PRODUCTION AND CHARACTERIZATION OF CHITOSAN EXTRACTED FROM SHRIMP SHELLS WASTE AND CHITOSAN FILM DEVELOPMENT

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ABSTRACT

Chitosan is a natural biopolymer with important biomedical and antimicrobial applications. In the present study, chitosan was extracted from the shells of Litopenaeus vannamei collected from a shrimp farm. The extraction process involved demineralization using hydrochloric acid, deproteinization using sodium hydroxide, and deacetylation to convert chitin into chitosan. From 10 g of shrimp shell, approximately 7 g of chitin was obtained, and 1 g of chitin was further converted into chitosan. The prepared chitosan was used to formulate a wound dressing Film by dissolving it in acetic acid, resulting in a homogeneous and stable film with suitable pH for skin application. Fourier Transform Infrared (FTIR) analysis was carried out at an external institution to confirm the presence of functional groups, and the results showed a reduction in amide peaks and the appearance of amine groups, confirming successful conversion. The study demonstrates that shrimp shell waste can be converted into valuable chitosan with potential applications in wound healing and antimicrobial fields.

Key words: Chitosan, Biopolymer, Litopenaeus vannamei, Fourier Transform Infrared Spectroscopy.

I. INTRODUCTION

Chitosan is a natural biopolymer acquired from chitin, which is one of the most plentiful natural polysaccharides originate in nature. Chitin is existing in the exoskeletons of crustaceans such as crabs, shrimps, and lobsters, as well as in the cell walls of fungi and some insects. The alteration of chitin into chitosan is attained through a chemical process called deacetylation, where acetyl groups are detached using strong alkaline solutions. (Antonino *et al.*, 2017). The chief species with the maximum manufacture produced by the Malaysian shrimp farming industry in 2018 were Pacific white leg shrimp, *LitoPenaeus vannamei* followed by Black tiger shrimp, *Penaeus monodon*, worth 203.82 million USD with a amount of 36,007 tons and 70.19 million USD with 9906 tons, respectively (Waiho *et al.*, 2020). In Malaysia, a seafood processing plant marketed shrimp as a fresh or treated product. According to their demand, maximum processed shrimp are traded to Europe, Japan, New Zealand, and Australia. Peeled-off shrimp or headless shell-on are favoured by the American and Japanese markets, while peeled and cooked shrimp are favoured by New Zealand and Australia (Hashim *et al.*, 2005).

Chitosan is a linear polysaccharide of natural source composed basically of β-(1,4)-linked glucosamine units (2-amino-2-deoxy- β -D-glucopyranose) together with some proportion of N-acetylglucosamine units (2-acetamino-2-deoxy- β -D-glucopyranose) Chitosan is characterized by its biocompatibility, biodegradability and non-toxicity (Dash *et al*., 2011). Its excellent biological properties (antimicrobial, antibacterial and coagulating activities, bio adhesivity and wound healing capacity) have made it an admirable applicant for claims in cosmetics (Vo *et al.*, 2013) medicine (Dash *et al*., 2011; Khor *et al*., 2013; Bouhenna *et al*., 2015) and pharmacy (Ahmed *et al*,.2016) agriculture (Sharp et al., 2013), chitosan in the preservation of agricultural supplies (Bautista-Banos *et al.*, 2016), the food industry (Luo *et al*., 2013) and waste water treatment (No *et al*., 2000), amidst numerous additional industrial applications. Chitosan has increased important attention in the fields of biotechnology, medicine, agriculture, and environmental science due to its distinctive properties such as biocompatibility, biodegradability, non-toxicity, and antimicrobial activity. Because of these characteristics, chitosan is broadly used in drug delivery systems, wound healing materials, water purification, food preservation, and agricultural applications. (Abebe *et al., 2016*).

Chitin is a normal polymer with white and firm appearances, nitrogenous, and has low chemical reactivity. Moreover, this polymer has a highly organized crystalline structure and counts as the second most abundant polysaccharide in nature, later cellulose (Vanessa *et al.*, 2020). Therefore, chitin and its by-products have an admirable economic worth due to their agrochemical uses and biological actions. Chitin by-products called chitosan are linear polysaccharides composed of N-acetylglucosamine and glucosamine. Chitosan can be removed using the traditional technique consisting of three main steps. They are demineralization, deproteination, and deacetylation of shell waste (Iber *et al*., 2021). Demineralization uses an acidic solution, while deproteination uses an alkaline solution. Lastly, deacetylation was performed to eliminate the acetyl group from the chitin polymer to attain chitosan. During deacetylation, chitin is cured using greatly concentrated alkaline (Kandile *et al*., 2018).

The mechanisms of action of chitosan against bacteria and fungi have been examined and stated in several articles (Chen, C.P *et al*., 2015; Felt, O *et al*., 2000). Whereas the antimicrobial properties of chitosan are massively linked with its structure, physicochemical characteristics, and environmental circumstances, in addition to the responsive hydroxyl groups at the C-3 and C-6 positions (Xing, K *et al*., 2015; Dutta, J *et al*., 2012), the method of action of chitosan against microbes can be classified as extracellular effects, intracellular effects, or together based on the directing site of the antimicrobial effects (Varlamov, V.P *et al*., 2018; Sharif, S *et al*., 2019). Meanwhile high-MW chitosan is commonly incapable to pierce the cell wall and cell membrane, its potential antimicrobial effects involved acting as a chelator of vital metals, avoiding nutrients from being taken up from cells extracellularly, and changing cell permeability (Sharif, S *et al*., 2019; Kong, M *et al*., 2010)However, low molecular weight chitosan not only has extracellular antimicrobial activity but also intracellular antimicrobial activity, thereby affecting RNA, protein synthesis, and mitochondrial function (Kong, M *et al*., 2010; Sudarshan, N.R *et al*., 1992).

In current years, chitosan has develop a vital biomaterial for research and industrial uses because it carries a bearable way to utilize seafood waste and produce value-added products. Therefore, studying the preparation and properties of chitosan is essential for understanding its potential applications in biotechnology and associated fields.

2. MATERIALS AND METHODS

2.1. Collection and Pre-treatment of Shrimp Shells:

Shells of *LitoPenaeus vannamei* were collected from a shrimp farm. The shells were washed thoroughly with tap water to remove adhering organic matter and impurities, followed by rinsing with distilled water. The cleaned shells were sun dried for 24 hours. The dried shells were ground into fine powder and stored in sterile airtight containers.

2.2. Demineralization:

10grams of powdered shell sample were treated with 1M HCl in a ratio of 1:10 (w/v). The mixture was stirred for 45 minutes at room temperature. The release of carbon dioxide indicated removal of calcium carbonate. The sample was filtered and washed repeatedly with distilled water until neutral pH was achieved.

2.3. Deproteinization:

The demineralized sample was treated with 1M NaOH (1:10 w/v) and heated at 70°C for 2 hours with continuous stirring using a magnetic stirrer. This step removed proteins present in the shell. The sample was washed thoroughly with distilled water until neutral pH was obtained.

2.4. Decolourization:

The sample was treated with 95% ethanol for 30 minutes to remove pigments, followed by washing with distilled water and drying in a hot air oven.

2.5. Deacetylation (Conversion to Chitosan):

Deacetylation of chitin was carried out by treating the chitin with 12.5 M sodium hydroxide (NaOH) at a solid-to-liquid ratio of 1:15 (g/mL). The reaction mixture was cooled and kept frozen at −83°C in an ultra-freezer for 24 hours. Subsequently, the temperature of the mixture was increased to 115°C, and the reaction was allowed to proceed with continuous agitation at 250 rpm for 4–6 hours. The resulting chitosan was filtered, washed with distilled water until neutral pH was achieved, and dried in an oven at 70°C.

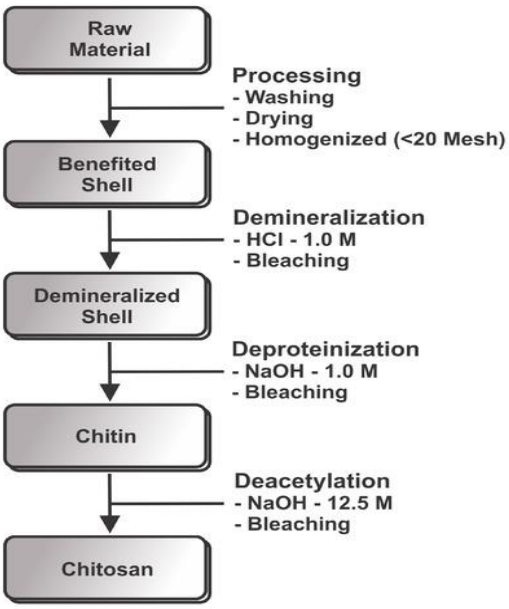


Fig 1: Schematic representation of the production of chitosan from shrimp.

2.6 Characterization of Chitosan:

2.6.1 Fourier Transform Infrared (FTIR) Analysis:

Chitosan samples were analysed using Fourier Transform Infrared (FTIR) spectroscopy to confirm their functional groups. The analysis was performed at an external institution due to the unavailability of FTIR facilities in the home laboratory. The obtained spectra were recorded and analysed to identify characteristic functional group peaks.

3. CHITOSAN FILM PREPARATION

3.1. Preparation of Chitosan Solution:

1–2 g of chitosan powder was dissolved in 100 mL of 1% acetic acid solution. The mixture was stirred continuously using a magnetic stirrer for 4–6 hours until a homogeneous solution was obtained. The pH of the solution was adjusted to 5.5–6.0 using NaOH. Glycerol (1–2%) was added as a plasticizer to improve flexibility of the dressing material.

3.2. Synthesis of Nanoparticles:

Silver nitrate solution was prepared in distilled water. The solution was heated at 60–70°C under continuous stirring. A reducing agent such as tris – sodium trihydrate as added dropwise. Formation of nanoparticles was indicated by colour change of the solution. The suspension was centrifuged at 10,000 rpm for 10 minutes. The obtained nanoparticles were washed with distilled water and dried.

3.3. Incorporation of Nanoparticles into Chitosan Matrix:

Prepared nanoparticles were added slowly into the chitosan solution. The mixture was stirred for 2 hours to ensure uniform distribution. The resulting mixture formed a nanocomposite chitosan solution.

3.4. Fabrication of Chitosan Wound Dressing Film:

The nanoparticle–chitosan mixture was poured into sterile Petri plates or Molds. The solution was dried at 40–50°C in a hot air oven for 24 hours. After drying, a thin flexible film was obtained. The films were carefully peeled and stored in sterile containers

3.5. Sterilization of Wound Dressing Material:

The prepared chitosan Nano composite films were sterilized using 70% ethanol washing, followed by drying under sterile conditions

4. RESULT AND DISCUSSION

4.1. Drying and grinding of Shrimp:

The collected shrimp shells were thoroughly washed and dried using sunlight until all moisture was removed. After drying, the shells became brittle and lightweight, indicating effective dehydration and prevention of microbial growth. A reduction in weight was observed due to the loss of water content. The dried shells were then ground into a fine, uniform powder using a mechanical grinder. The resulting powder appeared off-white to pale pink in color with a consistent texture, making it suitable for further processing such as chitin and chitosan extraction.

4.2. Extraction of Chitin:

The shells of *Litopenaeus vannamei* collected from a shrimp farm were used for the extraction of chitin. The shells were cleaned, dried, and powdered. During demineralization,

effervescence was observed due to the removal of calcium carbonate. After deproteinization, the sample became softer and lighter in colour, indicating removal of proteins and impurities. From 10 g of shrimp shell, approximately 1 g of chitin was obtained.

4.3. Conversion of Chitin to Chitosan:

The extracted chitin was converted into chitosan by deacetylation using concentrated sodium hydroxide. After the reaction, the material changed from a rigid structure to a more flexible and light cream-colored substance. This indicated the successful conversion of chitin into chitosan.

Fig 2: Collected Shrimp shell

Fig 3: Extracted Chitin from Shrimp shells

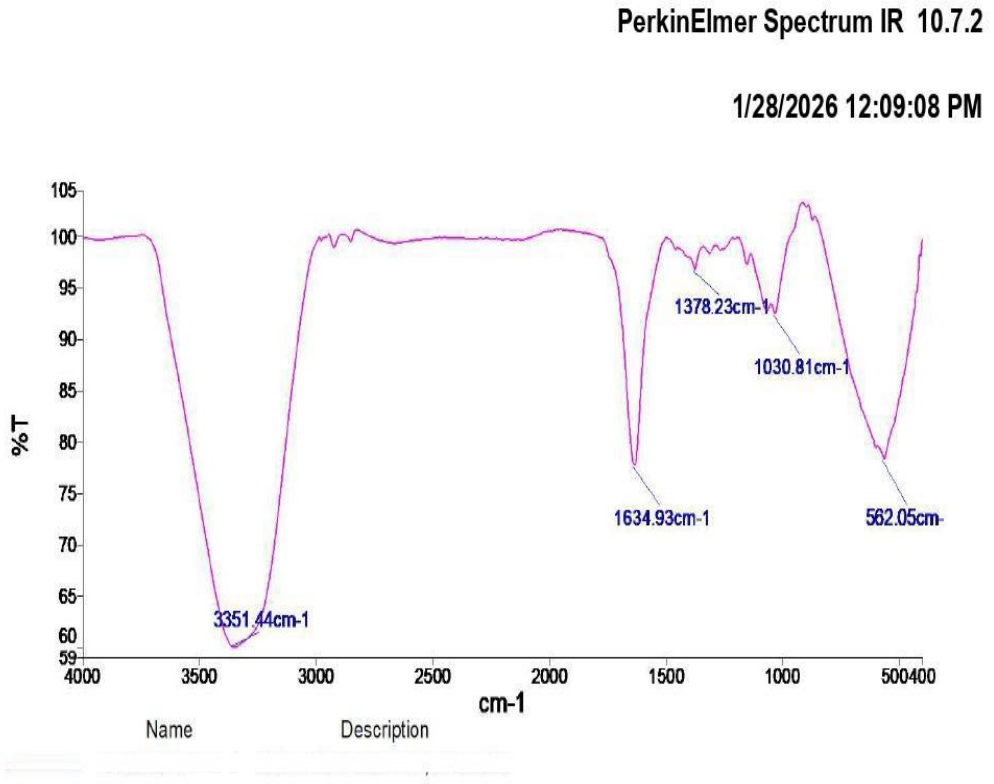
Fig 4: Improved Chitin to Chitosan

Fig 5: Filtrated Chitosan solution

4.4. FTIR Analysis:

FTIR analysis was carried out at an external institution to confirm the functional groups. The spectrum showed characteristic peaks corresponding to O–H, amide, and amine groups. The reduction in amide peaks and the appearance of amine peaks confirmed the conversion of chitin into chitosan. The FTIR analysis of the prepared chitosan film revealed characteristic

absorption peaks confirming the presence of functional groups associated with chitosan. A broad peak observed at 3351.44 cm^{-1} corresponds to O–H and N–H stretching vibrations, indicating the presence of hydroxyl and amine groups. The peak at 1634.93 cm^{-1} is attributed to the amide I (C=O stretching) group, which confirms the polymeric backbone of chitosan. The absorption band at 1378.23 cm^{-1} represents C–H bending vibrations, while the peak at 1030.81 cm^{-1} corresponds to C–O stretching, indicating the polysaccharide nature of chitosan. Additionally, a peak at 562.05 cm^{-1} suggests structural vibrations within the polymer matrix. Overall, the FTIR spectrum confirms the successful formation of chitosan and retains its essential functional groups, demonstrating its suitability for biomedical applications such as wound dressing.



Peak Table

Peak Number	X (cm-1)	Y (%T)
1	3351.44	59.97
2	1634.93	77.81
3	1378.23	96.89
4	1030.81	92.63
5	562.05	78.42

Graph 1: FTIR Analysis for Chitosan

5. PREPARATION OF CHITOSAN FILM

5.1. Preparation of Chitosan Solution:

The chitosan solution was successfully prepared by dissolving chitosan powder in 1% acetic acid. Continuous stirring for 2–3 hours resulted in a clear and viscous solution, indicating

complete dissolution of chitosan. The absence of visible particles confirmed the effectiveness of the solvent system.

5.2. Addition of plasticizer:

The incorporation of glycerol as a plasticizer improved the flexibility of the solution. After adding glycerol and stirring, the solution exhibited enhanced homogeneity and reduced brittleness, which is essential for forming a flexible wound dressing film.

5.3. pH Adjustment:

The pH of the chitosan solution was successfully adjusted to the range of 5.0–5.5 using sodium hydroxide. This pH range is suitable for biomedical applications and ensures stability of the polymer without causing precipitation.

5.4. Filtration and Purity:

Filtration using Whatman filter paper resulted in a clear and uniform solution free from undissolved particles and impurities. This step ensured better film quality and uniformity.

5.5. Film Formation (Casting Method):

The filtered chitosan solution was cast onto petri plates and spread evenly to form a thin layer. Upon drying at 40°C for 24–48 hours, a uniform film was formed, indicating good film-forming ability of chitosan.

The results confirmed that shrimp shell waste can be effectively converted into chitosan and used for antimicrobial applications.

Fig 6: Prepared Chitosan Film

6. DISCUSSION

The extraction of chitin from the shells of *Litopenaeus vannamei* was successfully carried out using chemical methods. During demineralization, the release of carbon dioxide indicated the removal of calcium carbonate. Deproteinization further removed proteins, resulting in a clean and soft material. A high yield of chitin (about 7 g from 10 g shell) was obtained, indicating effective processing. The conversion of chitin into chitosan by deacetylation showed clear physical changes such as increased flexibility and solubility, confirming successful transformation.

FTIR analysis confirmed the presence of important functional groups in both chitin and chitosan. The reduction of amide peaks and the appearance of amine peaks in the chitosan spectrum indicated successful deacetylation. These results confirmed that the extracted product was chitosan and contained the functional groups responsible for its biological activity. The prepared chitosan film showed good physical properties such as smooth texture, viscosity and stability. The film was homogeneous and maintained a suitable pH range for skin application.

These properties indicate that chitosan can be effectively used as a wound dressing material. The film forming ability of chitosan is an important characteristic for biomedical applications.

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