acid and a catalyst. In our study, we used 0.5g of copper sulfate $CuSO_4$ (Kjeltabs CT, Thompson and Capper LTD), heated at 450°C for 4 hours.

III.5.4.2. Distillation

The digested sample is then placed in an automatic distillation system, Kjeldahl VELP Scientifica UDK 129. After converting the ammonium sulfate into ammonia using a strong base (35% NaOH), the ammonia is carried by steam and collected in a boric acid solution containing a color indicator.

III.5.4.3. Titration

The distillate obtained is titrated with a 0.05N H_2SO_4 solution. The nitrogen content of the dry matter is calculated using the following equation:

$$Total\ nitrogen\ (N) en\ mg/g = \frac{(V_1 - V_0) * M * 14 * 100}{m}$$

Where:

- V_0: Volume in ml of sulfuric acid used for the blank;
- V_1: Volume in ml of sulfuric acid used for the sample;
- M: Concentration of the sulfuric acid;
- m: Mass in grams of the sample;

- 14: Molar mass of nitrogen.

The total protein content is obtained by multiplying the nitrogen content by 6.25 and expressed in g/100 g of dry matter (DM).

III.5.5. Determination of Lipid Content

A 1g sample is placed in a cellulose cartridge of a Soxhlet extraction system. 25 ml of hexane is added to pre-washed, dried, and tared aluminum (or Pyrex) crucibles. Extraction is carried out by boiling the solvent and condensing its vapors using a condenser [78]. This extraction lasts about 1 hour. The crucibles are then placed in an oven at 105°C for 24 hours to completely remove any remaining solvent.

Figure III.3 : Soxtherme used to determine lipid content.

The crucibles are then cooled in a desiccator and weighed again. The fat content is calculated by:

$$\boldsymbol{Lipid\ content = \frac{(P_2 - P_1)}{P_0} * 100}$$

- M_0: the mass in grams of the sample;
- M_1: the mass in grams of the empty crucible;
- M_2: the mass in grams of the crucible containing the fat.

III.5.6. Fourier Transform Infrared Spectroscopy (FTIR)

III.5.6.1. Principle

Fourier Transform Infrared (FTIR) Spectroscopy is used to identify the presence of specific chemical groups. This analytical method quickly provides IR spectra of solid and liquid samples without requiring extensive pre-treatment. The principle is based on the vibrations between atoms and various groups within a molecule when excited by a wavelength from the infrared spectrum [79]. The device measures these vibrations and provides a characteristic spectrum of the molecule (when pure), enabling identification and recognition, particularly through the use of databases (Pominville RacetteMathie, 2015).

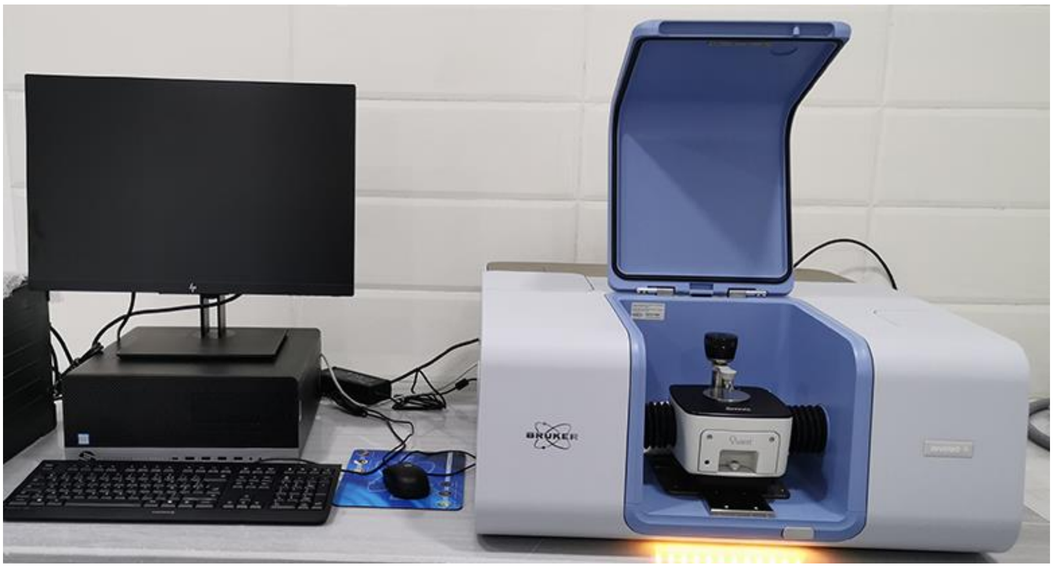


Figure III.4 : Infrared spectroscopy IR

III.5.6.2. Characterization of Chitosan by FTIR Spectroscopy

This straightforward technique is widely used to study the composition and structure of chitin, to distinguish the α form from the β form, and sometimes to determine the degree of acetylation. Samples of shrimp shells, extracted chitin, and extracted chitosan were all ground into a fine powder. An infrared spectrophotometer was used to record the IR spectra of the samples (extracted chitin, shrimp shells, and extracted chitosan) in absorption mode in the range of 400 to 4000 cm^{-1}.

III.5.7. Degree of Deacetylation (DD) of Chitosan

The chosen method to determine the degree of deacetylation is infrared (IR) spectroscopy. IR spectroscopy is the most common method for determining the DD. This analysis provides the vibrational spectral fingerprints of the groups constituting a molecule. The degree of deacetylation can be determined using the following formula:

$$DD = \left(1 - \frac{A_{1655}}{A_{3450} * 1.33}\right) * 100$$

Where:

- A_{1655} is the absorbance at 1655 cm⁻¹ (amide I band);
- A_{3450} is the absorbance at 3450 cm⁻¹ (hydroxyl band).

III.5.8. Solubility Test of Chitosan

To study the effect of acetic acid concentration on the dissolution of chitosan, a series of 25 ml solutions at different acid concentrations (0.1, 0.5, 1, 1.5, and 2 mol/l) are prepared, in which 0.08 g of extracted chitosan is dissolved under magnetic stirring for 24 hours at room temperature. The polymer solutions were vacuum filtered using filter paper, and the resulting residues were dried in an oven for 24 hours. The insoluble fractions were weighed after drying, and their weights (m) were used to calculate the solubility rates using the formula proposed by Mohy Eldin et al. (2008) below.

$$\text{Solubility Rate} = [1 - \left(\frac{\text{Weight of the insoluble fraction}}{\text{Total weight of the sample}}\right)$$

III.5.9. X-ray Fluorescence Spectrometry (XRF)

X-ray Fluorescence Spectrometry (XRF) is an analytical method used to determine the elemental composition of a sample. XRF spectroscopy is primarily employed for the analysis of inorganic elements and is based on the principle of characteristic X-ray emission from excited atoms [79]. Approximately 2g of chitosan was formed into a pellet before introduction into the X-ray fluorescence spectrometry dev bàice (XRF).

Figure III.5 : X-ray fluorescence spectroscopy device

III.5.10. Optical Microscope

Observing the morphology of chitosan under an optical microscope is a commonly used method to visualize the structure and shape of chitosan samples. During optical microscope observation, features such as the shape of chitosan particles, their size, arrangement, and distribution can be observed. Chitosan can form aggregates, fibers, films, or other structures depending on preparation conditions and material properties [80]. For this purpose, a small amount of powder is placed on a glass slide and covered with a coverslip before proceeding to microscopic observation.

Figure III.6 : Optical microscope

III.5.11. X-ray Diffraction (XRD)

When subjected to a beam of X-rays, a crystalline sample reflects diffracted beams whose angular deviations from the incident beam provide information about the crystalline planes it contains and their characteristic spacings [81]. The results obtained can be analyzed using Bragg's law:

$$2\ d \sin \theta = n.\lambda$$

Where,

- θ: the angle of the incident beam relative to the plane
- n: a positive integer called the order of diffraction
- λ: the wavelength of the incident beam
- d: the interplanar spacing, characteristic of the materi

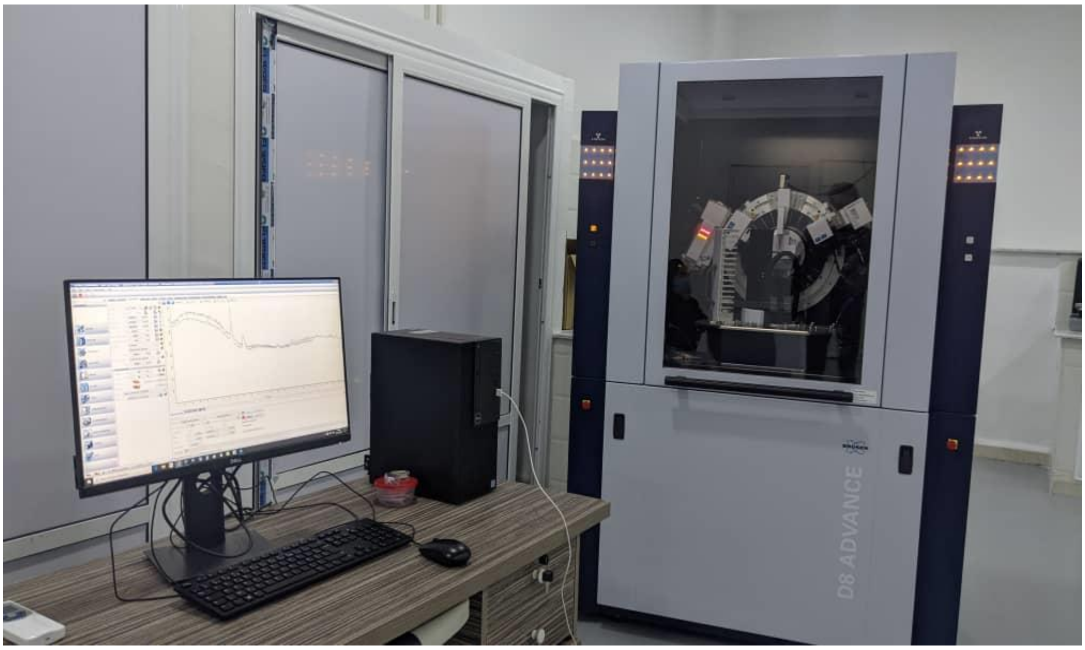


Figure III.7 : X-ray diffraction equipment

X-ray diffraction characterization was performed at Salah Boubnider University of Constantine using a BRUKER D8 ADVANCER instrument, utilizing the Cu $K\alpha_1$ line with a wavelength of η = 1.540598 Å. Diffractograms were recorded from 2° to 70° with a step size of 0.016°. The crystallinity percentage (degree) is calculated from the ratio of the area under the crystalline phase to the total area, according to the following equation:

$$X_C = \frac{A_C}{A_C + A_A} * 100$$

- X_C : the crystallinity percentage ;
- A_C : the area of the crystalline phase ;
- A_A : the area of the amorphous phase.

III.5.12. Thermogravimetric Analysis

Thermogravimetric analysis is a quantitative analytical method that measures the variation in mass of a sample subjected to thermal treatment, as a function of temperature and time. TGA curves can provide extensive information regarding chemical reactions: thermodynamics, kinetics, mechanisms, intermediate and final products, [82] etc. They also provide insights into the thermal stability of materials. The apparatus used is a thermogravimetric analyzer, primarily composed of a furnace where the sample is placed, and a microbalance for measuring mass changes. Thermograms of various samples were recorded using a Toledo-type device, equipped with coupled TGA/DTG capabilities and controlled by a microcomputer. This model can operate in the temperature range of 20 to 900°C, with a mass measurement precision ranging from 10 mg to 20 mg. Measurements are conducted under a nitrogen atmosphere with a heating rate of 10°C/min.

Figure III.8 : Thermal mass loss analyzer

III.6. Conclusion

The third chapter highlighted the physico-chemical and biological properties of chitosan, as well as extraction and purification methods. The results obtained demonstrate that chitosan possesses remarkable characteristics that make it an ideal candidate for a variety of applications. The detailed analyses carried out in this chapter provide a solid basis for future research and technological development, confirming chitosan's significant potential in a variety of application areas.

Chaptre IV

IV.1. Introduction

This section is essentially devoted to the presentation and discussion of the various results obtained. We begin with the interpretation of the different yields, followed by the physico-chemical analysis of chitosan, then the structural composition of the biopolymer is determined by FTIR, FRX, DRX optical microscopy and ATG/DTG.

IV.2. Chemical composition

The chemical composition of chitosan is determined through a series of analyses that include extraction yield and physico-chemical characterization.

IV.2.1. Extraction yield

The table IV.1 shows the different yields of chitosan extraction from shrimp shells through the demineralization, deproteinization, bleaching and deacetylation steps.

Table IV.1 : Extraction yields in the various stages

Stage	Demineralization	Deproteinization	Bleaching	Deacetylation
Yield (%)	31,85	80,93	79,68	74,63

The demineralization yield was 31.85%, indicating that approximately 31.85% of the initial material was recovered after the removal of minerals, mainly calcium carbonate. The deproteinization yield is 80.93%, showing significant material recovery after protein removal, essential for chitosan purification. The bleaching yield is slightly lower, at 79.68%, which may be due to the loss of some unwanted organic components or to further purification, improving the color and purity of the final product. Finally, the deacetylation yield was 74.63%, indicating substantial recovery of material after conversion of chitin to chitosan by removal of acetyl groups, essential for obtaining high-quality chitosan. These yields demonstrate the efficiency of each step in the chitosan production process, with substantial removal of unwanted mineral matter during demineralization and efficient purification with significant retention of useful material during the deproteinization and bleaching steps, culminating in efficient conversion of chitin to chitosan during deacetylation.

IV.2.2. Physico-chemical characterization

The table IV.2 shows analyses of various chitosan properties, including dry matter, water, ash, volatile matter, protein and lipid contents.

Table IV.2 : Physico-chemical analysis of chitosan

Extract	Dry matter content (%)	Water content (%)	Ash content (%)	Volatile matter content (%)	Protein content (%)	Lipid content (%)
Chitosan	95.17±0.57	4.83±0.64	0.7±0.46	99.3±0.46	3.9 ± 0.01	Nd

The dry matter content of chitosan is 95.17% ± 0.57, indicating that the majority of the material consists of non-volatile solids. The water content is 4.83% ± 0.64, which is relatively low and indicates that chitosan is mainly dry, with little residual moisture.

The ash content, at 0.7% ± 0.46, represents the mineral fraction of chitosan after incineration. This shows that chitosan contains very little mineral residue, which is typical for a purified biopolymer. Volatile matter content is 99.3% ± 0.46, suggesting that almost all the material is susceptible to volatilization or decomposition at high temperatures, a common feature of biopolymers.

Protein content was 3.9% ± 0.01, indicating the presence of trace amounts of protein, probably residual from the biological sources used for chitosan extraction. Finally, lipid content is not detected (Nd), meaning that lipids are either absent or present in quantities too small to be accurately measured by the methods used.

In summary, these analyses show that chitosan is mainly composed of dry matter with low water and ash content, a high proportion of volatile matter, minimal presence of protein and no detectable lipids. These characteristics confirm the purity of chitosan and its suitability for applications requiring a biopolymer with these specific properties.

IV.3. Solubility test

The graph in figure IV.1 shows the solubility of chitosan in acetic acid solutions at different concentrations (0.1%, 0.5%, 1%, 1.5% and 2%). It can be seen that at an acetic acid concentration of 0.1%, the solubility rate of chitosan is 84%. This relatively low value indicates that the low acetic acid concentration is not sufficient to effectively dissolve chitosan. When the acetic acid concentration is increased to 0.5%, the chitosan solubility rate improves considerably, reaching 92%. This increase indicates that acetic acid is beginning to have a stronger effect on chitosan dissolution. By further increasing the acetic acid concentration to 1%, chitosan solubility reached 95%, showing continued improvement in solubility. At 1.5%, solubility reached 96%, indicating a further slight increase over 1%. Finally, at the maximum concentration tested of 2%, chitosan solubility reaches 99%, showing that almost all the chitosan is dissolved in this concentration of acetic acid. In summary, the solubility of chitosan in acetic acid increases with the concentration of acid, reaching almost complete dissolution at a concentration of 2%. This suggests that to achieve maximum chitosan solubility, an acetic acid concentration of at least 2% is required.

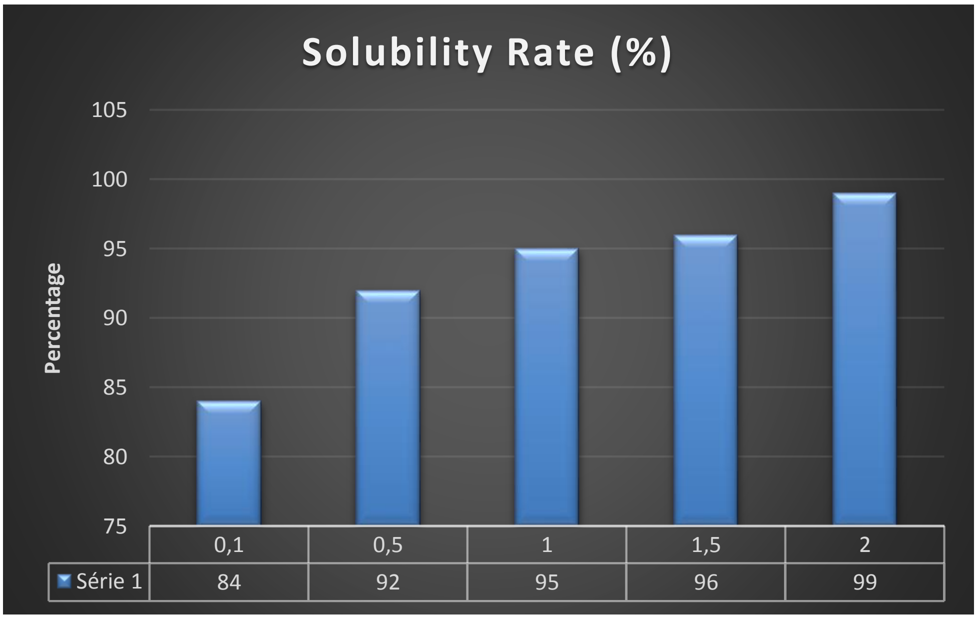


Figure IV.1 Figure IV.8 : Solubility ratio profile of chitosan

IV.4. X-ray fluorescence spectroscopy

The table IV.3 of elements detected in chitosan by X-ray fluorescence spectroscopy (XRF) reveals that light elements, representing mainly carbon, oxygen, nitrogen and hydrogen, make up 99.63% of the composition, which is characteristic of the organic structure of chitosan, a polysaccharide derived from chitin. Impurities include aluminum (0.221%), silicon (0.083%), copper (0.040%), zinc (0.022%), iron (0.005%), calcium (0.003%), nickel (0.001%) and lead (0.001%). Aluminum and silicon may originate from laboratory equipment or chemical reagents used, while copper, zinc and iron, although present in small quantities, could indicate slight metallic contamination from materials used during chitosan production. Calcium, detected in very small quantities, could be residual from crustacean shells if chitosan is extracted from chitin, or from contaminants in the reagents. Traces of nickel and lead, even in minute quantities, underline the importance of monitoring environmental contamination and chemical reagent residues, particularly lead, which poses health risks, especially in biomedical and pharmaceutical applications where chitosan purity is crucial.

Table IV.3 : Chemical composition of chitosan by XRF

Elements	Percentage (%)
Lights elements	99.63
Aluminum	0.221
Silicon	0.083
Copper	0.040
Zinc	0.022
Iron	0.005
Calcium	0.003
Nickel	0.001
Lead	0.001

IV.5. Structural and chemical characterization of chitosan

This section explores the structural and chemical characterization of chitosan using several analytical techniques. Fourier transform infrared spectroscopy (FTIR) identifies functional groups, while X-ray diffraction (XRD) reveals the crystalline and amorphous structure. Thermogravimetric analysis (TGA) and derived thermogravimetry (DTG) examine thermal stability, and optical microscope imaging shows particle morphology.

IV.5.1. Fourier transform spectroscopy

The FTIR spectrum IV.2. of chitosan reveals the presence of numerous characteristic functional groups, such as hydroxyl, amine, amide, methyl, methylene and ether groups. The broad, intense band at 3435 cm^{-1} corresponds to the elongation vibrations of the O-H and N-H bonds, indicating the presence of hydroxyl and amine groups, responsible for chitosan's hydration capacity and hygroscopic properties. Another band at 3256 cm^{-1} is also attributed to elongation vibrations of the N-H bonds of primary amines, confirming the presence of free amine groups. The peak at 3097 cm^{-1} is associated with C-H elongation vibrations of methyl and methylene C-H bonds, while the band at 2880 cm^{-1} corresponds to C-H elongation vibrations of aliphatic CH and CH_2 groups. The less intense peak at 2330 cm^{-1} could be related to vibrations of C≡N or C≡C bonds, although this is less common in pure chitosan. The peak at 1626 cm-¹ is characteristic of C=O elongation vibrations of amide I groups, present in the N-acetylglucosamine units of chitosan, while the band at 1554 cm-¹ is due to N-H and C-N deformation vibrations of amides II. The peak at 1381 cm-¹ can be attributed to C-H deformation vibrations of the methyl and methylene groups, reflecting the aliphatic structure of chitosan. The peak at 1009 cm-¹ is associated with the C-O-C strain vibrations of glycosidic bonds, indicating the presence of ether bonds in the polysaccharide structure. Finally, the band at 526 cm-¹ may be linked to out-of-plane deformation vibrations of C-H bonds in the polymer's pyranic rings. This information is essential for understanding the chemical and physical properties of chitosan, influencing its potential applications in the biomedical, pharmaceutical and materials fields.

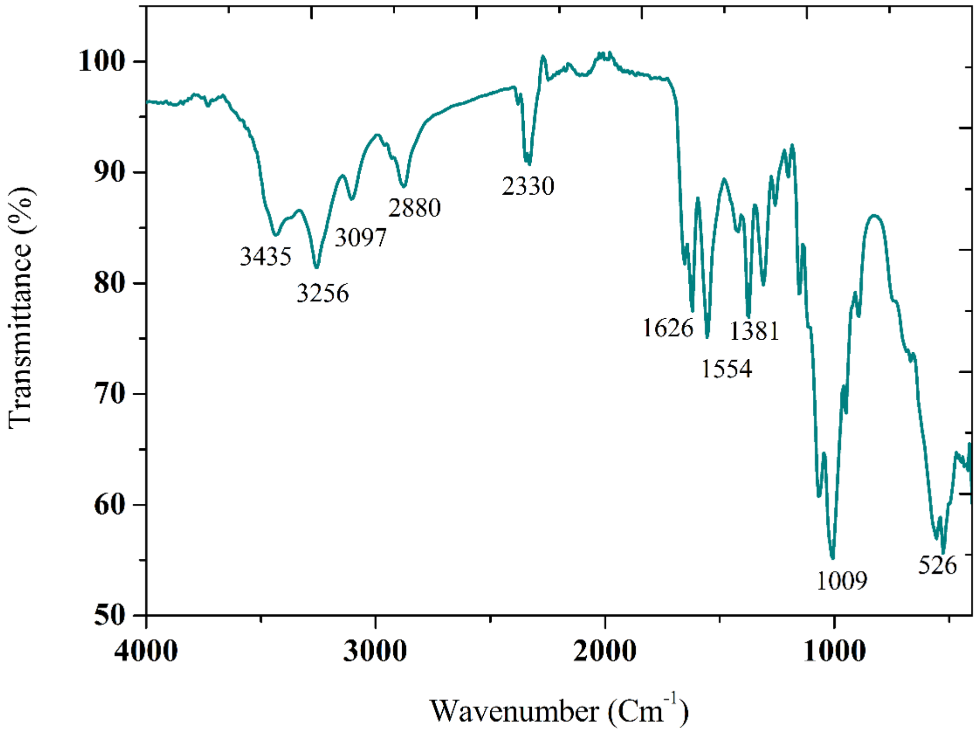


Figure IV.2 : Infrared spectrum of chitosan

IV.5.2. Determination of degree of deacetylation (DD%)

To calculate the degree of deacetylation (DD) of chitosan from the FTIR spectrum, the ratios of the intensities of certain peaks characteristic of the functional groups present in chitosan are generally used. Here's the common formula for calculating DD from FTIR spectra:

$$DD = \left(1 - \frac{A_{1655}}{A_{3450} * 1.33}\right) * 100$$

Where:

- A_{1655} is the absorbance at 1655 cm^{-1} (amide I band);
- A_{3450} is the absorbance at 3450 cm^{-1} (hydroxyl band).

$$DD = \left(1 - \frac{0.25}{0.75 * 1.33}\right) * 100$$

$$DD = 74.94\ \%$$

Therefore, the degree of deacetylation of chitosan is 74.94%.

IV.5.3. X-ray Diffraction (XRD) analysis of Chitosan

The graph IV.3 shows the X-ray diffractogram (XRD) of chitosan, which provides information on the crystalline and amorphous structure of the material.

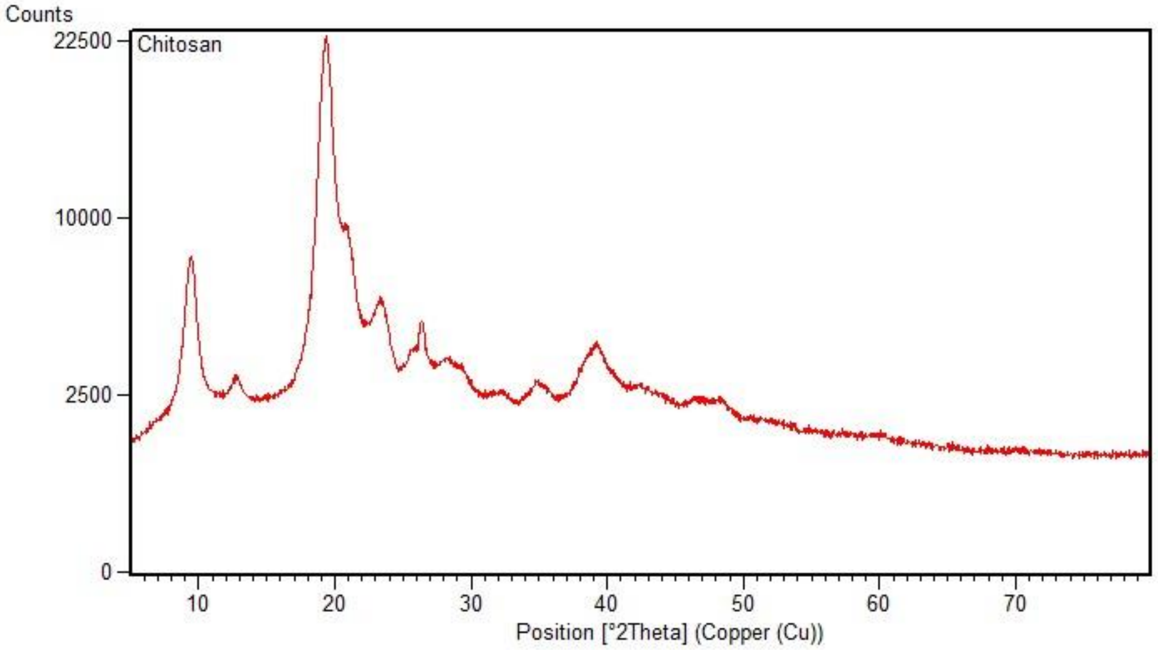


Figure IV.3 : XRD spectrum of chitosan

The intense peak around 2θ = 20° is characteristic of chitosan and indicates a certain crystallinity in its structure, corresponding to the ordered arrangement of polymeric chains.

Chitosan, derived from chitin, retains a semi-crystalline structure with crystalline and amorphous zones. A less intense peak observed around 2θ = 10° can be attributed to a second crystalline phase or to smaller ordered domains in the polymeric structure. The width of the peaks and a relatively high baseline indicate a strong amorphous component in chitosan, due to the disordered arrangement of the molecular chains. This disorder contributes to chitosan's flexibility. The XRD of chitosan thus shows a semi-crystalline structure with diffraction peaks revealing ordered zones in the polymer matrix, while the amorphous nature is highlighted by the width of the peaks and a high baseline. This structure is typical of chitosan, which exhibits a balance between crystalline parts, conferring rigidity and thermal stability, and amorphous parts, offering flexibility and better interaction with solvents and other chemicals. This structural profile is crucial for many chitosan applications, such as protective films, biomedical devices and matrices for controlled drug release, where specific properties of crystallinity and amorphism are required.

IV.5.4. Thermogravimetric Analysis (TGA) and Derived Thermogravimetry (DTG) of Chitosan

The graph IV.4 shows the results of thermogravimetric analysis (TGA) and derived thermogravimetry (DTG) for chitosan, a biopolymer derived from chitin. The TGA curve shows weight loss in percent, while the DTG curve represents the derivative of this loss with respect to temperature in degrees Celsius.

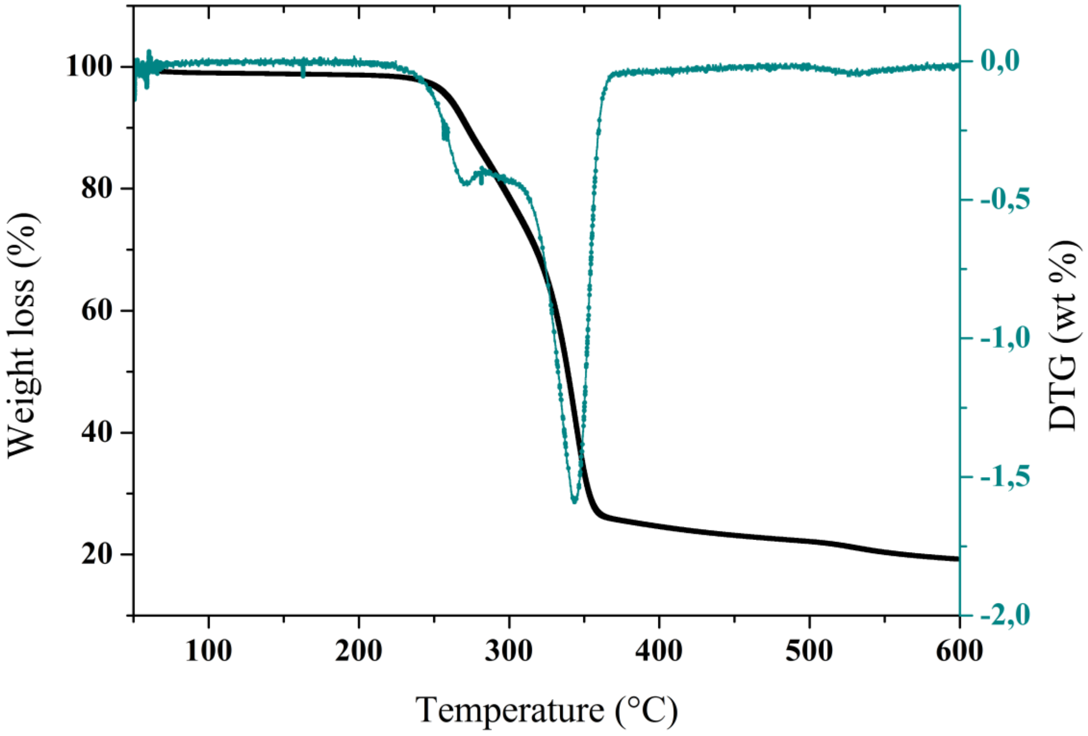


Figure IV.4 : ATG and DTG curves for Chitosan: Thermal Degradation Profile

Initial weight loss up to 200°C corresponds to the release of physically adsorbed water and water chemically bound to chitosan. Significant weight loss between 200°C and 300°C is due to the decomposition of functional groups present in chitosan, such as amine and acetamido groups, with the breaking of bonds between these groups and the main chain and the volatilization of the resulting fragments. Between 300°C and 400°C, degradation of the main polymeric backbone of chitosan is observed, with the breakdown of glycosidic bonds and the decomposition of glucosamine and N-acetylglucosamine units, accounting for most of the mass loss. Above 500°C, the weight loss curve stabilizes, indicating that the majority of the decomposable components have been volatilized or broken down, leaving a more thermally stable or inert residue.

The first peak of the DTG curve, around 300°C, corresponds to the maximum rate of decomposition of the first degradation phase, indicating the speed at which the functional groups of chitosan decompose and volatilize, while the second peak, around 450°C, corresponds to the rapid decomposition of the main polymeric backbone, reflecting a high rate of weight loss. The TGA and DTG results for chitosan show a multi-stage degradation, typical of biopolymers, with volatilization of water and decomposition of functional groups at lower temperatures, followed by degradation of the main polymer chain at higher temperatures. Stabilization above 500°C indicates the presence of thermally stable residues, providing crucial information for the use of chitosan in various applications requiring knowledge of its thermal stability, such as biomedical applications, protective films, and matrices for controlled drug release, making it possible to determine heat treatment conditions and possible applications for chitosan.

IV.5.5. Morphological observation of Chitosan by Optical Microscopy

The light microscope image shows chitosan particles with a granular, fragmented structure. The particles appear irregular in size with jagged contours, indicating grinding or fragmentation of the material. Variations in hue, with dark and light areas, suggest heterogeneity in particle thickness, where dark areas could represent thicker or more densely packed regions of chitosan. This granular morphology may result from mechanical or chemical processes applied to chitosan, and may have implications for the material's physical and chemical properties, influencing its behavior in various industrial, pharmaceutical or biomedical applications.

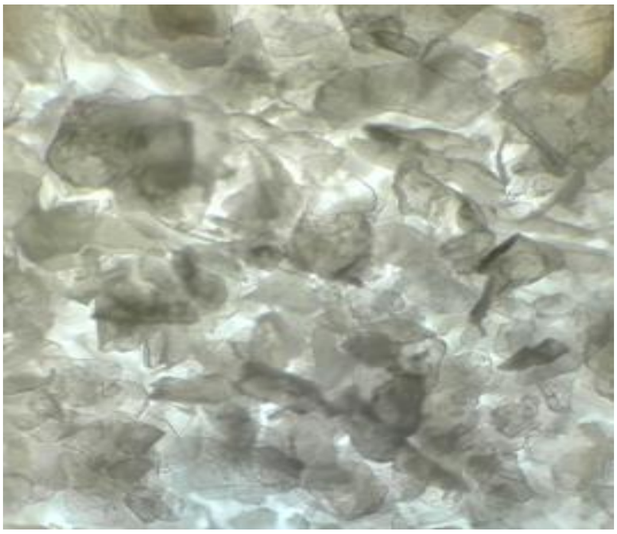


Figure IV.5 : Microstructure of Chitosan: Optical Microscope Image

IV. Conclusion

In short, chitosan's unique properties open up a wide range of applications in a number of industrial sectors. The case studies presented in this chapter illustrate the potential of this biopolymer to meet specific challenges, from water treatment and food preservation to regenerative medicine. Implementing these applications will require further research to optimize processes and ensure economic and environmental viability.

General Conclusion

General conclusion

The potential applications for chitosan are vast and varied. In the biomedical field, its biocompatible and antibacterial properties make it an ideal candidate for applications such as wound dressings, controlled drug delivery systems and biomedical implants. In the environmental field, chitosan shows promising efficacy in wastewater treatment and heavy metal recovery. Finally, its use in the food industry as a natural preservative and in agriculture as a plant growth stimulant and disease protectant demonstrates its versatility.

The valorization of marine crustacean waste through the production of chitosan not only meets an urgent environmental need, but also offers significant economic opportunities. The results obtained pave the way for future research and innovation, aimed at improving and diversifying the applications of chitosan, while strengthening the circular economy and environmental sustainability.

This research has shed light on the physico-chemical characteristics and functional properties of chitosan extracted from shrimp shells. The various analyses carried out have confirmed the quality and purity of the product obtained, and its multiple potential applications in various fields.

- XRD analysis revealed a semi-crystalline structure of chitosan, with diffraction peaks indicating ordered zones in the polymeric matrix, while highlighting a significant amorphous component due to the disordered arrangement of molecular chains. This is crucial for chitosan applications in protective films, biomedical devices and controlled drug release matrices.
- XRF analysis has shown that chitosan is composed mainly of light elements such as carbon, oxygen, nitrogen and hydrogen, with small amounts of metallic impurities such as aluminum, silicon, copper, zinc, iron, calcium, nickel and lead(Arslene Manuscript). This purity is essential for its use in biomedical and pharmaceutical applications.
- ATG and DTG curves revealed a multi-step mass loss typical of biopolymers, with volatilization of water and decomposition of functional groups at lower temperatures, followed by degradation of the main polymer chain at higher temperatures. This information is crucial for determining heat treatment conditions and potential applications for chitosan.

- FTIR analysis confirmed the presence of characteristic functional groups such as hydroxyls, amines, amides, methyls, methylenes and ethers. This helps to identify the composition and structure of chitosan.
- The solubility test showed that the solubility of chitosan in acetic acid solutions increases with the concentration of the acid.
- Physico-chemical analysis revealed that chitosan is mainly composed of dry matter with low water, ash and protein content and no detectable lipids. These characteristics confirm the purity of chitosan and its suitability for applications requiring a biopolymer with these specific properties.

Perspective

Perspectives

L'optimisation des processus d'extraction et de purification de la chitine et du chitosane passe par l'amélioration des techniques d'extraction pour augmenter le rendement et la pureté, ainsi que par le développement de méthodes de purification plus efficaces et respectueuses de l'environnement. Les applications de la nanotechnologie ouvrent des perspectives intéressantes, notamment dans la fabrication de nanoparticules pour des applications médicales telles que la délivrance ciblée de médicaments et la thérapie génique, ainsi que dans le développement de nanocomposites à base de chitosane pour des applications industrielles avancées. En médecine régénérative, il est essentiel de tester l'efficacité du chitosane dans la cicatrisation des plaies et la réparation des tissus. L'impact environnemental et la biodégradabilité du chitosane doivent également être étudiés dans divers environnements naturels pour garantir sa compatibilité écologique. Enfin, le développement de nouvelles applications industrielles innovantes, telles que les emballages alimentaires biodégradables, est crucial. Ces perspectives visent à approfondir les connaissances sur le chitosane et à maximiser son potentiel en tant que matériau durable et polyvalent, contribuant ainsi à la résolution des problèmes environnementaux et à la création de produits innovants et respectueux de l'environnement.

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Résumé

Cette étude se concentre sur l'extraction et la purification du chitosane à partir de déchets de crustacés marins. Elle est structurée en deux parties principales. Le premier chapitre théorique fournit une revue détaillée des polysaccharides, en particulier de la chitine et du chitosane, et discute de leurs diverses applications industrielles, biomédicales et environnementales. Le second chapitre théorique traite des méthodes d'extraction et de purification, ainsi que des techniques d'analyse pour caractériser le chitosane. Les résultats expérimentaux montrent des rendements significatifs à chaque étape du processus : 31,85 % pour la déminéralisation, 80,93 % pour la déprotéinisation, 79,68 % pour le blanchiment et 74,63 % pour la déacétylation. Ces résultats indiquent l'efficacité des procédures utilisées. Les analyses physico-chimiques du chitosane extrait révèlent une composition principalement en matière sèche, avec une faible teneur en eau et en cendres, une proportion élevée de matière volatile, une présence minimale de protéines et une absence de lipides, confirmant ainsi la pureté du produit final. En conclusion, cette recherche démontre que l'extraction de chitosane à partir de déchets marins non seulement aide à réduire les déchets mais permet également de produire un matériau de haute valeur ajoutée, tout en respectant les principes du développement durable. Les perspectives futures incluent l'amélioration des procédés d'extraction et l'exploration de nouvelles applications pour maximiser l'efficacité et la qualité du chitosane produit.

Mots clés : Biodégradabilité, Biomédicales, Biopolymère, Chitosane, Déchets marins, Polysaccharide.

Abstract

This study focuses on the extraction and purification of chitosan from marine crustacean waste. It is structured in two main parts. The first theoretical chapter provides a detailed review of polysaccharides, in particular chitin and chitosan, and discusses their various industrial, biomedical and environmental applications. The second theoretical chapter deals with extraction and purification methods, as well as analytical techniques for characterizing chitosan. Experimental results show significant yields at each stage of the process: 31.85% for demineralization, 80.93% for deproteinization, 79.68% for bleaching and 74.63% for deacetylation. These results indicate the effectiveness of the procedures used. Physico-chemical analyses of the extracted chitosan revealed a predominantly dry matter composition, with low water and ash content, a high proportion of volatile matter, minimal protein and an absence of lipids, confirming the purity of the final product. In conclusion, this research demonstrates that the extraction of chitosan from marine waste not only helps to reduce waste, but also enables the production of a high value-added material, while respecting the principles of sustainable development. Future prospects include improving extraction processes and exploring new applications to maximize the efficiency and quality of the chitosan produced.

Key words: Biodegradability, Biomedical, Biopolymer, Chitosan, Marine waste, Polysaccharide.

ملخص

تركز هذه الدراسة على استخراج وتنقية الكيتوسان من نفايات القشريات البحرية. وهي منظمة في جزأين رئيسيين. يقدم الفصل النظري الأول مراجعة مفصلة للسكريات المتعددة، وخاصة الكيتين والكيتوسان، ويناقش تطبيقاتها الصناعية والطبية والبيئية المختلفة. يتناول الفصل النظري الثاني طرق الاستخراج والتنقية، بالإضافة إلى التقنيات التحليلية لتوصيف الكيتوسان. تُظهر النتائج التجريبية مردودًا كبيرًا في كل مرحلة من مراحل العملية: 31.85% لإزالة المعادن، 80.93% لإزالة البروتين، 79.68% للتبييض و74.63% لإزالة الأسيتيل. تشير هذه النتائج إلى فعالية الإجراءات المستخدمة. كشفت التحليلات الفيزيائية الكيميائية للكيتوسان المستخرج عن تركيبة غالبها مادة جافة، مع محتوى منخفض من الماء والرماد، ونسبة عالية من المواد المتطايرة، وكمية ضئيلة من البروتين وغياب الدهون، مما يؤكد نقاء المنتج النهائي. في الختام، تُظهر هذه الأبحاث أن استخراج الكيتوسان من النفايات البحرية لا يساعد فقط في تقليل النفايات، بل يمكّن أيضًا من إنتاج مادة ذات قيمة مضافة عالية، مع احترام مبادئ التنمية المستدامة. تشمل الآفاق المستقبلية تحسين عمليات الاستخراج واستكشاف تطبيقات جديدة لزيادة كفاءة وجودة الكيتوسان المنتج.

الكلمات المفتاحية: القابلية للتحلل البيولوجي، الطب الحيوي، البوليمر الحيوي، الكيتوسان، نفايات بحرية، السكريات المتعددة.