

Extraction and Characterization of Chitin and Chitosan from Shrimp Shell and Squid Pen Waste: Application in Biofilm Production

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Abstract—The objective of this study was to extract and characterize α -chitosan from *Penaeus Vannamei* shrimp shell waste and β -chitosan from *Loligo Vulgaris* squid pen waste using a chemical extraction method, thereby providing a theoretical basis for the improved development and utilization of chitosan-based biomedical materials. Extraction involved sequential deproteinization, demineralization, and deacetylation, followed by structural characterization using Fourier Transform Infrared (FT-IR) spectroscopy. FT-IR spectra confirmed the successful conversion of chitin to chitosan and revealed characteristic bands corresponding to O-H/N-H stretching, amide groups, and glycosidic linkages. The degree of deacetylation (DD) was calculated as 77.82% for α -chitosan and 94.45% for β -chitosan, indicating that β -chitosan undergoes deacetylation more readily due to its parallel chain arrangement and weaker intermolecular hydrogen bonding. These structural differences significantly influence physicochemical properties such as solubility and crystallinity, which are directly linked to biological activity. Given that higher DD enhances erythrocyte and platelet aggregation, the high-DD β -chitosan obtained from squid pens demonstrates strong potential for use in hemostatic gel biomaterials. Furthermore, biofilms produced from both α - and β -chitosan underline their promise for biomedical applications, particularly in wound healing and surgical hemostasis.

Keywords—Chitin/Chitosan, degree of deacetylation, biofilm, biomaterial applications

I. INTRODUCTION

Globally, shellfish processing generates an estimated 12–14 million tons of waste annually, of which approximately 20–40% is composed of chitin, a naturally occurring biodegradable polymer [1][2].

Chitin, also known as poly(β -(1 $\rightarrow$ 4)-N-acetyl-D-glucosamine), is a valuable raw material for the commercial production of chitosan. It is primarily sourced from marine waste such as squid pens, shrimp shells, and crab shells, and is recognized as the second most abundant natural biopolymer after cellulose [3].

Chitin and chitosan differ mainly in their degree of deacetylation (DD) and solubility. Partial deacetylation of chitin in an alkaline medium, where acetyl groups are removed and replaced with amino groups (NH_2), yields chitosan, a linear polysaccharide with a greater application potential [4].

Chitosan consists of two types of sugar units: glucosamine (deacetylated) and N-acetylglucosamine (acetylated). The proportion of acetylated units, known as the degree of acetylation (DA), is an important parameter used to describe and classify chitosan [5][6].

α , β , and γ are the three primary polymorphic structures of chitin, which can be found in fibrous, crystalline forms. The α -form, which is primarily found in crab and shrimp shells, is structurally different from β -chitin, which is primarily found in squid pens, and γ -chitin, which is found in yeast and fungi. Because of its weaker intermolecular hydrogen bonds and parallel polymer chains, β -chitin is more open to deacetylation during the chitosan production process and exhibits higher reactivity and affinity in a variety of physical and chemical treatments [7].

Chitosan's unique biological properties, such as its non-toxicity, biocompatibility, solubility in acidic media, and anti-inflammatory activity, make it incredibly valuable in biomedical applications [8]. It is a versatile and promising bioactive polymer, according to recent studies. Since it is the main derivative of chitin, its free amino groups make it extremely susceptible to chemical modification, which allows the synthesis of a variety of derivatives while maintaining biodegradability. [9]. These readily synthesized and commercially available derivatives have been applied to drug delivery [10], wound healing [11], and tissue engineering [12]. Apart from their biomedical applications, chitin and chitosan have also been used in food, textiles, cosmetics, water treatment, pharmaceuticals, and agriculture [13]. A key biomedical use of chitosan is in hemostatic agents that aid wound care and promote healing, with several products already approved by the United States Food and Drug Administration (USFDA) [5].

Karahaliloğlu et al. (2017) [14] evaluated the performance of active nano/microbilayer hemostatic dressings in a diabetic rat femoral artery bleeding model. The hemostatic dressing containing chitosan and bacterial cellulose, reinforced with protamine sulfate and coated with silk fibroin (SF-coated PS/BC/CTS), significantly reduced bleeding time to 80 ± 0.3 seconds compared to standard gauze ($p < 0.005$).

Recently, drug-loaded chitosan nanoparticles have been

widely studied for cancer therapy. Asiri et al. [15] reported that glutaraldehyde-conjugated chitosan nanoparticles significantly reduced HCT-116 colorectal cancer cell viability in a dose-dependent manner and altered cell morphology.

This study aimed to prepare different types of chitosan and evaluate their application in producing biopolymeric films. Chitosan was extracted from shrimp shell and squid pen waste using a chemical extraction method. β -Chitin was successfully isolated and characterized from squid pens, while α -chitin was obtained from shrimp shells. The resulting chitosan powders were characterized using FT-IR spectroscopy. Afterwards, biofilms were produced from both α - and β -chitosan with the objective of creating a hemostatic and a blood-clotting biomaterial for surgical applications, utilizing chitosan—a biopolymer well-documented- for its antibacterial and hemostatic activities.

II. EXPERIMENTAL PROCEDURE

A. Materials

The squid pens and the raw shrimp shell waste were collected from a local fish market 'Mola Balıkçılık' in İzmir, Turkey. All chemicals and solvents used in this study were analytical grade or the highest available purity and all solutions were freshly prepared using distilled water.

B. Sample Preparation

Chitosan isolation includes 3 basic stages, namely deproteinization, demineralization and deacetylation. Figure 1 shows the chitin and chitosan extraction stages and biofilm production from shrimp and squid waste shells. Before extracting the chitin and chitosan, the shells must be free from undesired substances, so collected wastes were washed with running water and distilled water then dried in an oven at 40 °C for 24h. The dried samples were grounded before starting further extraction.

1) *Deproteinization*: The process of deproteinization involves breaking the chemical bonds that hold chitin and proteins together, which causes the biopolymer to depolymerize when exposed to an alkaline solution. In order to extract the proteins from the shells, chemical deproteinization is a necessary step [16].

2) *Demineralization*: Demineralization is the process of removing minerals from shells and pens, mostly calcium carbonate. Usually, demineralization is accomplished by acid treatment with HCl. Because demineralization entails the breakdown of calcium carbonate into water-soluble calcium salts and the release of carbon dioxide, as indicated in [8] by the (1):



3) *Deacetylation*: The deacetylation process converts chitin into chitosan by removing acetyl groups through treatment with concentrated NaOH, leaving free amine groups ($-\text{NH}_2$) [17].

C. Production of Chitosan

First step of extraction of chitin was deproteinization. The dried shell and squid pen fragments are immersed in a 5% NaOH solution ratio of 1:10 (w/v) and heated at 70°C for 2.5 hours with stirring to remove residual proteins and fats. The sample is then subjected to repeated washing with distilled water until the pH reaches a neutral level of approximately 7. The second step of extraction was demineralization which is the deproteinized material is then subjected to treatment with 1 M hydrochloric acid (HCl) at room temperature for a period of 60 minutes. This process serves to eliminate calcium carbonate and other mineral components. The material was then filtered, and the washing process was repeated with distilled water until a neutral pH was achieved. The extracted chitin was subjected to deacetylation using a 50% (w/v) NaOH solution at 100 °C for 6h, with a solid-to-solution ratio of 1:20 (w/v), to remove acetyl groups. Following the reaction, the material was filtered and repeatedly washed with distilled water until reaching a neutral pH. The resulting chitosan was then dried and prepared for subsequent biofilm production.

D. Preparation of Chitosan Bio-Films

For each sample, 0.2 g of chitosan was dissolved in 1% (v/v) acetic acid (CH_3COOH) under constant stirring for 1–2 hours, after which the solution was poured into flat, flexible plastic molds. Glycerin is used as a plasticizer to obtain a film with good mechanical properties. The samples were then left to dry at room temperature until solid films were formed.

III. CHARACTERIZATION OF α - AND β -CHITOSAN

A. FT-IR Analysis and Deacetylation Degree of Produced Chitosan

FT-IR was used to characterize the obtained chitosan powder in order to identify functional groups and use the baseline method to determine the DD. The analysis was also performed to observe structural differences between the two types of chitosan. Infrared spectra of chitosan were recorded using a Perkin Elmer Frontier™ FT-IR spectrometer (Perkin Elmer, USA) with attenuated total reflectance (ATR) mode. Each spectrum was obtained by averaging 32 scans at a resolution of 4 cm^{-1} over the range 400–4000 cm^{-1} covering both the functional group region and the fingerprint region. The amide III band at 1320 cm^{-1} was used as the analytical band, with 1420 cm^{-1} selected as the reference band for determining the DD. This approach minimizes interference from water, residual acids, or alkalis. DD was calculated from the absorption ratio of the 1320 to 1420 cm^{-1} peaks (A_{1320}/A_{1420}) for both α - and β -chitosan. Brugnerotto et al. (2001) [18] reported that this ratio provides the lowest error and is highly sensitive to the chemical composition of chitin and chitosan, regardless of technique, state, or secondary structure. Moreover, this method has been widely used in other studies and shown to yield reliable results consistent with alternative approaches [19]. The degree of deacetylation was calculated from the obtained FT-IR spectra from (2) proposed by Brugnerotto et al (2001) [18] :

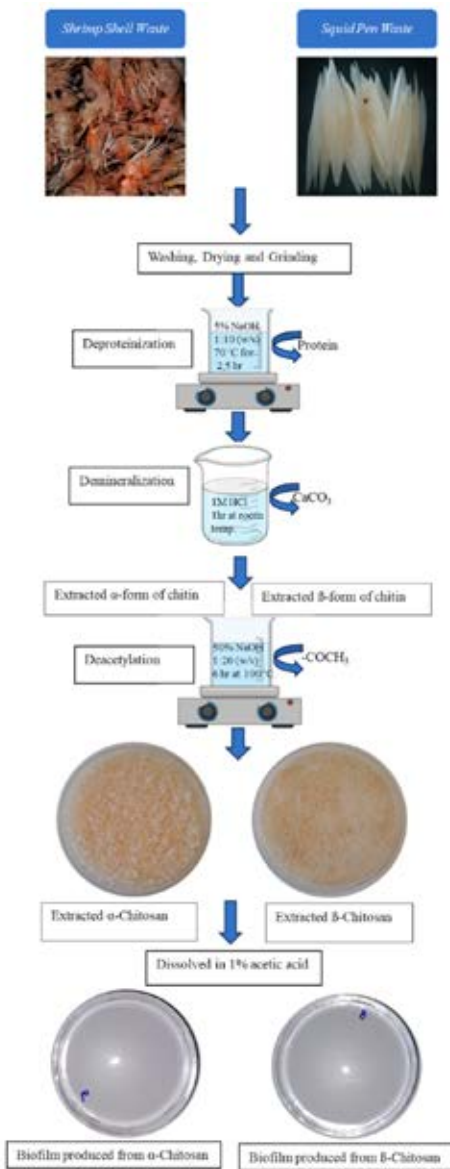


Fig. 1: Schematic representation of the extraction and production process of chitin and chitosan from shrimp shell and squid pen waste.

$$DA(\%) = [((A_{1320})/(A_{1420}) - 0.3822)/0.03133] \quad (2)$$

DA: Degree of Acetylation

$$DD(\%) = 100 - DA(\%) \quad (3)$$

The A_{1320} band reflects N-acetylation, while the A_{1420} band serves as a reference for comparing D-glucosamine and N-acetylglucosamine units [18].

IV. RESULTS AND DISCUSSIONS

A. FT-IR Characterization

Figure 2 shows the FT-IR spectra of the produced α - and β -chitosan, both of which closely match typical literature profiles. Both samples display the characteristic broad O–H/N–H

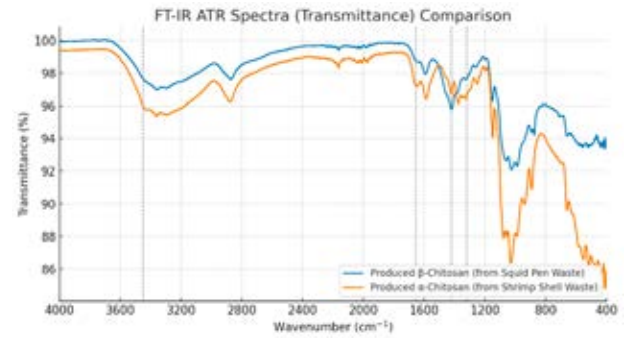


Fig. 2: FT-IR Spectra of α - and β -Chitosan

stretching band at $\sim 3400\text{--}3200\text{ cm}^{-1}$, indicative of hydrogen bonding in the chitosan structure. The amide I band ($C=O$ of residual N-acetyl groups) appears at 1658.78 cm^{-1} for α -chitosan and 1655 cm^{-1} for β -chitosan, with greater intensity in α -chitosan, suggesting higher residual acetylation. Similarly, the amide II band at $\sim 1560\text{ cm}^{-1}$ (N–H bending) is more intense in α -chitosan, reinforcing this observation. The CH_3 stretching vibration at $\sim 2920\text{ cm}^{-1}$ is present in both samples but slightly stronger in α -chitosan, consistent with a more ordered/crystalline matrix. CH_2 bending at 1420 cm^{-1} is relatively higher in β -chitosan, whereas the CH_3 bending at $\sim 1380\text{ cm}^{-1}$ is slightly stronger in α -chitosan; these bands are commonly referenced in DD estimations. Both spectra also show the glycosidic β -(1 $\rightarrow$ 4) linkage signal near 895 cm^{-1} , confirming the expected polysaccharide backbone.

Overall, β -chitosan (from squid pens) exhibits weaker amide I and II bands than α -chitosan (from shrimp shells), indicating a higher degree of deacetylation, while α -chitosan retains more acetyl groups and displays features typically associated with greater crystallinity.

B. Degree of Deacetylation

The degree of deacetylation is the most crucial factor defining a chitosan sample. Chitosan is generally defined as partially or fully deacetylated chitin, with DD values typically above 50% [20]. In this study, the DD of chitosan extracted from shrimp shells was calculated as 77.82%, whereas chitosan obtained from squid pens exhibited a markedly higher DD of 94.45%, based on FT-IR analysis. These results confirm that β -chitosan (from squid pens) undergoes deacetylation more readily than α -chitosan (from shrimp shells). This observation is consistent with the FT-IR spectra, where β -chitosan showed weaker amide I and II bands, reflecting a lower residual acetyl content. The higher reactivity of the β -form, linked to its parallel chain arrangement and weaker hydrogen bonding, explains its greater susceptibility to deacetylation. As reported in the literature, the DD is strongly influenced by the morphology and origin of chitin, which in turn significantly affects key properties of chitosan such as solubility, hydrophilicity, crystallinity, and biological response [21], [22].

V. CONCLUSION

α - and β -chitin were extracted from shrimp shell and squid pen waste and subsequently converted into α - and β -chitosan through deacetylation. The chemical composition of the obtained chitosan was characterized by FT-IR, which showed DD values of 77.82% for α -chitosan and 94.45% for β -chitosan. FT-IR spectra indicated that β -chitosan exhibited weaker amide I and II bands than α -chitosan, confirming a higher DD(%), while α -chitosan retained more acetyl groups and displayed features associated with higher crystallinity. These differences are attributed to the parallel chain arrangement and weaker intermolecular hydrogen bonding of β -chitin, which make it more reactive. Since the degree of deacetylation directly affects hemostatic performance by promoting erythrocyte and platelet aggregation [23], high-DD β -chitosan from squid pens demonstrates strong potential for developing hemostatic gel biomaterials. In addition, the chitosan biofilms produced in this study further highlight the biomedical potential of chitosan, particularly in wound healing and hemostatic applications.

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