CHITOSAN EXTRACTION USING GREEN TECHNOLOGY AND IT'S APPLICATION FOR PRESERVATION OF FRESH COCONUT SLICES

A thesis submitted towards the partial fulfilment of the requirements for the degree of Master of Technology in Food Technology and Biochemical Engineering course affiliated to Faculty of Engineering and Technology, Jadavpur University

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The thesis has been evaluated by me and found satisfactory. It is therefore, being accepted in partial fulfilment of the requirement for awarding the degree of Master of Technology in Food Technology and Biochemical Engineering course affiliated to Faculty of Engineering and Technology, Jadavpur University.

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The foregoing thesis is hereby approved as a creditable study in Master of Technology in Food Technology and Biochemical Engineering and presented in a manner satisfactory to warrant its acceptance as a prerequisite to the degree for which it has been submitted. It is understood that by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein but approve the thesis only for the purpose for which it is submitted.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

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All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

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ABSTRACT

The extraction of chitosan from shrimp shells using environmentally friendly methods offers a sustainable alternative to conventional techniques that rely on harsh chemicals like hydrochloric acid and sodium hydroxide. This thesis explores the extraction of chitosan through a green technology approach involving a two-step demineralization process utilizing citric acid and Deep Eutectic Solvent (DES). The chitosan thus obtained was employed as a coating agent for the preservation of fresh coconut slices, a perishable commodity with a notoriously short shelf life.The extracted chitosan was formulated into a coating solution consisting of 25% glycerol, 1% acetic acid, and 1% chitosan. The application of this coating on fresh coconut slices resulted in a shelf life extension to approximately 20 days when stored in zip lock bags under refrigerated (8 °C) conditions. Comparatively, coconut slices coated with commercially purchased chitosan from Loba Chemie PVT LTD demonstrated an extended shelf life of nearly one month under similar conditions.This research also undertakes a comprehensive comparison between the lab-extracted chitosan and the commercially obtained counterpart. Analytical techniques such as Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) were employed to characterize the chitosan samples. Furthermore, the efficacy of the chitosan coatings was evaluated based on microbial spoilage, sensory properties and moisture loss of the treated coconut slices.The findings underscore the potential of green technology in producing high-quality chitosan, which is effective in extending the shelf life of fresh produce. This study not only contributes to the field of sustainable biopolymer extraction but also highlights practical applications in food preservation, promoting both environmental and economic benefits.

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CHITOSAN EXTRACTION FROM SHRIMP SHELL WASTE AND ITS APPLICATION FOR COCONUT FLESH PRESERVATION

1. INTRODUCTION-

Chitosan is the main derivative of chitin, which was first observed in mushrooms by French professor Henri Braconnot in 1811. Braconnot named chitin "fongine" and Ojer later renamed it "chitine". 1876, Ledderhose discovered glycoamine, the first derivative of chitin. In 1878, Ledderhose also revealed that chitin is made up of glucosamine and acetic acid.

Charles Rouget discovered chitosan in 1859 by heating chitin in an alkaline medium. In 1894, Felix Hoppe-Seyler named the material chitosan, but its chemical structure wasn't determined until 1950.

Year	Important figures	Description
1811	Henri Bracannot (Director of the Botanical Garden in Nancy, France; Professor of Natural History)	Conducted research on mushrooms and extracted chitin Hypothesis: chitin did not dissolve in sulfuric acid
1823	Ojer	Named "chitin," based on Greek word "khiton" meaning "envelope"
1832	Opperman	Chitin was extracted from insects-similar substances as chitin can also be found in the structure of insects
1843	Lassaigne	Demonstrated the presence of nitrogen in chitin
1859	Rougeut	Discovered chitosan Observed that the substances in chitin could be manipulated through chemical and temperature treatments for it to become soluble Treated chitin with hydroxide potassium concentrated at higher temperature
1878	Ledderhose	Identified chitin as made of glucosamine and acetic acid
1894	Hoppe-Seyler (German scientist and physiologist)	Proposed the name of the chitosan
1930	Rammelburg	Identified more chitin sources apart from insects and fungi Chitosan can be extracted from marine arthropods. E.g., crab, shrimp, lobster Hydrolyzed chitin in several ways Detected that chitin is a polysaccharide of glucosamine
1950	Darmon and Rudall	Structure of chitosan discovered X-ray analysis advanced the study on the discoveries of chitin and chitosan X-ray, the most advanced technology at that period, recorded the existence of chitin and cellulose in the cell wall The absorption spectra of chitin, chitosan nitrate, and wood cellulose have been recorded in the region 3600-750 cm^{-1} using polarized radiation
1951	First book was published 140 years after the initial observation of Braconnot, which was then confirmations were done by many researchers on the discovery of chitosan biomaterials	
1960 Till present	Many researchers have conducted research using modified and unmodified chitosan derivatives in the biomedical field	

(1) *Figure 1*

Chitin is a natural mucopolysaccharide, derived naturally and found to be produced abundantly (second to cellulose) through biosynthesis. Chitins are characterized as white, nonelastic, hard, nitrogenous polysaccharides that have been estimated to be synthesized in approximately one billion tons annually. There are three forms of chitin: alpha chitin which in antiparallel in arrangement and found abundantly, beta chitin which in parallel in arrangement and found mainly in squids, gamma chitin which is antiparallel and found mainly in fungi and yeasts. Chitosan is derived from the N-deacetylation form of chitin. Chitosan is composed of β (1→4)-linked 2-acetamido-2-deoxy-β-D-glucose (N-acetylglucosamine). Chitin is structurally identical to cellulose, but it has acetamide groups (-NHCOCH3) at the C2-portion. On the other hand, chitosan is a linear polymer formed by α (1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose and derived by N-deacetylation, characterized by the degree of deacetylation, which is the copolymer of N-acetylglucosamine and glucosamine. Chitosan are the major elements derived from the shells of arthropods such as crabs, shrimps, lobsters, and insects, also produced extracellularly by the cell walls of fungi and brown algae. Shrimp shells, being thinner, are easier to purify. Shrimp shells

mainly consist of CaCO3, protein, and chitin. We extract chitin by various methods including demineralization, deproteinization. Chitosan is rarely found in nature but does occur in dimorphic fungi, such as Mucor rouxii, by the action of the deacetylase enzyme on chitin.

Chitosan is an aminopolysaccharide molecule which is cationic, enabling it to form electrostatic complexes or multilayer structures with other negatively charged synthetic or natural polymers. Its notable characteristics, including biocompatibility, non-toxicity, low allergenicity, biodegradability, antimicrobial functionality, renewability and bioabsorbability make it suitable for various applications. These properties are influenced by the degree of deacetylation, defined by the molar fraction of deacetylated units, and the molecular weight of chitosan.

Recently, chitosan has found extensive use in biological and biomedical applications due to its unique properties. It is utilized in water treatment, wound-healing materials, pharmaceutical excipients or drug carriers, obesity treatment, and as scaffolding for tissue engineering. Interest in the pharmaceutical and biomedical applications of chitosan and its derivatives has grown, leading to significant advancements in the field.

Chitosan is insoluble in water and organic solvents however it is soluble once mixed with acetic, nitric, hydrochloric, perchloric, and phosphoric acids. The solubility of chitosan derivatives can be observed especially in aqueous acidic solutions, which has a pH ratio lower than 6.5 (2). At the same time, the solubility range also can be altered upon depolymerization and chemical modification of primary and secondary hydroxyl groups. Recently, carboxymethyl chitosan and oligochitosan have become widely studied groups due to their characteristics of promising synthesis and rich diversity of applications in biomedical and biopharmaceutical areas of study.

To obtain chitosan typically, shells of the same size and species are collected, cleaned, dried, and ground into small pieces. There is no standardized purification method due to the structural diversity of different chitin sources, necessitating varied treatments. The conventional purification protocol includes demineralization, deproteinization, and decolorization steps, which can be performed using chemical methods or biological methods like enzymatic treatment or fermentation. For biomedical or pharmaceutical applications, the end-products must be highly purified to eliminate residual proteins, minerals, or pigments that could cause severe side effects. Chitin can be converted to chitosan through enzymatic or chemical deacetylation, with chemical deacetylation being more commonly used in commercial production due to its cost-effectiveness and suitability for mass production.

1.1Properties

1. SOLUBILITY

   Chitosan is produced by deacetylating chitin, during which some N-acetylglucosamine units are transformed into glucosamine units. The abundance of protonated -NH2 groups in chitosan's structure makes it soluble in acidic aqueous solutions due to its pKa of about 6.5. Chitosan becomes soluble when around 50% of its amino groups are protonated.

   The solubility of chitosan is influenced by factors like polymer molecular weight, degree of acetylation (DD), pH, temperature, and polymer crystallinity. Homogeneous deacetylation of chitin at low temperatures (alkali treatment, 0°C) can produce polymers that are soluble in aqueous acetic acid solutions even with a DD as low as

28%, a result not achieved with heterogeneous deacetylation (alkali treatment, high temperatures). At a DD of 49%, chitosan samples are water-soluble. This solubility is due to homogeneous deacetylation increasing the number of glucosamine units and altering the polymer's crystalline structure. These structural changes range from reduced crystal size and perfection to the emergence of a new crystal structure similar to β-chitin, depending on the polymer's DD.

Sogias et al (3) studied the impact of crystallinity and inter- or intramolecular forces on chitosan solubility. They found that re-acetylating a chitosan sample to half its original state with acetic anhydride or fully deacetylating it under homogeneous conditions affected solubility. After re-acetylation, the polymer's solubility extended to pH 7.4, while fully deacetylated chitosan showed a slight reduction in solubility range. The lower solubility was attributed to increased polymer crystallinity following deacetylation, which counteracted the effect of more glucosamine units. Conversely, the half-acetylated sample exhibited reduced crystallinity. Using hydrogen bond disruptors like urea or guanidine hydrochloride can also change chitosan's solubility range, and combining chemical and physical disruption of hydrogen bonds can achieve broad solubility.

2. Viscosity-

The viscosity of polymers is a crucial parameter from a technological perspective because highly viscous solutions are difficult to handle. Viscometry, while not an absolute method, is an effective and quick technique for determining chitosan's molecular weight, requiring the determination of constant that are solvent-specific . The Mark-Houwink-Sakurada equation, which relates molecular weight to intrinsic viscosity:

$$\eta = KMV^{\alpha}$$

Where K and alpha as experimentally determined constants. Various values for K and alpha exist based on solvent composition, pH, and ionic strength.Chitosan viscosity is influenced by its molecular weight and degree of deacetylation (DD), decreasing as the molecular weight decreases. Viscosity measurements can indicate polymer stability in solution, as a decrease suggests polymer degradation over time. Shear viscosity increases with higher deacetylation degrees. For instance, when comparing two chitosan samples with 91% and 75% DD, the sample with higher DD exhibited greater shear viscosity at the same shear rate, indicating that higher DD results in more significant chain expansion due to increased glucosamine units and charge density. To adjust chitosan viscosity, the addition of co-solvents has been studied. Kassai et al. found that adding isopropanol and ethanol to a chitosan solution in 1% acetic acid reduced the intrinsic viscosity of the polymer.

3. Chemistry of Chitosan-

Chitosan contains several reactive groups: a primary amino group (C2) and primary and secondary hydroxyl groups (C6, C3), along with glycosidic bonds and acetamide groups. These functional groups facilitate numerous modifications, yielding polymers with new properties and behaviours. Chitosan derivatives are developed to enhance properties like solubility and biodegradability or to introduce new functions. For example, deacetylation, depolymerization, or quaternization can improve solubility in water. Modified chitosan, such as 6-O-sulfated chitosan for promoting neuronal differentiation and phosphorylated chitosan for inhibiting corrosion, showcases the diverse potential of these derivatives. Here we focus on chitosan phosphorylation and degradation processes. A group of scientists developed a water-soluble phosphorylated

chitosan by mixing chitosan with phosphorus acid and adding formaldehyde at 70°C, preserving the film-forming properties. A similar method produced N-methylenephenyl phosphonic chitosan, soluble in water, and N-lauryl-N-methylene phosphonic chitosan, which is less soluble in water but better in organic media, forming micelles. N-methylene phosphonic N-methylene carboxylic chitosan was also synthesized, acting as a bivalent metal chelating agent due to multidentate ligands. Although chitosan has been used as a gene carrier, its relatively low transfection efficiency limits its application. Phosphorylated derivatives, which improve transfection by 100-fold and exhibit enhanced metal ion chelating activity, are more suitable. Chitosan degradation, reducing its molecular weight, can be achieved through various methods like acid hydrolysis, oxidative-reductive depolymerization, ultrasonic degradation, or enzymatic degradation using specific and non-specific enzymes. Different processes preferentially break specific glycosidic linkages (D-D, A-A, A-D, and D-A), producing varied samples from the same parent chitosan. Ultrasonic degradation maintains the degree of acetylation and polydispersity, offering moderate polymer degradation, while hydrogen peroxide causes rapid, random degradation, yielding monomers and chitooligosaccharides. Nitrous acid selectively degrades chitosan by attacking the primary amine in glucosamine, resulting in 2,5-anhydro-D-mannose at the reducing end, which is a disadvantage. HCl hydrolysis is less selective, primarily breaking A-A and A-D linkages.Controlled precipitation with methanol can produce chitooligosaccharides with DPs up to 16. Enzymatic degradation by chitosanases and chitinases offers specificity, producing chitooligosaccharides with minimal monomer release. Non-specific enzymes like lysozyme, proteases, cellulases, and hemicellulases also degrade chitosan, with each enzyme showing unique patterns of action.The method chosen for degradation affects the resulting product's properties. Chemical and physical methods are less selective than enzymatic ones, which are more precise. For instance, lysozyme and other proteolytic enzymes like pepsin, papain, and pronase yield low molecular weight chitosans (4-10 kDa) and chitooligosaccharides & small amount monomers. Hemicellulase acts endo-wise, not producing N-acetylglucosamine, while lipases, although slower, offer control over final product types by adjusting reaction conditions. Thus, selecting the appropriate degradation methodology is crucial for targeting specific chitosan products, influencing their biological and technological behaviours based on degradation patterns rather than size or acetylation degree.

4. Biological properties –
   Chitin, chitosan, oligosaccharides, and their derivatives possess various biological activities such as antitumoral, antimicrobial, antioxidant, and anti-inflammatory effects, making them potential therapeutic polymers. However, it is noteworthy that, to date, regulatory agencies only recognize chitosan and chitosan hydrochloride as excipients, not as drugs for disease treatment.

5. Antimicrobial activity- Bacterial resistance to antibiotics is a significant public health issue, necessitating the search for antibiotic alternatives. Chitosan, its derivatives, and chitooligosaccharides exhibit antimicrobial activity against various microorganisms, including bacteria, filamentous fungi, and yeast. Chitosan appears to inhibit microbial growth, as bacteria can resume growth once the polymer is removed from the medium. This is crucial because resistant populations could develop if cells adapt to chitosan.

6. Antioxidant activity - Antioxidants are increasingly important due to their link with diseases such as Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis, and cancer, as well as complications in conditions like diabetes. Chitosan, which has amino and hydroxyl groups, can neutralise free radicals, demonstrating antioxidant properties. Certain derivatives, such as chitosan sulfates and N-2 carboxyethyl chitosan, show enhanced antioxidant activity. Additionally, chitooligosaccharides have been modified with compounds like gallic acid and phenolics to boost their antioxidant properties. Various methods are used to evaluate the antioxidant activity of chitosan and its derivatives, including DPPH, ABTS, and FRAP assays, as well as peroxide and hydroxyl radical scavenging tests and macrophage models. The DPPH and ABTS assays rely on electron and hydrogen atom transfer, while the ORAC assay is also commonly employed. Differences in the polymers and testing methods lead to significant variations in the effective polymer concentrations, ranging from 50 µg/mL to 400 mg/mL. Lower molecular weight samples generally exhibit stronger antioxidant activity due to fewer intramolecular hydrogen bonds, making reactive groups more accessible for radical scavenging. Increased acetylation degree, however, tends to reduce antioxidant activity. (4)

1.2 Different methodology of Chitosan Extraction

1. Biochemical: - Shrimp shells were first sent to a washing unit for cleaning, then dried and crushed to reduce particle size. In a filtration process, treated shell waste with particles larger than 0.5 mm was separated and removed from the stream. The processed exoskeleton was then subjected to depigmentation using ethanol and demineralization with hydrochloric acid. These steps eliminated minerals and pigments (such as astaxanthin) while avoiding the hydrolysis of chitin. The material was then deproteinized using sodium hydroxide, converting it into chitin, which is the main precursor to chitosan. Partial deacetylation of chitin was achieved using sodium hydroxide to produce chitosan.

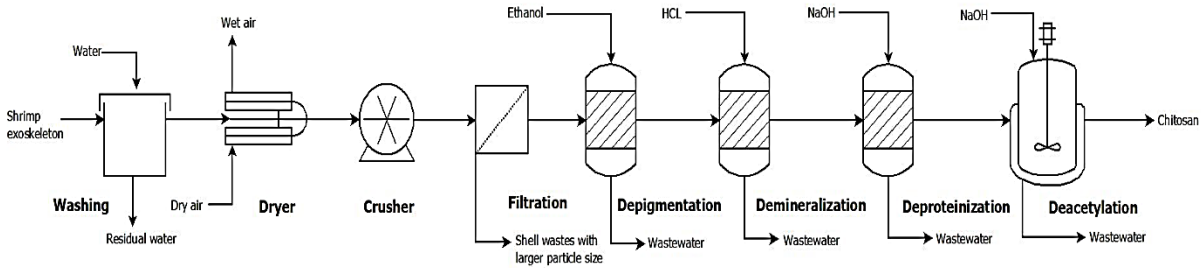

Figure 2 *(5)*

2. **Chemical:** Chitin is extracted by treating it with acid to dissolve calcium carbonate, followed by an alkaline treatment to dissolve proteins, and then a depigmentation step to remove astaxanthin and obtain a colourless product (Acosta et al., 1993). Chitosan is produced by hydrolyzing the acetamide groups of chitin. This typically involves strong alkaline hydrolysis at high temperatures, due to the resistance of these groups caused by the trans arrangement of the C2–C3 substituents in the sugar ring. Sodium or potassium hydroxides are usually used in concentrations of 30%–50% (w/V) at high temperatures (100 °C). (6)

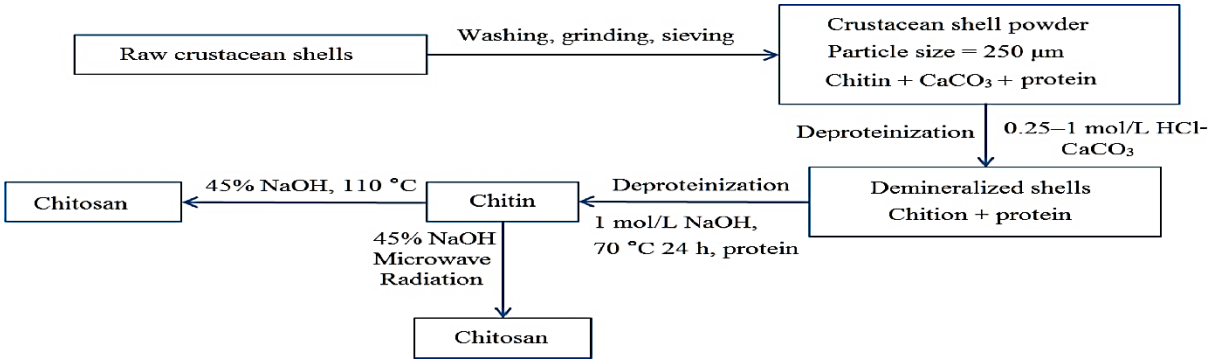

Figure 3 (7)

3. Fermentation (From Fungi):- Traditionally, chitosan production involves a two-step extraction process (acid and base treatment) from the cell wall of fungi after fermentation. The mycelia are treated under alkaline conditions (2%-4% NaOH) at elevated temperatures (90-121°C for 15-120 minutes), resulting in an alkali-insoluble material (AIM) that includes the degraded cell wall with proteins and chitosan. The chitin-glucan complex is then dissociated, releasing chitin, which is subsequently converted to chitosan through a deacetylation process. The AIM is treated with an acid, such as hydrochloric, lactic, or acetic acid (specifically 2%-10% acetic acid at 25-95°C for 1-24 hours) to remove phosphates or insoluble materials from the cell wall. The remaining cell wall material, rich in "fungal chitosan," is precipitated by raising the pH to 9-10, followed by centrifugation and washing with acetone and ethanol (8). This method does not require the deproteinization or demineralization processes needed for extracting chitosan from crustacean sources. Additionally, fungal chitosan is free of proteins that can cause allergic reactions, such as tropomyosin, myosin light chain, and arginine kinase, which are present in crustaceans. (7)

4. Enzymatic:

Sample Collection and Pre-treatment -Mealworm breeding was conducted in plastic trays containing a mixture of various raw materials, such as ground corn, wheat bran, and rice bran, to ensure adequate levels of protein, lipids, carbohydrates, vitamins, and minerals. Additionally, different vegetables were provided to supplement the diet and supply water. The food substrate was replaced every two weeks, and the larvae, pupae, and adults were separated. Mealworm cuticles were collected from all breeding boxes over six months. The collected samples were stored in plastic containers, protected from light, and kept at room temperature until pre-treatment.

The mealworm cuticles were dried overnight in an air circulation oven (Fanem 520, Brazil) at 50°C. The dried cuticles were then ground using a knife mill (Marconi Wiley Mill Standard no. 03, USA) to reduce particle size. The resulting powder was sieved to standardize particle size (Tyler 42) and remove any remaining impurities. After pre-treatment, the final sample was stored in plastic containers, protected from light, and kept at room temperature until use.

Deproteinization - To remove protein from the sample, an enzymatic hydrolysis method was used. The pre-treated mealworm cuticle sample was homogenized in distilled water at a 3% (m/v; protein/water) ratio. The dispersion conditions were adjusted to pH 8 (using 1 M sodium hydroxide) and a temperature of 50°C for 10 minutes. Enzymatic hydrolysis was initiated by adding Alcalase enzyme at a 2% (w/w; enzyme/substrate) ratio. The degree of hydrolysis (GH) was monitored every 10 minutes until it became constant. At the end of the process, the enzyme was inactivated at 90°C for 15 minutes. The resulting product was centrifuged at 9000 × g for 20 minutes at 4°C to separate the soluble (protein hydrolysate) and insoluble (chitin and minerals) fractions. The insoluble fraction was dried overnight in an air circulation oven at 50°C, ground in a knife mill (Marconi Wiley Mill Standard no. 03, USA), and stored in plastic containers at room temperature until use. Demineralization was not necessary due to the low mineral content in the sample. The deproteinization efficiency was calculated as described in (9) and expressed as a percentage using the following equation: Deproteinization Efficiency (%) = {(Po*O)-(PR*R)/Po*O }*100 where Po and PR are protein concentration % before and after hydrolysis . O and R represent mass of original sample and hydrolysed residue on dry weight basis respectively.

Deacetylation - The sample was mixed with a 40% (w/v) NaOH solution at 90°C under constant mechanical agitation (500 rpm). After 8 hours, the solution was filtered and washed with distilled water. The suspension was neutralized to pH 7.0 with 1 M HCl and filtered again to separate the supernatant. The obtained chitosan was dried overnight in an air circulation oven (Fanem 520, Brazil) at 50°C, ground in a knife mill (Marconi Wiley Mill Standard no. 03, USA), and stored in plastic containers at room temperature until use. (10)

5. Microbial - Lactobacillus plantarum subsp. plantarum ATCC 14917 was inoculated in 5 mL of De Man, Rogosa, and Sharpe (MRS) broth and incubated overnight at 200 rpm and 37°C. Similarly, Bacillus subtilis subsp. subtilis ATCC 6051 was inoculated in 5 mL of Luria broth (LB) and incubated overnight at 200 rpm and 30°C. The following day, 5 g of prawn shell waste was placed in a conical flask, covered with a cloth cap, and autoclaved at 121°C. After autoclaving, 100 mL of a 20% sterile glucose solution (1:20 w/v ratio) was added to the prawn waste. The optical densities (OD) of the bacterial cultures were measured to determine their concentrations. The desired culture concentration (1 × 10^6 colony-forming units (CFU) per milliliter of overnight culture) was inoculated into the sterile fermentation flask and incubated at 200 rpm and 30°C. After 5 days, the fermented supernatant was filtered off, and the prawn shell material was washed with deionized water and sterilized with 70% (v/v) ethanol. The prawn shell remains were then dried in a vacuum oven at 60°C for 24 hours before being ground into smaller pieces for analysis. (11)

2.1 Advantages of Chitosan Coating

1.Microencapsulation:- The microcapsule must remain stable and intact throughout its journey through the digestive tract, disintegrating only upon reaching its intended destination to release its contents . Applying a coating to the microcapsule adds a protective layer, enhancing its mechanical strength and providing a robust barrier function. This process involves immersing hydrogel particles into a solution containing the coating polymer.

Chitosan coating offers several key benefits, including its unique cationic properties, high biocompatibility, non-toxicity, and biodegradability, making it well-suited for applications in the food and pharmaceutical industries. Chitosan is derived from the shell waste of crabs, shrimp, and crawfish, and its molecular weight, which affects its crystallinity, degradation, tensile strength, and moisture content, is influenced by its source but can be adjusted through processing to increase deacetylation .This type of coating is particularly interesting for targeted probiotic release due to its high compatibility with living cells Chemically, chitosan is a polysaccharide consisting of (1, 4)-linked 2-amino-deoxy-b-d-glucan, a deacetylated form of chitin. In terms of natural availability, chitosan is second only to cellulose. The degree of deacetylation of chitin is a critical factor in chitosan's characterization. For instance, when the degree of deacetylation exceeds 50%, chitosan becomes soluble in aqueous acidic conditions. Additionally, the conditions under which deacetylation occurs, whether homogeneous or heterogeneous, significantly impact chitosan's microstructure, which in turn determines its solubility and suitability for various applications, such as drug or food carriers.

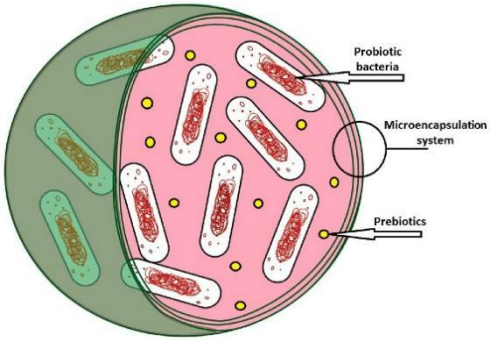

Figure 4 (12)

Chitosan coatings have been effectively utilized as active packaging materials and antimicrobial films to enhance the quality of agricultural products, particularly fruits and vegetables. This success is largely attributed to chitosan's excellent film-forming properties and biocompatibility The list below highlights that film-forming ability and biocompatibility are essential characteristics for its use as a coating material, enabling its application as edible films, coatings, or packaging materials for fruit and vegetable storage. According to Xing et al. chitosan coatings can form thin films with micropores, improving the storability of perishable foods by reducing transpiration losses and modifying the internal atmosphere of the package. Additionally, the biocompatibility of chitosan coating films makes them suitable carriers for antimicrobial agents. Chitosan has also been shown to possess unique biocompatibility for applications in emulsification and dye binding. Consequently, the application of chitosan, with its excellent film-forming properties and biocompatibility, has garnered interest as a natural coating material for preserving the quality of fruits and vegetables. (13)

Chitosan has also been found to have an antimicrobial effect in preserving fresh meat, effectively acting against spoilage bacteria such as Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas fragi. (14)

The use of a chitosan coating combined with phytic acid for preserving fresh-cut lotus root was studied. The results indicated that a composite coating with 1% chitosan and 1% phytic

acid could reduce the weight loss rate and malondialdehyde (MDA) content in fresh-cut lotus root, delay browning, inhibit the activities of peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL), and maintain higher levels of vitamin C and polyphenols. After 8 days, the weight loss rate of coated fresh-cut lotus root was half that of the control, the MDA content was 18.8% lower, and the L value was 51.2% higher than the control sample. Therefore, the composite coating of chitosan and phytic acid is a more effective method for preserving fresh-cut lotus root compared to using chitosan alone. (15)

Single berries or clusters that were artificially inoculated were immersed in chitosan, ethanol, or a mixture of both. Tests with small clusters were conducted to simulate the conditions of prolonged commercial cold storage of table grapes. The combination of reduced doses of chitosan and ethanol provided better control of gray mold in table grapes compared to using either substance alone. The combined effect was at least additive and sometimes synergistic. (16)

The combination of 1-MCP and chitosan coating can effectively extend the storage life and maintain the quality of Indian jujube fruit when stored at room temperature. (17)

2.2 Application of chitosan coating on fruits and vegetables

Chitosan has demonstrated efficacy against both gram-positive and gram-negative bacteria. Gram-negative bacteria like Escherichia coli possess an outer membrane (OM) composed of an asymmetric lipid-protein bilayer known as lipopolysaccharide (LPS). The divalent cations (e.g., Ca2+, Mg2+) within the OM are crucial for stabilizing the core anionic charges of LPS molecules. It is postulated that chitosan displaces these divalent cations from their binding sites, thereby reducing interactions among LPS molecules. This disruption weakens the membrane and leads to cell lysis, facilitated by the penetration of positively charged chitosan through the bacterial cell membrane via electrostatic interaction.

In contrast, Gram-positive bacteria lack an outer membrane. Thus, chitosan, being a polycationic long-chain molecule, can more effectively adhere to gram-positive bacterial species such as Staphylococcus aureus. Consequently, chitosan's inhibitory action is more potent against gram-positive bacteria compared to gram-negative ones. Literature suggests that gram-positive bacteria, which contain poly-anionic surface polymers like teichoic acid and lipoteichoic acid, interact with intracellular substances, leading to impairment of vital bacterial functions.

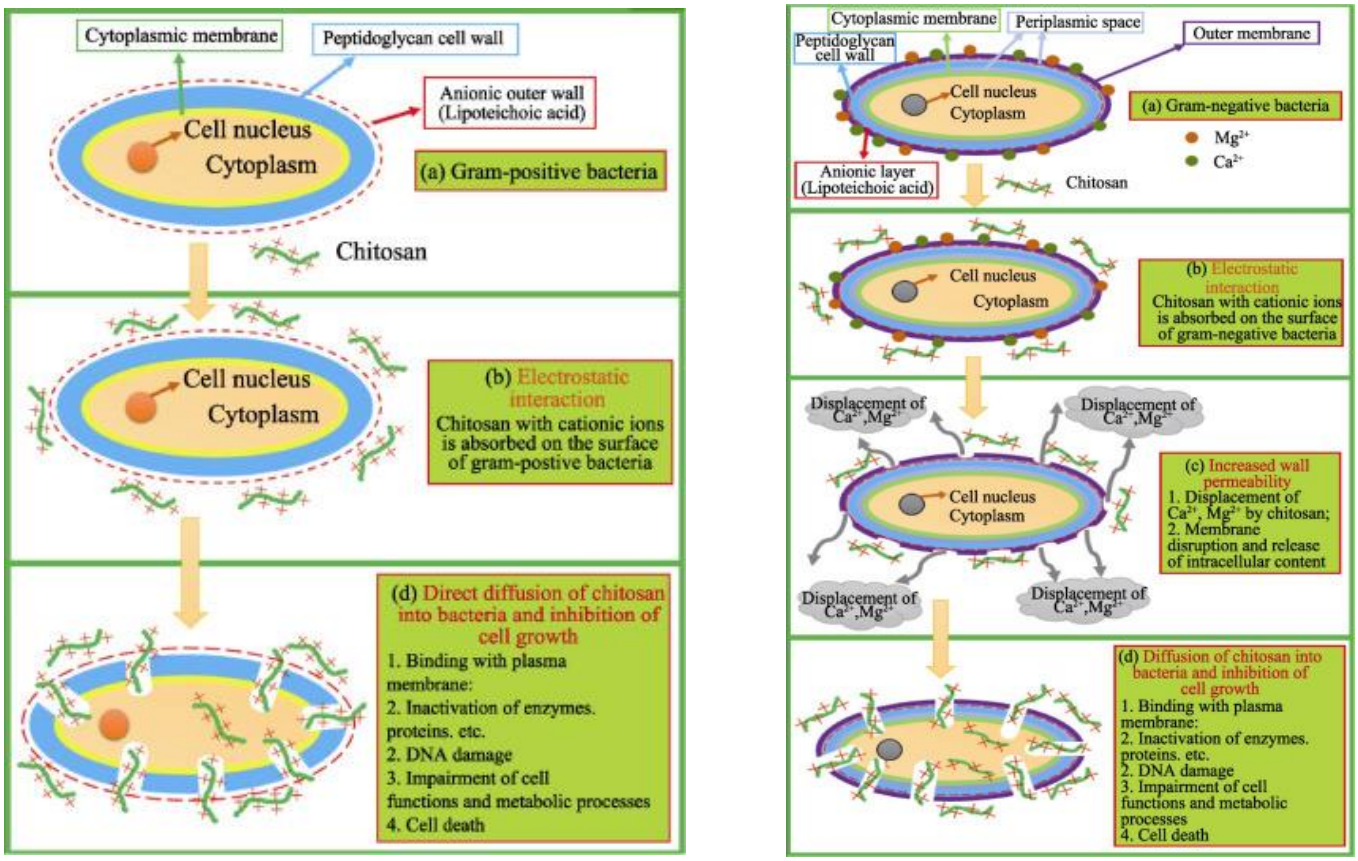

Figure 5

The utilization of chitosan is extensively explored as an edible coating, which involves forming a thin film directly on the surface of the intended product for protection. These edible coatings or films create a protective barrier around fruits and vegetables (F&V), which can be consumed along with the coated product. In applications aimed at preserving F&V, the establishment of a moisture and gas barrier can result in reductions in weight loss and respiration rate, thereby delaying spoilage and extending the shelf life of the product. Water vapor permeability (WVP) and oxygen permeability (OP) are commonly studied barrier properties to assess the ability to shield foods from the environment. Edible chitosan films excel in obstructing the permeation of oxygen while displaying relatively modest water vapor barrier properties .These films and coatings develop selective permeability characteristics, particularly towards O2, CO2, and ethylene, enabling some regulation of fruit respiration and inhibition of microbial growth . (18)

Here's how chitosan is utilized in different types of fruits:

1. **Citrus Fruits**: Chitosan coatings are frequently used on citrus fruits like oranges, lemons, and grapefruits to extend their shelf life. These coatings create a protective layer that reduces moisture loss, delays aging, and prevents fungal growth. Additionally, chitosan helps maintain fruit texture and minimizes spoilage.

2. **Apples and Pears**: Chitosan coatings have been extensively studied for their ability to prolong the storage life of apples and pears. By forming a thin layer on the fruit's surface, chitosan coatings decrease water loss, regulate gas exchange, and inhibit microbial growth. This results in decreased spoilage, retained firmness, and improved appearance during storage.

3. **Berries**: Chitosan applications benefit berries such as strawberries, blueberries, and raspberries by extending their post-harvest shelf life. These coatings preserve berry texture, color, and nutritional content by reducing moisture loss and microbial spoilage. Additionally, they enhance the overall visual appeal of the berries.

4. **Tomatoes**: Chitosan-based coatings are utilized to extend the shelf life of tomatoes by creating a barrier against moisture loss, physical damage, and microbial contamination. These coatings help preserve tomato firmness, color, and flavor during storage and transportation, thereby minimizing post-harvest losses.

5. **Stone Fruits**: Fruits like peaches, plums, and cherries benefit from chitosan applications to prolong shelf life and maintain quality. Chitosan coatings reduce post-harvest issues such as softening and browning, while also inhibiting fungal growth and extending storage duration.

6. **Bananas and Mangoes**: Chitosan-based coatings are applied to bananas and mangoes to extend shelf life by suppressing ethylene production and delaying ripening. These coatings prevent premature softening and decay, thus enhancing storage stability and marketability.

CHAPTER 2

Literature Review

2. LITERATURE REVIEW

Chitosan, a natural polysaccharide derived from chitin, has garnered significant attention due to its biocompatibility, biodegradability, and diverse applications in fields such as medicine, agriculture, and food preservation. Chitin is primarily extracted from the exoskeletons of crustaceans, especially shrimp shells, which are abundant byproducts of the seafood industry. The extraction of chitosan from shrimp shells involves several chemical processes including demineralization, deproteinization, and deacetylation. This literature review examines the various methods of chitosan extraction, focusing on the chemical and physical processes involved, and evaluates their efficiency and environmental impact.

2.1 Chitosan Extraction Processes

Demineralization

Demineralization involves removing inorganic components, primarily calcium carbonate, from shrimp shells. This process typically uses dilute hydrochloric acid (HCl). Studies, such as those by (19), have demonstrated that varying the concentration of HCl and the treatment time can significantly influence the efficiency of demineralization. For instance, a concentration of 1-3% HCl for 6-24 hours is commonly used, achieving high removal rates of minerals. (20) used two step demineralization technique by using citric acid of varying concentrations. (21) also demonstrated two-step demineralization.

Deproteinization

Deproteinization is aimed at removing proteins from the shrimp shells. Sodium hydroxide (NaOH) is commonly used for this purpose. Research in (22) indicates that 1-5% NaOH at temperatures ranging from 60-100°C for 1-3 hours is effective in protein removal. The degree of deproteinization impacts the purity and quality of the resulting chitin and chitosan. (23) used DES for deproteinization .They demonstrated on synthesis of DES . ChCl and Glycerol were mixed in a 1 : 2 molar ratio and heated in an oil bath with continuous magnetic stirring at 90 °C for 0.5 h to produce a transparent and homogeneous DES which was used for deproteinization. The DES used was further recovered.

Deacetylation

Deacetylation converts chitin to chitosan by removing acetyl groups. This is typically achieved using concentrated NaOH solutions at elevated temperatures. (24) highlighted that deacetylation degrees above 70% are required for the material to exhibit chitosan properties. Conditions such as 40-50% NaOH at 90-100°C for 1-3 hours are frequently employed to achieve high degrees of deacetylation.

Optimization and Environmental Impact

Recent studies have focused on optimizing these processes to improve yield and reduce environmental impact. For instance, (25)investigated the use of enzymatic methods for deproteinization, which could potentially reduce the reliance on harsh chemicals and lower the environmental footprint. Enzymatic treatments using proteases, while effective, still face challenges in terms of cost and process efficiency.

Another area of focus has been the development of integrated processes that combine demineralization and deproteinization steps. Research by (26) suggests that the sequential or simultaneous application of acids and bases can streamline the extraction process, reducing processing time and chemical usage.

2.2 Applications of Chitosan

The extracted chitosan has found applications across various industries due to its unique properties. In agriculture, it is used as a biopesticide and soil enhancer. In medicine, it has applications in wound healing, drug delivery, and tissue engineering. The food industry utilizes chitosan for its antimicrobial properties, making it valuable for food preservation and packaging.

In Mat et al.'s study from 2013, chitosan was employed to enhance the firmness and minimize the browning of peach fruits. Similarly, Xu Qinghai et al. utilized chitosan as a coating agent to maintain the freshness of Nanguo pears at room temperature. Their findings indicated that Nanguo pears coated with 1.5% chitosan exhibited hardness and vitamin C content akin to fresh ones, with significantly reduced weight loss and improved preservation compared to the control group. These coated pears could be stored for over 50 days at room temperature.

Chen Tian et al.'s research on kiwi fruit preservation with chitosan at room temperature revealed a notable enhancement in fruit freshness. Kiwi fruits were immersed in chitosan solution for 30 seconds, air-dried, sealed, and packed in polyethene film bags within corrugated boxes. While the control samples lasted 10-12 days at room temperature, chitosan-treated fruits extended their shelf life to 70-80 days.

Huang Xianghong et al. investigated the preservation efficacy of chitosan film on four-season grapefruit, demonstrating its capacity to reduce nutrient loss and inhibit fruit respiration. A 2.0% chitosan membrane proved the most effective in curbing fruit decay.

Chen Ningsheng prolonged the freshness of cucumbers for 20 days and green peppers for at least 25 days using a 2.0% chitosan treatment. Furthermore, experiments on amaranth, leek, and other green vegetables indicated varying degrees of preservation effectiveness.

The extraction of chitosan from shrimp shells is a well-established process, yet ongoing research continues to optimize and refine the methods to enhance efficiency and

sustainability. The use of alternative, environmentally friendly methods such as enzymatic treatments hold promise but requires further development. The diverse applications of chitosan underscore its importance and the need for continued innovation in extraction techniques. As environmental concerns grow, the focus on sustainable extraction methods will likely intensify, driving future research in this field.

2.3 Objectives of this work:

1. **Maximize Yield**: One primary objective is to optimize the extraction process to obtain the highest possible yield of chitosan from shrimp shells. This involves selecting the most efficient extraction method and optimizing various parameters such as acid concentration, reaction time, and temperature to maximize chitosan recovery.

2. **Ensure Purity**: Another key objective is to ensure the purity of the extracted chitosan. This involves minimizing contamination with other shell components such as proteins, minerals, and pigments. Selecting appropriate demineralization, deproteinization, and deacetylation methods is crucial to achieve high-purity chitosan.

3. **Minimize Environmental Impact**: The extraction process should be designed to minimize its environmental impact. This includes reducing energy consumption, solvent usage, and waste generation. Utilizing green extraction methods, such as enzymatic or environmentally friendly solvents, can help achieve this objective. We used environment friendly solvents like DES which reduces primary energy consumption, increases energy efficiency, and reduces GHG emissions.

4. **Explore Novel Extraction Techniques**: Research in chitosan extraction aims to explore and develop novel extraction techniques that offer advantages over traditional methods. This includes techniques like two stage demineralization, microwave-assisted extraction (MAE) which have shown promise in enhancing extraction efficiency and reducing processing time.

5. **Characterize Extracted Chitosan**: Characterization of the extracted chitosan is essential to evaluate its quality and properties. This involves analyzing parameters such as degree of deacetylation and molecular stability . Understanding the characteristics of chitosan extracted from different sources and under different conditions is crucial for its successful application in various fields. We used various methods like FT-IR, SEM, EDX, XRD to characterize chitosan.

6. **Explore Applications**: Application of chitosan coating in coconut as an antifungal agent and also as source of antioxidant. Evaluation of its effectivity was done for the above parameters.

CHAPTER 3

Materials & Methodology

3. MATERIALS & METHODOLOGY -

The aim of this research is to explore the extraction of chitosan using green solvents and to evaluate its efficacy in preserving fresh coconut flesh. This study is driven by the need for sustainable and environmentally friendly methods in food preservation, aligning with the broader goals of reducing chemical residues and enhancing food safety. Chitosan, a biopolymer derived from chitin, has shown significant potential in food preservation due to its antimicrobial and film-forming properties. However, conventional extraction methods often involve harsh chemicals that can be detrimental to the environment. By employing green solvents, this research seeks to develop a more sustainable approach to chitosan extraction, thereby minimizing environmental impact while maintaining or enhancing the preservation qualities of the biopolymer.

The methodology comprises two primary phases: (1) the extraction of chitosan using green solvents, and (2) the application of the extracted chitosan in the preservation of fresh coconut flesh.

In the first phase, the focus is on identifying and optimizing the use of green solvents for the efficient extraction of chitosan from chitin. Green solvents, which are characterized by their low toxicity and environmental friendliness, offer a promising alternative to traditional chemical solvents. This phase involves a series of experiments to determine the most effective green solvent, considering factors such as extraction yield, purity of the extracted chitosan, and the solvent's recyclability and biodegradability.

The second phase involves applying the extracted chitosan to fresh coconut flesh to assess its preservation efficacy. Fresh coconut flesh is prone to rapid spoilage due to its high moisture content and nutrient-rich composition, making it an ideal candidate for testing the preservative effects of chitosan. This phase will include the preparation of chitosan-based coatings and their application to the coconut flesh, followed by the evaluation of various preservation parameters such as microbial load, moisture retention, texture, and sensory attributes over a defined storage period.

By integrating these two phases, this research aims to demonstrate the feasibility and effectiveness of using chitosan extracted through environmentally benign processes for the preservation of perishable food items. This approach not only addresses the environmental concerns associated with traditional chitosan extraction methods but also provides a practical solution for extending the shelf life of fresh produce, thereby contributing to food security and sustainability.

3.1 MATERIALS

Prawn shell, grinder, magnetic stirrer, strainer, muslin cloth, citric acid, acetone, ChCl, Ethylene Glycol, microwave, centrifuge, NaOH, filter paper.

3.2 CHITOSAN EXTRACTION PROCESS:

Preprocess:

1) Prawn shells were taken and were washed with water.
2) Boiled in water (water: shrimp = 3:1) for 1 hour
3) Dried at 160 C for 2 hours in hot air oven.
4) The dried shells were ground

2-stage Demineralization:

1) Powdered shells and distilled water in a ratio of 1:7 were mixed in a beaker
2) 50% citric acid solution 118.74 ml was added to 200gm powder.
3) Strirred using a magnetic stirrer for 35mins
4) Straining was done
5) Washed to neutral
6) Processed powdered shells and distilled water in a ratio of 1:10 were taken
7) 50% citric acid 79.16ml was added.
8) Strirred using magnetic stirrer for 60mins
9) Then this was filtered and was again washed to neutral
10) Bleached with Acetone(1:10 w/v) for 10mins
11) This was filtered
12) Dried at 70 C

Deproteinization

1) ChCl and Ethylene glycol mixture in 1:2 ratio was stirred using magnetic stirrer at 60 C until it becomes transparent to form DES.
2) Shell and DES mixture in the ratio of 1:20 was microwaved (2-3 pulses for 10mins) accompanied with continuous stirring.
3) Centrifugation was done.
4) Washed with distilled water until neutral.
5) Bleached with Acetone (1:10 w/v) for 10mins
6) This was filtered
7) Dried at 80 C. We get chitin after deproteinization.

Deacetylation

1) 70% NaOH solution was prepared.
2) NaOH solution and Chitin were taken in the ratio of 14ml:1gm
3) This was kept at room temperature and continuously stirred for 75 hours
4) Then the solid fraction was collected and washed with distilled water. We get chitosan after this step.

3.3 FLOW CHART FOR CHITOSAN EXTRACTION

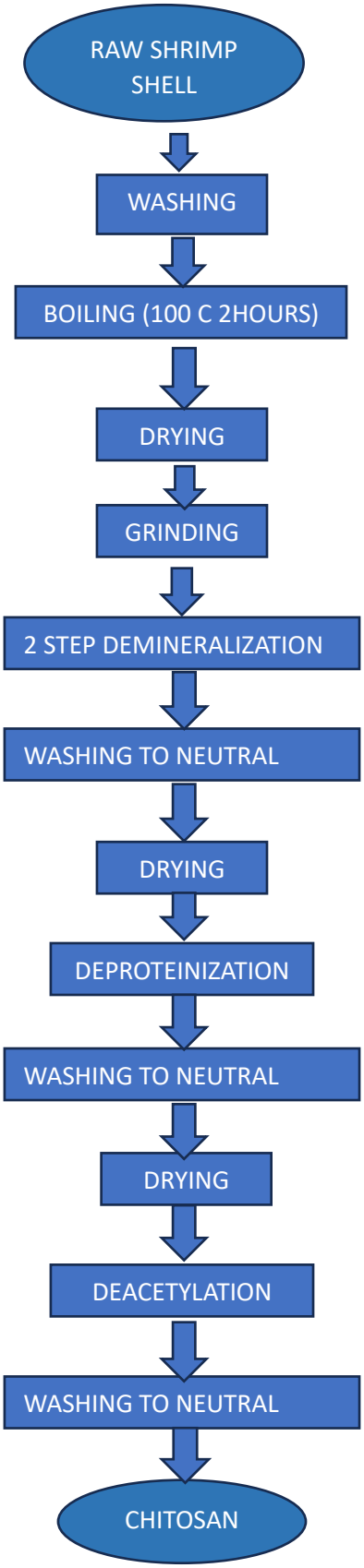

Figure 6

3.4 MATERIALS:

Coconut, chitosan, glycerol, acetic acid,3% hydrogen peroxide solution, zip lock bags

3.5 METHOD FOR CHITOSAN COATING ON COCONUT

1) Coat solution is prepared which consists of 1% chitosan, 25% glycerol, 1% acetic acid. Two coat solutions are prepared one using lab-grade chitosan and the other using purchased chitosan for better comparison.
2) Fresh coconut was sliced and sterilized using 3% hydrogen peroxide.
3) Sterilized coconuts were dipped into the coating solutions for 60 seconds.
4) Straining and air drying were done.
5) Coated coconuts were stored in zip-lock bags under refrigerated conditions (8°C)

3.6 FLOW CHART FOR CHITOSAN COATING ON COCONUT

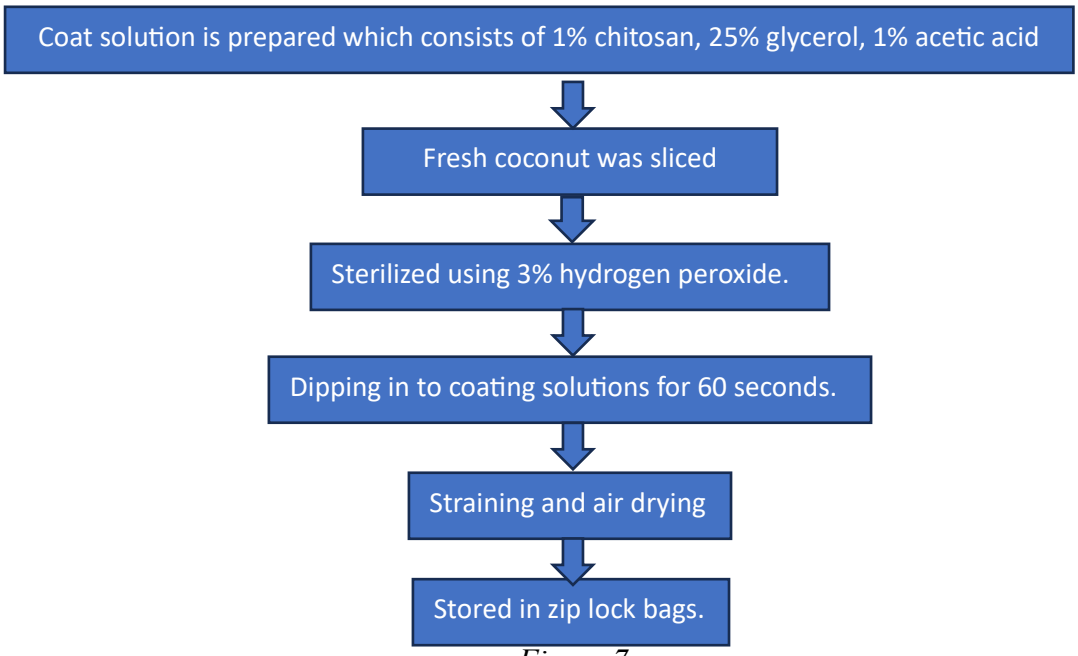

Figure 7

3.7 ANALYSIS OF SHRIMP SHELL PRODUCTS AT VARIOUS STAGES -

The analysis of shrimp shell products such as raw shrimp shell flakes, dried shrimp shell flakes, demineralized shrimp shell flakes, chitin, and chitosan is pivotal for understanding their structural and compositional properties. These products, derived from shrimp shell waste, are valuable sources of biopolymers, particularly chitin and its derivative chitosan, which have diverse applications in various industries including biomedical, pharmaceutical, agricultural, and food sectors. Advanced characterization techniques such as Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDX), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR) are essential tools for thoroughly analyzing these materials.

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX)

SEM-EDX provides detailed insights into the surface morphology and elemental composition of shrimp shell products. SEM allows for high-resolution imaging, revealing the microstructural features and surface texture of the samples, which are crucial for understanding the impact of various processing stages, from raw to chitosan. EDX complements this by offering quantitative and qualitative elemental analysis, which is particularly useful for assessing the effectiveness of demineralization and the purity of chitin and chitosan.

X-ray Diffraction (XRD)

XRD is employed to investigate the crystalline structure of the shrimp shell products. This technique helps in identifying the crystalline phases present in the samples and evaluating the degree of crystallinity, which influences the mechanical and thermal properties of chitin and chitosan. The analysis of diffraction patterns enables the differentiation between amorphous and crystalline regions, providing insights into the structural transitions that occur during the conversion from raw shrimp shells to purified chitin and chitosan.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is utilized to determine the chemical structure and functional groups present in the shrimp shell products. This technique provides vital information about the molecular interactions and changes in chemical bonds during the processing stages. FTIR spectra can identify specific functional groups characteristic of chitin and chitosan, such as amide, hydroxyl, and acetyl groups, thus confirming the successful extraction and deacetylation processes.

The primary objective of this analysis is to comprehensively characterize shrimp shell products at various stages of processing using SEM-EDX, XRD, and FTIR. By employing these advanced techniques, the study aims to elucidate the morphological, structural, and chemical transformations that occur during the conversion of raw shrimp shells into chitin and chitosan. This detailed characterization will enhance the understanding of the material properties, which is essential for optimizing the extraction processes and expanding the applications of chitin and chitosan in various industries.

This research holds significant importance in the valorization of shrimp shell waste, promoting sustainable practices by converting waste into valuable bioproducts. The findings from this study will contribute to the development of more efficient and environmentally friendly extraction methods, ensuring high-quality chitin and chitosan production. Additionally, the comprehensive analysis will aid in tailoring the properties of these biopolymers for specific applications, thereby broadening their industrial utility and fostering innovation in material science.

3.8Analysis of Chitosan-coated Coconuts –

The application of chitosan as a natural preservative for fresh produce has gained significant attention due to its biocompatibility, biodegradability, and antimicrobial properties. In this context, chitosan-coated coconuts represent a promising approach to extending the shelf life and maintaining the quality of this highly perishable commodity. This research focuses on analyzing the efficacy of lab-made chitosan coatings and purchased chitosan from LOBA CHEMIE PVT LTD on fresh coconut flesh and comparing the two by evaluating its microbial spoilage, moisture loss and conducting sensory evaluations. We will also observe fresh coconut slices without any coating kept under the same conditions for better understanding of the chitosan's role as a preservative.

Fresh coconut flesh is a nutritious and popular food product, but it is prone to rapid spoilage due to its high moisture content and rich nutrient composition. Traditional preservation methods often involve the use of synthetic chemicals, which can pose health risks and environmental concerns. Chitosan, derived from chitin found in shrimp shells and other crustacean exoskeletons, offers a natural alternative. Its ability to form a protective barrier and its inherent antimicrobial properties make it an excellent candidate for preserving fresh produce.

Microbial Spoilage

Assessing the antimicrobial efficacy of chitosan coatings in inhibiting the growth of spoilage-causing microorganisms, thereby extending the shelf life of the coconut slices.

Sensory Evaluation

Sensory evaluation is critical for assessing the consumer acceptability of chitosan-coated coconuts. This involves evaluating attributes such as appearance, texture, flavour, and overall acceptability. Sensory evaluations provide insights into any potential changes in the organoleptic properties of the coconut flesh due to the chitosan coating. Maintaining or enhancing these sensory attributes is essential for the commercial success of the preservation method.

Moisture Loss

Investigating the ability of chitosan coatings to reduce moisture loss from the coconut slices, thereby maintaining their freshness and preventing desiccation during storage.

The primary objective of this research is to assess the impact of chitosan coating on the preservation quality of fresh coconut flesh. This will be achieved by observing microbial

spoilage, sensory evaluation and moisture loss. Through these analyses, we aim to determine the effectiveness of chitosan as a natural preservative in maintaining the nutritional, microbiological, and sensory qualities of fresh coconut flesh.

The significance of this study lies in its potential to provide a natural, safe, and effective method for preserving fresh coconut flesh. By demonstrating the efficacy of chitosan coatings, this research could contribute to reducing food spoilage and waste, enhancing food safety, and promoting sustainable preservation practices. Furthermore, the findings could facilitate the broader application of chitosan in the preservation of other perishable food items, thereby advancing the field of food science and technology.

CHAPTER 4

Result & Discussion

4.1Proximate Analysis of Dried Shrimp Shell Flakes –

The proximate analysis of dried shrimp shell flakes is a critical aspect of evaluating their nutritional and industrial potential.

Firstly, the analysis provides valuable information on the moisture, ash, protein and fat.

Secondly, the analysis aids in assessing the potential of shrimp shells as a source of chitin and chitosan. Chitin extraction efficiency largely depends on the initial composition of the shrimp shells, particularly the protein and ash content. Therefore, understanding these parameters can optimize extraction processes and improve yield and quality.

In this section, we present the results of the proximate analysis of dried shrimp shell flakes. The discussion interprets these findings in the context of their potential applications as coating material. Through this analysis, we aim to highlight the significance of utilizing shrimp shell waste, contributing to more sustainable and economically viable raw materials for chitosan extraction.

Raw Shrimp Shell Moisture content :- 69.88%

COMPONENT %	DRIED SHRIMP SHELL
MOISTURE	14.23
ASH	25.34
CRUDE FAT	1.99
PROTEIN	31.35

Table 3 Proximate analysis of dried shrimp shell flakes

4.2 ANALYSIS OF DRIED SHRIMP SHELL, DEMINERALIZED SHELL, LAB-MADE CHITOSAN AND PURCHASED CHITOSAN USING SEM WITH EDX –

The Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX) analysis provided detailed insights into the surface morphology and elemental composition of the dry shrimp shell, demineralized shrimp shell, our lab-made chitosan, and commercially purchased chitosan.

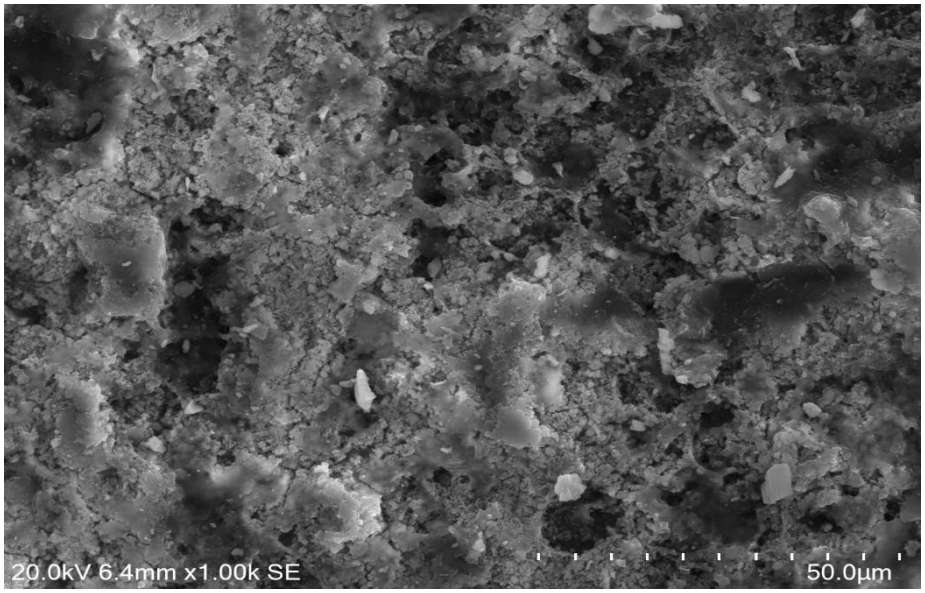

Figure 8 IMAGE OF DRY SHRIMP SHELL FLAKES

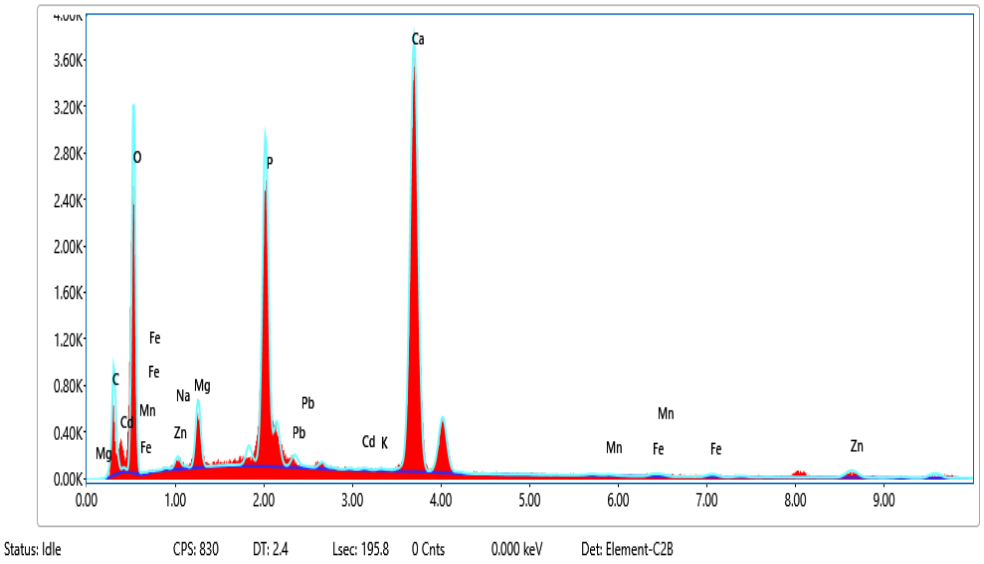

Figure 9 EDX GRAPH OF DRY SHRIMP SHELL FLAKES

Element	Weight %
C K	1.76
O K	19.20
Na K	0.41
Mg K	4.16
P K	11.48
K K	0.00
Ca K	60.29
Mn K	0.08
Fe K	0.20
Zn K	1.31
Cd L	0.12
Pb M	0.99

Table 4 ELEMENT WEIGHT % FOR DRY SHRIMP SHELL

Figure 10 SEM IMAGE OF DEMINERALIZED SHRIMP SHELL

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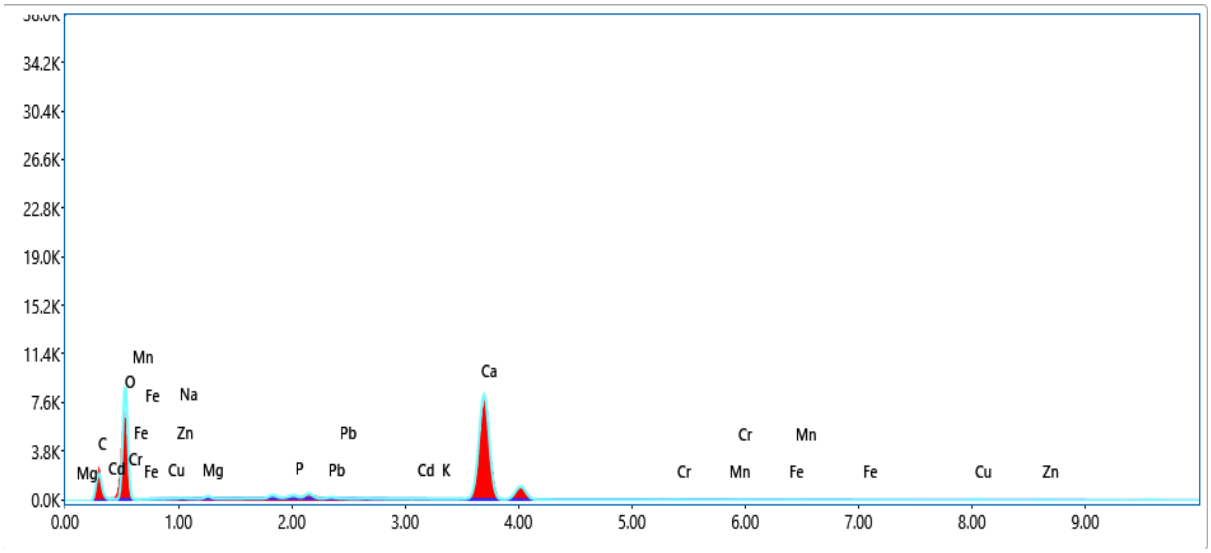

Figure 11 EDX GRAPH FOR DEMINERALIZED SHRIMP SHELL

Element	Weight %
Na K	5.30
Mg K	8.41
P K	7.25
K K	0.00
Ca K	44.40
Cr K	0.60
Mn K	0.61
Fe K	0.59
Cu K	7.41
Zn K	9.15
Cd L	0.41
Pb M	1.88

Table 5 ELEMENT WEIGHT % FOR DEMINERALIZED SHRIMP SHELL

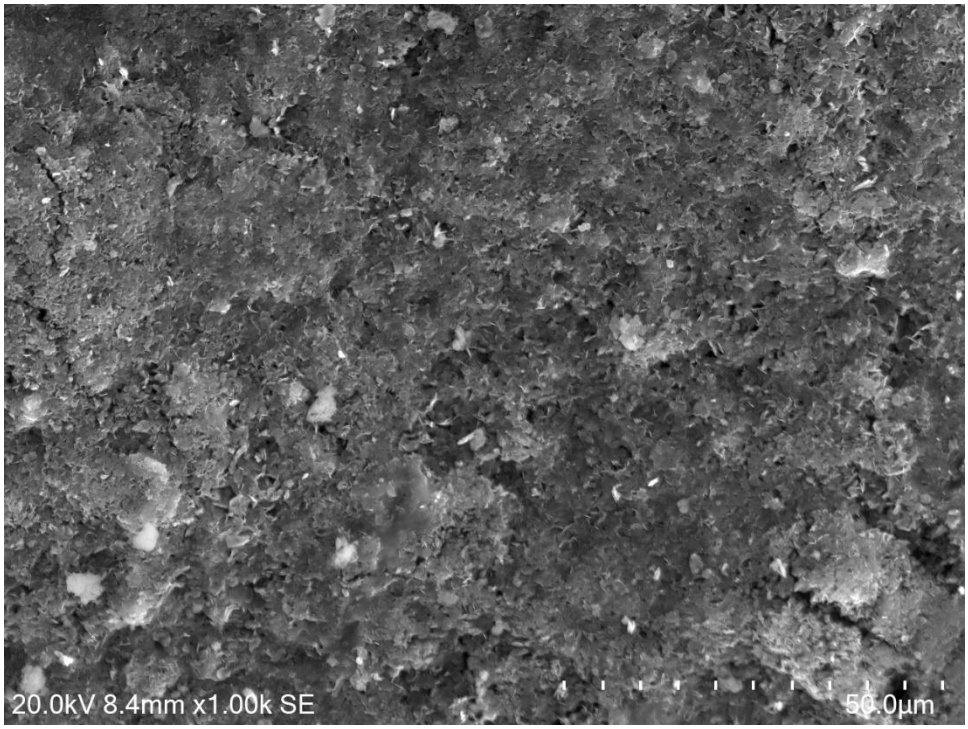

Figure 12 SEM IMAGE OF LAB-MADE CHITOSAN

Figure 12

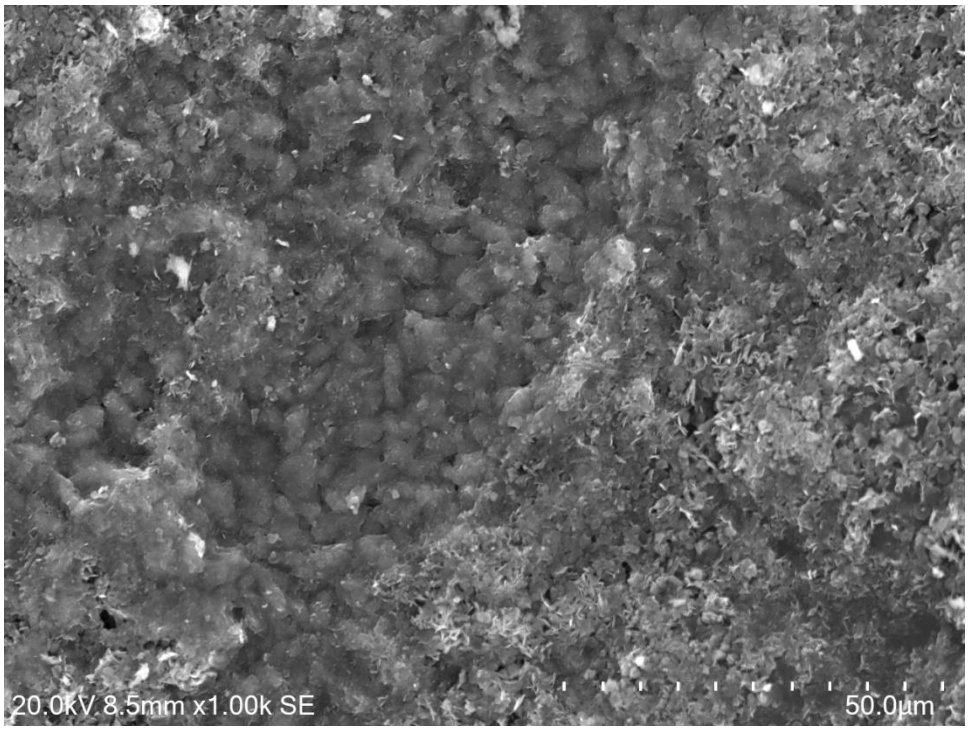

Figure 13

Figure 13 A DIFFERENT IMAGE OF SEM OF LAB-MADE CHITOSAN

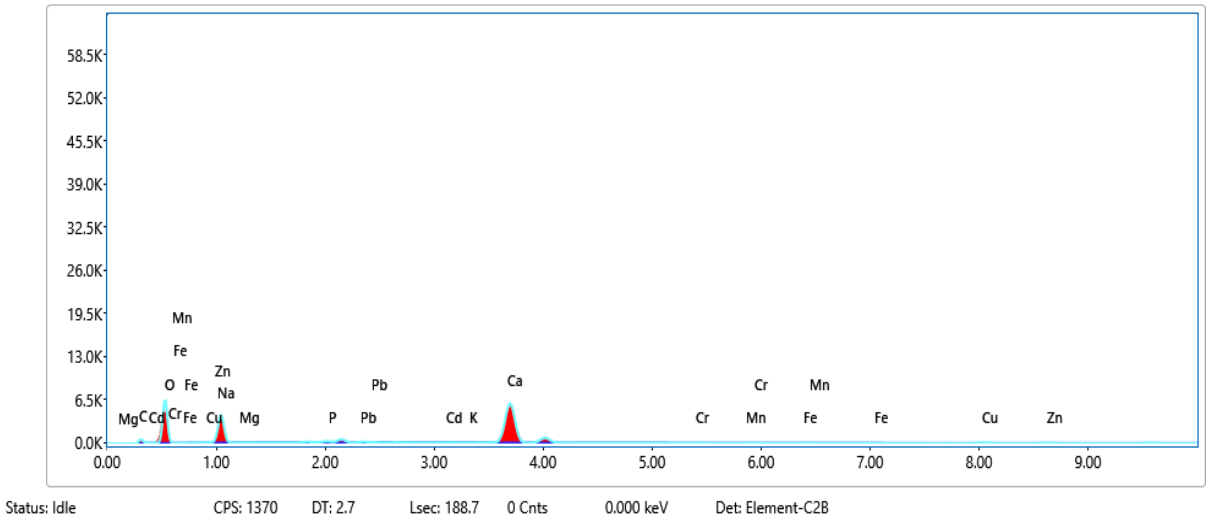

Figure 14 EDX GRAPH FOR LAB-MADE CHITOSAN

Element	***Weight %***
Na K	*46.21*
Mg K	*0.66*
P K	*1.75*
K K	*0.00*
Ca K	*33.15*
Cr K	*0.45*
Mn K	*0.51*
Fe K	*0.43*
Cu K	*4.61*
Zn K	*3.79*
Cd L	*0.37*
Pb M	*2.08*

Table 6 ELEMENT WEIGHT % FOR LAB-MADE CHITOSAN

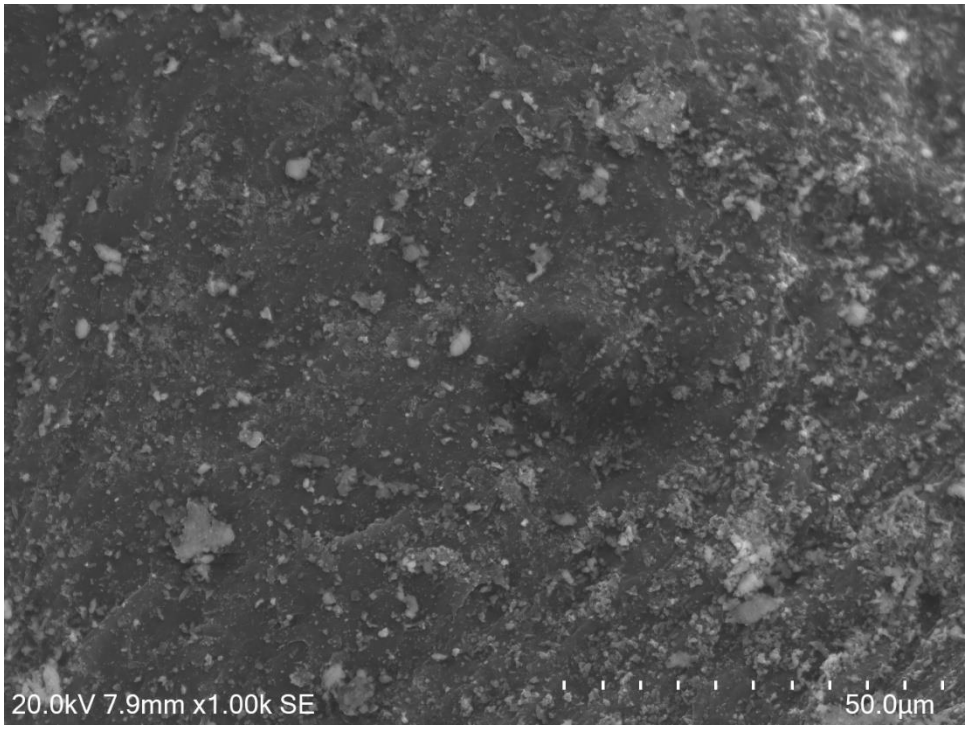

Figure 15 SEM IMAGE OF PURCHASED CHITOSAN

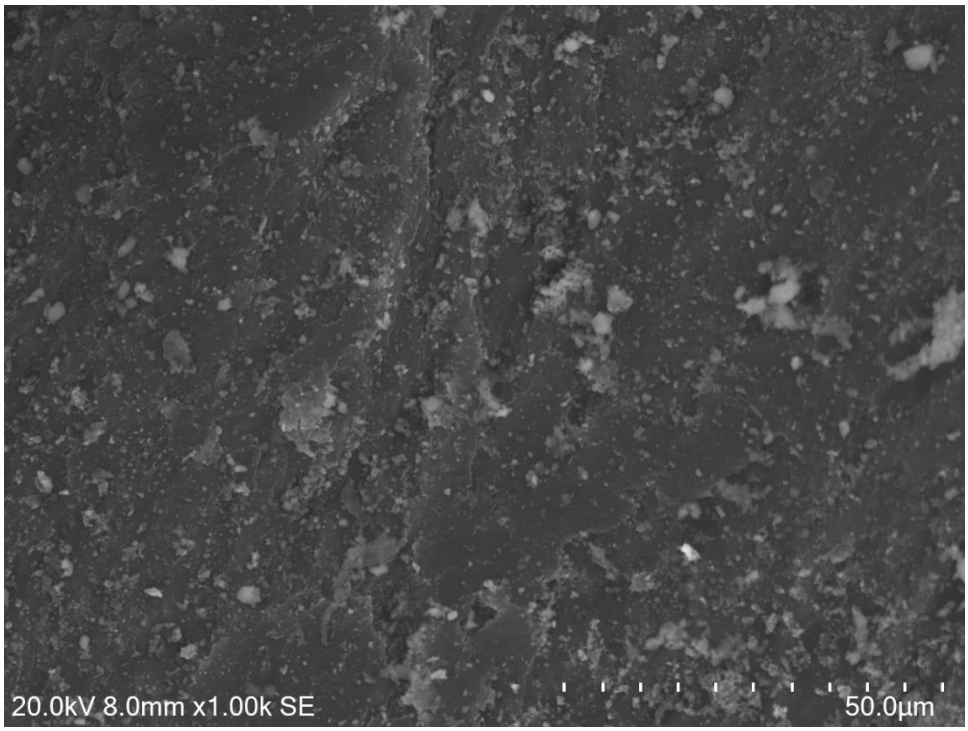

Figure 16 SEM IMAGE OF PURCHASED CHITOSAN

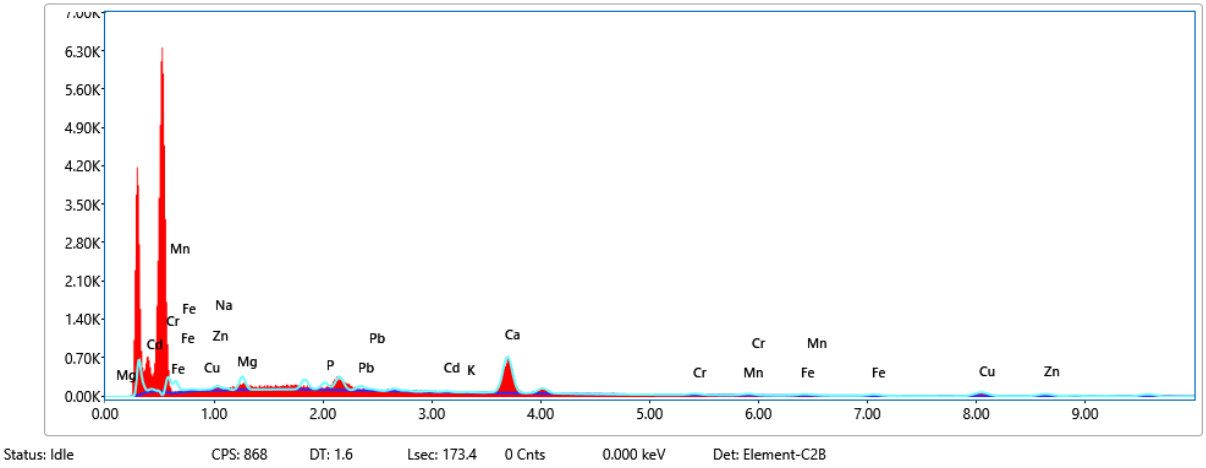

Figure 17 EDX GRAPH OF PURCHASED CHITOSAN

Element	Weight %
Na K	3.12
Mg K	19.63
P K	6.60
K K	0.00
Ca K	35.72
Cr K	1.95
Mn K	2.13
Fe K	2.01
Cu K	11.15
Zn K	7.88
Cd L	1.60
Pb M	8.21

Table 7 ELEMENT WEIGHT % OF PURCHASED CHITOSAN

SEM images revealed that the dry shrimp shell exhibited a rough and irregular surface structure, characteristic of its unprocessed state, with a complex matrix interspersed with calcium carbonate deposits. Upon demineralization, the shrimp shell showed a more uniform and smoother surface, indicating the effective removal of mineral content, primarily calcium. The lab-made chitosan displayed a porous and fibrous structure, indicative of successful chitin deacetylation, which enhances its solubility and functional properties. Comparatively, the purchased chitosan exhibited a more refined and consistent texture, likely due to industrial purification processes. EDX spectra further corroborated these observations by highlighting significant differences in elemental composition: the dry shrimp shell contained high levels of calcium and other minerals, which were significantly reduced in the demineralized sample. Both the lab-made and purchased chitosan showed a marked decrease in calcium content, confirming efficient demineralization and conversion processes. These results validate the effectiveness of our demineralization and chitosan extraction protocols, providing a solid foundation for further applications. However, the increase in sodium % in the final lab-made chitosan could be because of the 70% NaOH solution used during deproteinisation, which remained in the chitosan matrix due to inadequate washing.

4.3 ANALYSIS OF DRY SHRIMP SHELL FLAKES AND LAB-MADE CHITOSAN USING XRD –

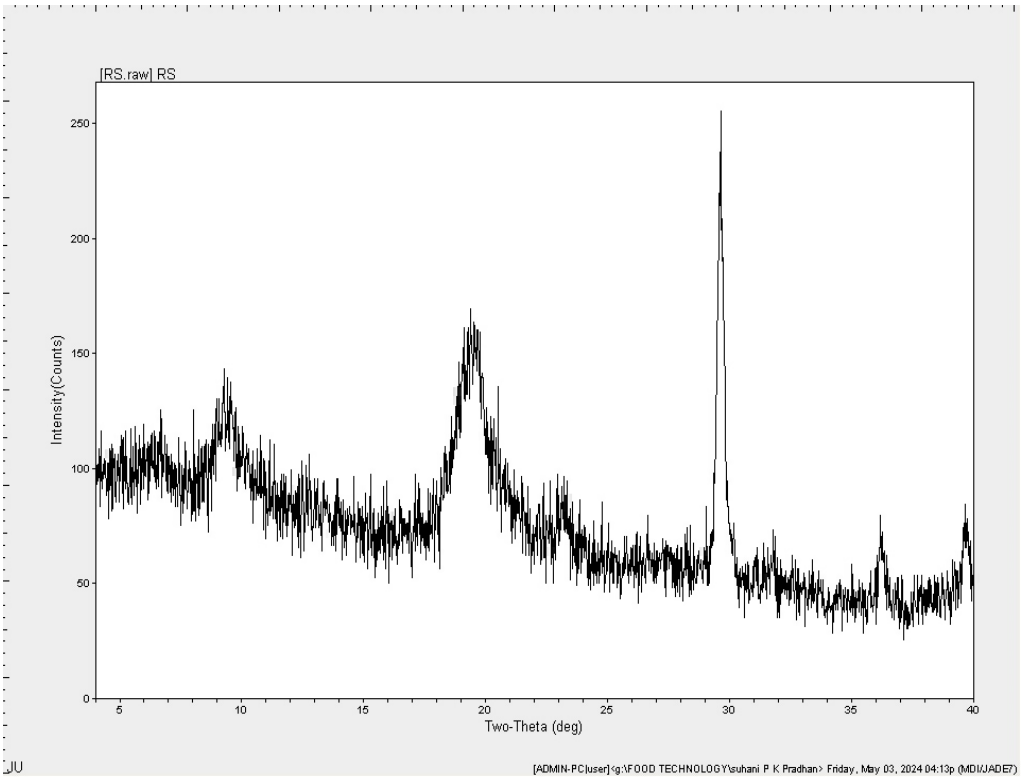

Figure 18 XRD GRAPH OF DRY SHRIMP SHELL FLAKES

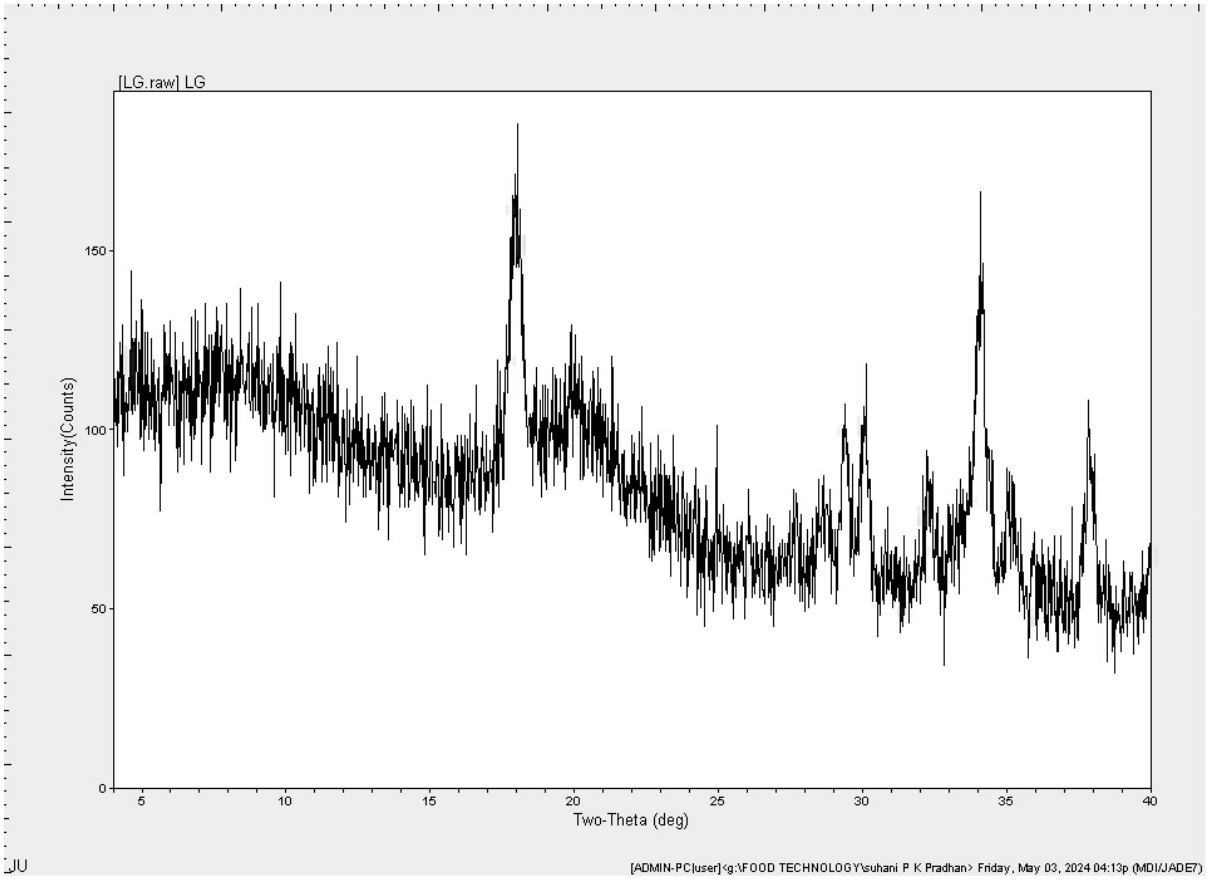

Figure 19 XRD GRAPH OF LAB-MADE CHITOSAN

The X-ray Diffraction (XRD) analysis of dry shrimp shell powder and lab-made chitosan provides crucial insights into the crystalline structure and phase composition of these materials. The XRD pattern of the dry shrimp shell powder typically exhibits distinct peaks corresponding to the crystalline regions of calcium carbonate, primarily in the form of calcite and aragonite, which are integral components of the shrimp shell matrix. These peaks indicate a high degree of crystallinity associated with the mineral content. In contrast, the XRD pattern of the lab-made chitosan shows a significant reduction in the intensity of these crystalline peaks, reflecting the removal of calcium carbonate during the demineralization process. Additionally, the chitosan exhibits broad diffraction peaks characteristic of amorphous regions, indicative of the deacetylated chitin polymer structure. The comparison of XRD data between the dry shrimp shell powder and the lab-made chitosan underscores the successful transformation from a crystalline mineral-rich material to an amorphous biopolymer. This shift in crystallinity not only confirms the efficiency of our chitosan production process but also highlights the enhanced properties of chitosan, such as solubility and reactivity, which are critical for its application in diverse industrial and biomedical fields.

4.4 ANALYSIS OF SHRIMP SHELL PRODUCTS USING FTIR-

4.4.1 COMPARISON BETWEEN RAW SHRIMP SHELL AND DRY SHRIMP SHELL -

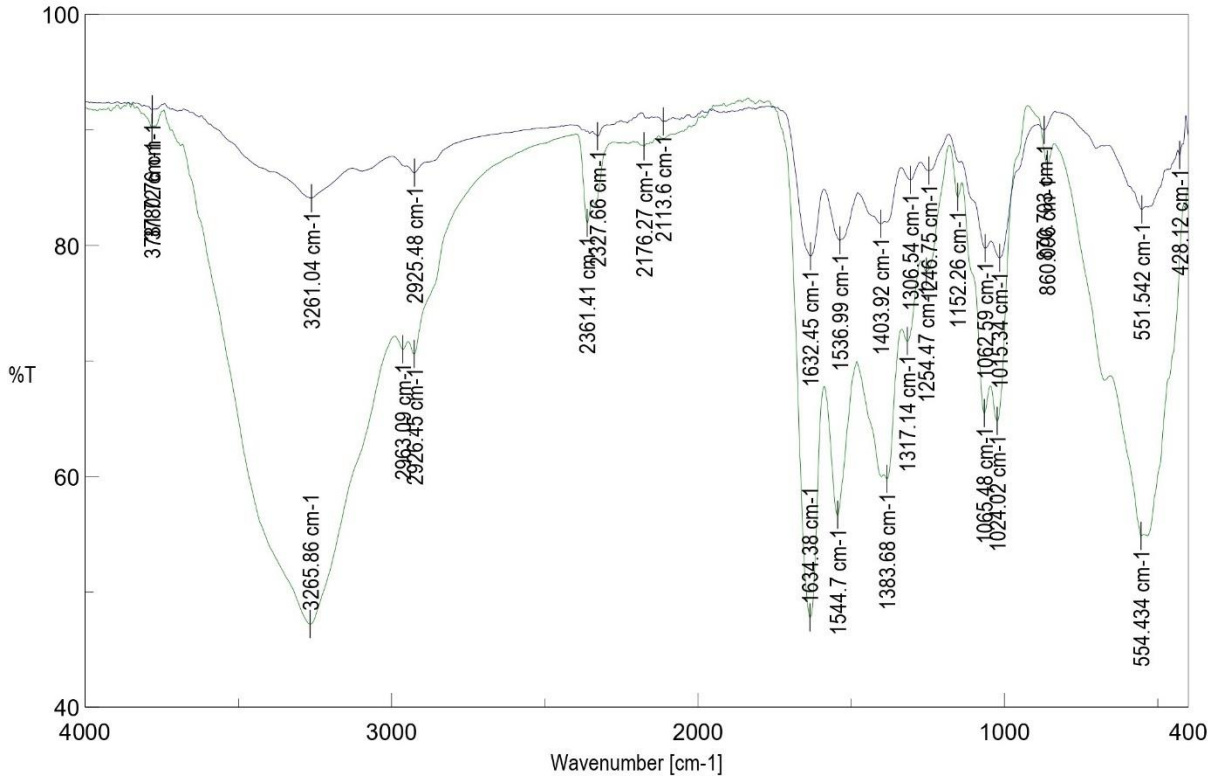

Figure 20 FTIR SPECTRUM OF RAW SHRIMP SHELL AND DRY SHRIMP SHELL

The Fourier Transform Infrared (FTIR) spectroscopy analysis of raw shrimp shell and dry shrimp shell provides valuable information on the functional groups present in these materials, which can be interpreted from their respective FTIR spectra in terms of wavenumber versus percentage transmittance.

For the raw shrimp shell, the FTIR spectrum exhibits several characteristic absorption bands. The broad peak around 3200-3400 cm^{-1} corresponds to the O-H stretching vibrations, indicating the presence of moisture and hydroxyl groups. The peaks near 2850-2950 cm^{-1} can be attributed to C-H stretching vibrations from aliphatic chains. Notable absorption bands at approximately 1650 cm^{-1} and 1550 cm^{-1} are associated with the amide I (C=O stretching) and amide II (N-H bending) vibrations, respectively, which are indicative of the chitin-protein complex in the shrimp shell. Additionally, the peak around 1400 cm^{-1} corresponds to the C-H bending vibrations, and the band near 1030 cm^{-1} is due to the C-O stretching vibrations, further confirming the polysaccharide nature of chitin.

In comparison, the FTIR spectrum of the dry shrimp shell shows similar absorption bands, with some notable differences in intensity and sharpness. The O-H stretching vibration peak around 3200-3400 cm^{-1} is less intense, indicating reduced moisture content due to drying. The amide I and II bands around 1650 cm^{-1} and 1550 cm^{-1}, respectively, are still present,

confirming the retention of the chitin structure. However, these peaks appear slightly sharper and more defined in the dry shrimp shell, suggesting a higher degree of organization in the chitin-protein matrix post-drying. The C-H stretching and bending vibrations, as well as the C-O stretching vibrations, remain prominent, confirming the structural integrity of the chitin polymer.

The comparison of the FTIR spectra of raw and dry shrimp shells indicates that drying primarily affects the moisture content and may enhance the ordering of the chitin structure, but does not significantly alter the fundamental chemical composition. This analysis underscores the stability of the chitin matrix in shrimp shells and validates the effectiveness of drying as a preparatory step without compromising the material's chemical integrity. These findings are crucial for subsequent processing steps, such as demineralization and chitosan extraction, where maintaining the structural fidelity of chitin is essential for yielding high-quality biopolymers.

4.4.2 ANALYSIS OF DEMINERALISED SHRIMP SHELL, CHITIN AND LAB-MADE CHITOSAN USING FTIR-

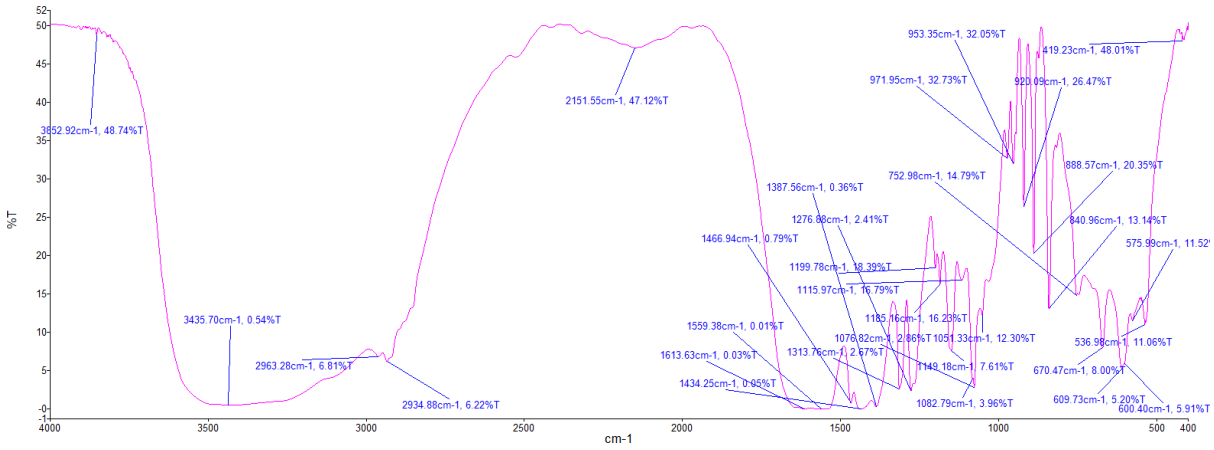

Figure 21 FTIR SPECTRUM OF DEMINERALISED SHRIMP SHELL

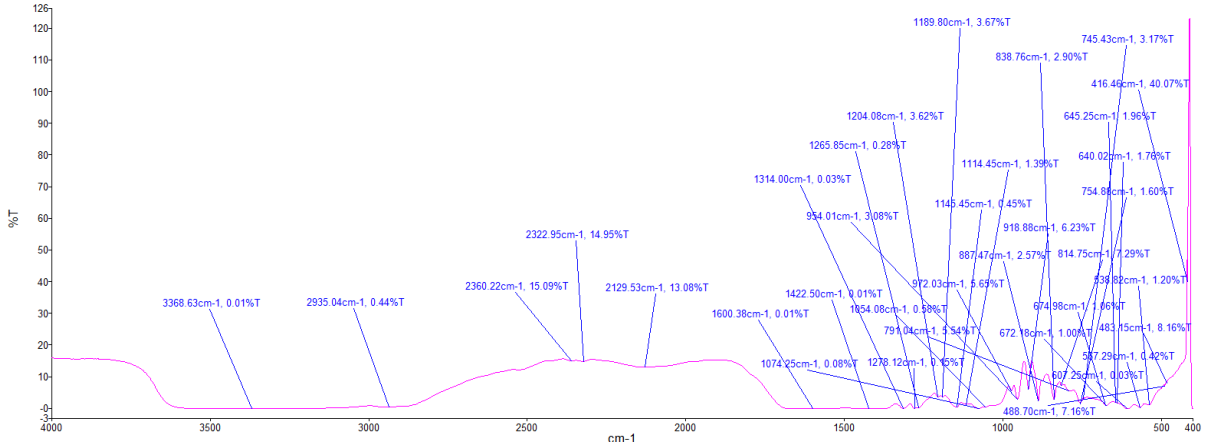

Figure 22 FTIR SPECTRUM FOR CHITIN

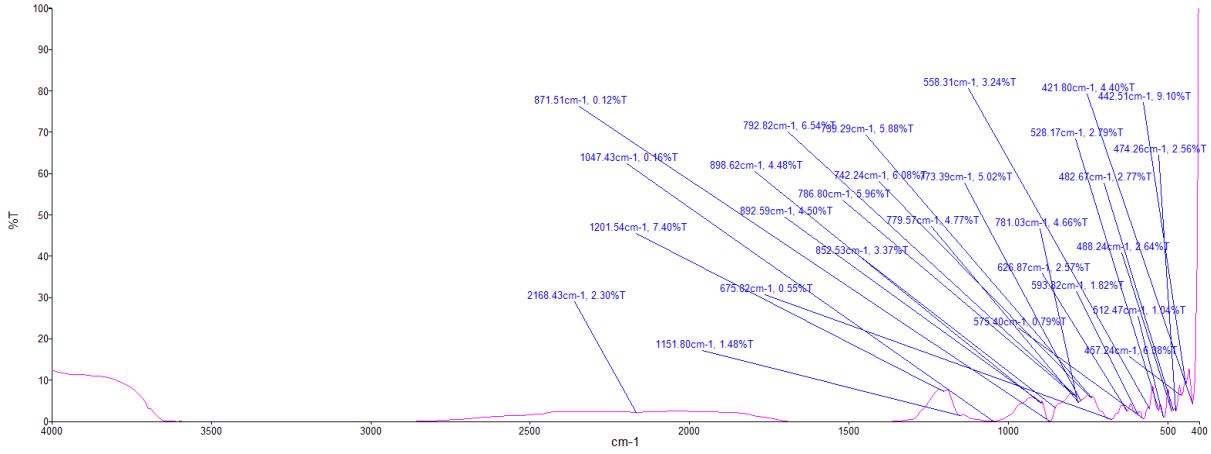

Figure 23 FTIR SPECTRUM FOR LAB-MADE CHITOSAN

The FTIR (Fourier Transform Infrared) analysis of demineralized shrimp shell, chitin, and lab-made chitosan provides significant insights into these materials' structural changes and chemical compositions. This section discusses the findings based on the FTIR spectra obtained and compares the characteristic peaks associated with each material, highlighting the transformation process from shrimp shell to chitin and finally to chitosan.

Demineralized Shrimp Shell

The FTIR spectrum of demineralized shrimp shell exhibited characteristic peaks indicating the presence of chitin and residual proteins. The major absorption bands were observed around 1655 cm^-1, 1550 cm^-1, and 1313 cm^-1, corresponding to the amide I (C=O stretching), amide II (N-H bending), and amide III (C-N stretching) vibrations, respectively. The presence of a broad band around 3420 cm^-1 indicated O-H stretching, which is typical for polysaccharides, suggesting the presence of chitin. The peaks around 2920 cm^-1 and 2850 cm^-1, corresponding to the C-H stretching of aliphatic groups, pointed to the presence of residual proteins or other organic matter.

Chitin

The spectrum of chitin showed enhanced peaks at 1655 cm^-1 (amide I), 1550 cm^-1 (amide II), and 1313 cm^-1 (amide III), which are indicative of the crystalline structure of chitin. The absence of significant peaks in the region 1000-1500 cm^-1 compared to the demineralized shrimp shell suggested the effective removal of proteins and other impurities. Additionally, the broad O-H stretching band at 3420 cm^-1 became more pronounced, reflecting the higher purity of the chitin sample. The peak at 896 cm^-1, corresponding to the β-glycosidic bond, further confirmed the presence of chitin.

Lab-Made Chitosan

The FTIR spectrum of lab-made chitosan displayed significant differences compared to chitin, particularly in the amide regions. The peak at 1655 cm^-1 (amide I) was significantly reduced and shifted to around 1600 cm^-1, indicating the conversion of acetamido groups (-NHCOCH3) to amino groups (-NH2). The peak at 1550 cm^-1 (amide II) was also reduced, further confirming the deacetylation process. A new peak around 1580 cm^-1 appeared, corresponding to the N-H bending of primary amines, characteristic of chitosan. The broad O-H stretching band at 3420 cm^-1 remained, but with increased intensity, reflecting the increased number of hydroxyl groups due to deacetylation.

The changes in the FTIR spectra from demineralized shrimp shell to chitosan highlight the successful removal of minerals and proteins and the subsequent conversion of chitin to chitosan. The presence of characteristic peaks associated with chitosan (such as the reduced amide I and II peaks and the new peak at 1580 cm^-1) confirms the effectiveness of the deacetylation process. These spectral changes validate the stepwise transformation process and the chemical modifications occurring at each stage.

Overall, the FTIR analysis effectively demonstrates the structural evolution from demineralized shrimp shells to chitosan, underscoring the chemical modifications and purification processes involved. This understanding is crucial for optimizing the production of chitosan for various applications, ensuring that the final product possesses the desired properties for its intended use.

4.5 ANALYSIS OF CHITOSAN COATED COCONUTS AND ITS COMPARISON WITH COCONUT WITHOUT ANY COATING

Figure 24 LAB-MADE CHITOSAN COATED COCONUTS ON DAY 24

Figure 25 PURCHASED CHITOSAN COATED COCONUTS ON DAY 24

Figure 26 COCONUT SLICES WITHOUT ANY COATING ON DAY 24

This study investigated the effectiveness of lab-made chitosan coating on coconut slices compared to purchased chitosan-coated and uncoated coconut slices over 24 days. The analysis focused on three key parameters: microbial spoilage, sensory evaluation, and moisture loss. The results provide insights into the preservation capabilities and quality maintenance of the different coatings.

Microbial Spoilage

- **Lab-Made Chitosan Coated Coconuts :** Visible mold growth was observed after 24^{th} day of storage in zip lock bags under refrigerated conditions.This could be due to lack of industrial inventory setup in an academic laboratory and hence inability to maintain accurate conditions during processing.
- **Purchased Chitosan Coated Coconuts :** Visible mold growth was observed after 26^{th} day of storage in zip lock bags under refrigerated (8deg C) conditions.
- **Uncoated Coconut Slices :** These turned yellow slimy within 10^{th} day of storage and hence conforming to the low shelf life of coconut flesh.

Sensory Evaluation

Sensory evaluation, encompassing taste, texture, and overall acceptability, is crucial for determining consumer preference and marketability.

- **Lab-Made Chitosan-Coated Coconuts:** These samples generally received slightly lower than those for purchased chitosan but still higher than uncoated samples. The chitosan coating likely contributed to a fresher taste and firmer texture by reducing moisture loss and microbial growth, thereby maintaining the quality of the coconut slices.
- **Purchased Chitosan-Coated Coconuts:** Sensory scores for these samples generally received the highest scores in sensory evaluations but still higher than uncoated samples. The commercial chitosan coating provided huge benefits in maintaining taste and texture throughout the storage of 24 days.
- **Uncoated Coconut Slices:** These samples received the lowest scores across all sensory parameters. The lack of coating led to quicker deterioration in taste and texture, likely due to higher moisture loss and faster spoilage. Visible yellow slimy texture change was observed within 10 days of storage.

Loss of Moisture

Moisture retention is a critical factor for the freshness and longevity of food products. Over the 24-day period, moisture loss varied significantly among the samples.

- **Lab-Made Chitosan-Coated Coconuts:** These samples showed reduced moisture loss with moisture % of 35 % compared to uncoated slices though not as effectively as the purchased chitosan suggesting that the lab-made chitosan coating required more improvement in terms of coating agent.
- **Purchased Chitosan-Coated Coconuts**. These sample showed the least moisture loss with moisture % of 46 % suggesting that the purchased chitosan coating was highly effective at forming a barrier to water vapor. This retention of moisture helped maintain the texture and overall quality of the coconut slices.
- **Uncoated Coconut Slices:** These slices experienced the highest moisture loss at a moisture % of 19% resulting in a dry and less appealing texture. The absence of a coating allowed for rapid water evaporation, leading to quality degradation.

CHAPTER 5

Conclusion

Conclusion

This thesis presents a comprehensive study on the extraction of chitosan from shrimp shells using environmentally friendly solvents, specifically citric acid and Deep Eutectic Solvents (DES). The adoption of these green technologies marks a significant departure from conventional extraction methods that rely on harsh chemicals such as hydrochloric acid and sodium hydroxide, offering a more sustainable and eco-friendly alternative.

The research demonstrates that chitosan extracted using these green solvents exhibits comparable, if not superior, properties to commercially available chitosan. This was evidenced through extensive characterization using techniques such as Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). The structural integrity and purity of the lab-extracted chitosan were validated, confirming its suitability for food preservation applications.

The application of this chitosan as an edible coating for fresh coconut slices revealed significant enhancements in shelf life. Coconut slices coated with the lab-made chitosan displayed an extended shelf life of up to 20 days under refrigerated conditions, while those coated with commercially purchased chitosan from Loba Chemie PVT LTD demonstrated a shelf life of nearly one month. This substantial improvement is attributed to the antimicrobial properties, moisture retention capabilities, and film-forming ability of chitosan.

Further analysis focused on microbial spoilage, sensory evaluation, and moisture loss, providing a holistic understanding of the coating's effectiveness. The chitosan coatings successfully inhibited microbial growth, preserved sensory qualities, and minimized moisture loss, thereby maintaining the freshness and quality of the coconut slices throughout the storage period.

In conclusion, this study not only establishes the feasibility of using green solvents for chitosan extraction but also underscores the practical benefits of chitosan as a natural preservative in extending the shelf life of perishable food products. The findings advocate for the broader adoption of green extraction technologies in the biopolymer industry, promoting sustainability while delivering high-performance materials. Future research can build on these results by exploring further applications and optimization of chitosan coatings in various food preservation contexts, contributing to the advancement of sustainable food processing and packaging solutions.

CHAPTER 5

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