Isolation and Characterization of Shrimp Shell Chitosan

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Abstract : Chitosan is a polysaccharide formed through the deacetylation of chitin. Chitosan is commonly produced from the waste of the fishing industry, such as shrimp, crab, or lobster shells with a process that first extracts the chitin and then goes to deacetylation. This research aims to isolate and characterize chitosan derived from shrimp to examine its physicochemical properties using various methods. The moisture content was determined by drying the sample in an oven (13.1%), indicating a relatively high moisture level compared to the Indonesian National Standard (SNI) scale (12%), while the ash content was obtained through combustion (8.68%). FTIR analysis identified characteristic peaks at 3500-3000 cm^-1 (OH group), 1600-1500 cm^-1 (amide C=O group), ±1600 cm^-1 (N-H group), and ±1000 cm^-1 (C-N group), which are typical features of chitosan's structure. The degree of deacetylation, indicating a reduction in acetylation groups, was calculated through acid-base titration (20.196%), confirming the formation of chitosan. The result of the deacetylation degree is way below the SNI standard (>75%). Information about these chitosan characteristics highlights its potential applications in the food, pharmaceutical, and agricultural industries.

INTRODUCTION

Chitosan is a polysaccharide formed by the deacetylation of chitin, a major component of the exoskeletons of crustaceans such as shrimp, crabs, and lobster (Marieta & Musfiroh, 2019). Shrimp shell chitosan, in particular, holds immense promise as it is abundantly available as a byproduct of the thriving fishing industry. One of the many things about chitosan properties is it can be used as a biopolymer that can easily be degraded (Cahyono, 2018).

The demand for sustainable and eco-friendly in recent years has driven the interest to utilize more biodegradable raw materials, such as shrimp shells, to produce valuable products like chitosan. Chitosan extracted from shrimp shells exhibits these properties, making it a promising solution to synthetic polymers for various applications (Dompeipen et al, 2016). By isolating and characterizing chitosan from shrimp shells, we can analyze its structure and chemical composition, leading to a better understanding of its potential.

Chitosan is isolated using various methods like maceration and demineralization (Purwanti & Yusuf, 2014). The isolation process plays a crucial role in determining the quality of chitosan. One of the many methods to isolate chitosan is a process called maceration which uses some chemical as a solvent to isolate the chitin first and the product will go through a process called deacetylation to remove the acetyl group in the chitin (Purgiyanti et al, 2022). The extraction method can influence the physicochemical properties of chitosan. There are many parameters of the quality which what chitosan should have. The standard that chitosan should meet according to 2013 SNI standard num7949 moisture level, ash content, and deacetylation level (Kusmiati & Hayati, 2020).

This research analyzes commercial chitosan extracted from shrimp shells by reviewing many articles that relate to the isolation and characterization of chitosan and comparing it with our already available chitosan. We aim to identify the properties and chemical composition of chitosan derived from shrimp shells. The findings of this study will make a clearer understanding of chitosan as a renewable biopolymer.

METHOD

Pectin Isolation

The isolation of chitosan from shrimp involves several steps to extract and purify the chitosan from the chitin-rich shells of the shrimp. Chitosan is derived from chitin through a deacetylation process. Here's a general overview of the isolation process:

1. Shell Preparation:

The shells of shrimp (and other crustaceans) contain chitin, which is a polymer made up of N-acetylglucosamine units. The first step is to collect and thoroughly clean the shells to remove any debris or contaminants.

2. Demineralization:

Shrimp shells usually contain minerals like calcium carbonate. To remove these minerals, the shells are treated with an acid solution, commonly hydrochloric acid. This process is known as demineralization and helps in making the subsequent steps more efficient.

3. Deproteinization:

The demineralized shells are then treated with an alkaline solution, often sodium hydroxide (NaOH), to remove proteins and other organic matter. This step breaks down the protein bonds and solubilizes them, leaving behind the chitin structure.

4. Deacetylation:

The chitin obtained from the previous step is then subjected to deacetylation. This involves treating the chitin with a strong base like sodium hydroxide at an elevated temperature. The acetyl groups on the chitin molecules are hydrolyzed, leading to the conversion of chitin into chitosan. The degree of deacetylation (DD) can be controlled by adjusting the conditions.

5. Neutralization and Filtration:

After deacetylation, the mixture is neutralized to pH 6-7 to stop the reaction. Insoluble chitosan begins to precipitate out of the solution. The mixture is then filtered to separate the chitosan from the liquid.

6. Washing and Drying:

The isolated chitosan is washed with water to remove any residual chemicals and impurities. It is then typically dried, either through air drying or using other drying methods.

7. Grinding and Milling (Optional):

Depending on the desired particle size and application, the dried chitosan can be ground or milled to achieve the desired physical characteristics.

FTIR Analysis of Chitosan from Shrimp Shells

Sample chitosan extracted from shrimp shells was characterized using Fourier Transform Infrared Spectroscopy (FTIR) to determine the functional groups present in the compound.

Moisture Content Test

A 1-gram sample of chitosan was weighed and placed into a crucible, which was then placed in an oven at 105°C for 1 hour. Subsequently, the sample was cooled in a desiccator and weighed until a constant weight was obtained (Pardede et al., 2013).

$$\%water\ content = \frac{Original\ weight\ -\ \text{Constant weight}}{\text{Original weight}}\ x\ 100\%$$

Ash Content Test

A 1-gram sample of chitosan was placed into a pre-weighed crucible and then placed in a muffle furnace at 600°C for 3 hours. Afterward, it was allowed to cool in a desiccator for one hour and weighed until a constant weight was achieved (Pardede et al., 2013).

$$\%\ Ash\ Content = \frac{\text{Ash weight}}{\text{Pectin weight}}\ x\ 100\%$$

Deacetylation degree

The deacetylation degree of chitosan refers to the extent to which the acetyl groups on the N-acetyl-D-glucosamine units of chitosan have been removed, resulting in the presence of glucosamine units. This parameter is important because it affects the physical, chemical, and biological properties of chitosan, influencing its potential applications. To calculate the deacetylation degree of chitosan, you can use several analytical methods, with the most common one involving Fourier Transform Infrared Spectroscopy (FTIR) or Proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy.

$$\%DD = \left[100 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{100}{1{,}33}\right)\right]$$

Explanation:
A1665=Absorbance at wavelength 1665cm^(-1)
A3450=Absorbance at wavelength 3450 cm^(-1)

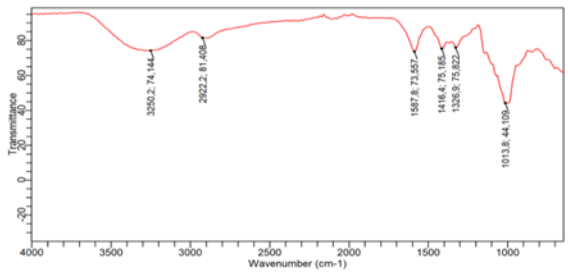
RESULTS AND DISCUSSION

Chitosan, a distinctive linear polysaccharide, presents a unique composition comprising randomly distributed units of β-(1→4)-linked D-glucosamine (in its deacetylated form) and N-acetyl-D-glucosamine (in its acetylated form). This intriguing molecule is synthesized by subjecting the chitin shells sourced from diverse crustaceans, such as shrimp, to an alkaline treatment, commonly utilizing substances like sodium hydroxide (Shahhidi & Synowiecki, 1991).

The versatility of chitosan becomes evident when exploring its multifaceted commercial and potential biomedical applications. In the realm of agriculture, its role as a seed treatment and biopesticide stands out, offering plants a natural means to defend against fungal invasions, thereby bolstering crop yields and quality. Its involvement in winemaking extends to acting as a fining agent, playing a pivotal role in not only enhancing the wine's clarity and visual appeal but also contributing to its longevity by mitigating the risk of spoilage (Lee et al, 2013). Branching into the industrial sphere, chitosan showcases its prowess by being incorporated into self-healing polyurethane paint coatings. This innovative application harnesses chitosan's inherent properties to contribute to the paint's durability and longevity, offering a promising solution in the field of advanced materials (Saputro & Ovita, 2017).

Table 1: Quality measurement of commercial chitosan

Measured Quality	Result	Indonesian National Standard (SNI No. 7949, Year 2013)
Water level content	13,1%	≤ 12%
Ash content	8,86%	≤ 5%
Deacetylation degree	20,196%	≤75%



Peak Number	Wavenumber (cm-1)	Intensity
1	1013,83589	44,10867
2	1326,93227	75,82173
3	1416,38838	75,18483
4	1587,84592	73,55667
5	2922,23286	81,40812

Figure 1: FTIR Spectra of commercial Chitosan

Water level content determination plays a pivotal role in the comprehensive evaluation of the quality and suitability of chitosan obtained from shrimp shells. This crucial step in the analytical process provides valuable insights into the amount of water present within the chitosan sample, a parameter that can significantly impact

its overall performance and potential applications (Djaenudin et al, 2019). In the specific case of the chitosan sample derived from shrimp shells, the measured moisture content of 13.1% has emerged as a noteworthy observation, indicating a marginally higher level when juxtaposed against the stipulated threshold of 12% as set by the Indonesian National Standard (SNI) (Kusmiati & Nurhayati, 2020). This disparity, while relatively modest, holds substantial implications for the utility and efficacy of the chitosan in diverse fields.

The Water content of chitosan is a fundamental attribute that affects its physical, chemical, and mechanical properties. In applications such as food production, pharmaceutical formulations, and agricultural practices, maintaining an optimal moisture level is imperative to ensure desired outcomes. The slightly elevated moisture content as indicated in the analysis can potentially influence the chitosan's stability during storage and transportation (Adamczuk et al, 2021). Excessive moisture can contribute to microbial growth, degradation, and undesirable changes in the material's texture, potentially diminishing its shelf life and overall quality. This divergence from the SNI standard could trigger considerations for adjusting processing techniques or implementing enhanced packaging strategies to mitigate the effects of higher moisture content (Kusmiati & Nurhayati, 2020).

The assessment of ash content, a method reliant on the process of combustion, offers value in understanding the composition of inorganic matter within the chitosan sample derived from shrimp shells (Liu, 2019). This analytical technique is performed by putting the sample under high temperatures thus combusting organic components and leaving behind an insoluble residue. In this study, the obtained ash content of 8.68% serves as a vital indicator hence the implication of minerals metals, and various non-organic substances intrinsic to the chitosan structure (Takarina et al., 2017).

The ash content is the tangible representation of the “non-volatile” fraction within the chitosan that encompasses both essential and incidental elements. The minerals and metals identified in the ash content may originate from either originating solely where crustaceans reside or from processing methods employed within chitosan extraction (Liu, 2019). Calcium is prevalent in crustacean shells and could contribute to the ash content (Wei et al, 2021). Other elements like magnesium, potassium, and phosphorus might also be present potentially influencing not only the physicochemical properties of the chitosan but also those of its parent form – shrimp.

The measured ash content of 8.68% calls for a judicious inquiry into its implications for chitosan’s applicability across diverse industries. In further investigation and through a meticulous comparison with established standards, such as those prescribed by the Indonesian National Standard (SNI), an imperative becomes Cartesian to measure. In the standard, the acceptable level is below or the same as about 5% (Kusmiati & Nurhayati, 2020). This points to a higher than standard ash content of the pectin that we acquired.

In the food industry, for example, elevated ash content might raise concerns about sensory attributes and overall product quality (Kusmiati & Nurhayati, 2020). In pharmaceuticals, the presence of certain minerals could influence drug interactions or alter the chitosan's behavior in drug delivery systems. Additionally, in agricultural applications, the ash content may impact soil health and plant growth. Therefore, a comprehensive understanding of the relationship between ash content and application-specific requirements is indispensable.

Fourier-transform infrared (FTIR) analysis is an effective spectroscopic method that delivers profound information about the complex molecular characteristics of chitosan derived from shrimp shells (Dutta, 2017). This non-destructive analytical technique allows scientists to examine the vibrational modes of different chemical groups within the structure of chitosan (Fadlelmoula et al, 2022). FTIR spectrum obtained from the chitosan sample shows a captivating array of peaks that unravel its underlying molecular architecture.

In the FTIR spectrum, the presence of peaks in the range of 3500-3000 cm^-1 provides a compelling indication of the hydroxyl (OH) functional groups (Sadeghi et al, 2003). These hydroxyl groups are integral components of chitosan's polysaccharide structure, contributing to its overall hydrophilic nature and interaction capabilities. The resonance observed in this region signifies the stretching vibrations of the OH groups, underscoring their significance in the material's chemical behavior and potential applications.

Further analysis of the FTIR spectrum unveils distinctive peaks in the region of 1600-1500 cm^-1, which is characteristic of the amide C=O stretching vibrations (Faix, 1998). This observation serves as a compelling confirmation of the presence of chitosan's core structure, validating the transformation of chitin into chitosan through the deacetylation process. The amide group is a hallmark of chitosan's composition, and its detection via FTIR reaffirms the successful conversion of chitin's acetyl groups to amino groups in the chitosan structure.

Moreover, the FTIR analysis offers a captivating revelation through the presence of peaks at approximately 1600 cm^-1 and 1000 cm^-1. These peaks serve as distinct markers for the N-H and C-N groups, respectively. The identification of these functional groups further strengthens the evidence of chitosan's molecular composition. The N-H groups highlight the presence of amino functionalities that contribute to chitosan's unique properties, including its ability to form complexes with various molecules. Similarly, the C-N groups signify the connectivity between carbon and nitrogen atoms, reaffirming the chitosan's complex yet well-defined molecular structure (Jackson & Mantsch, 1995).

The derived degree of deacetylation, as a parameter that was calculated from analysis, has importance in determining the amount of transformation that happened when chitin to chitosan was produced. The value below the threshold set stipulated by Indonesian National Standard (SNI) is to be deemed of good quality, it must be higher or the same as 75% (Kusmiati & Nurhayati, 2020). This proves that our chitosan has not yet formed properly, because it is just 20,196%.

The degree of deacetylation is indicative of the proportion of acetyl groups that have been successfully removed, resulting in the formation of glucosamine units within the chitosan polymer (Zakaria et al, 2012). In the context of this analysis, the calculated value signifies that a portion of chitin's acetyl groups remains intact, contributing to the observed lower degree of deacetylation. This insight prompts a proactive stance toward enhancing the chitosan production process.

Fine-tuning the deacetylation conditions offers a promising avenue for elevating the degree of deacetylation and consequently enriching the chitosan's properties. Process optimization can encompass a spectrum of variables, including reaction time, temperature, the concentration of deacetylating agents, and pH levels (Zakaria et al, 2012). By systematically experimenting with these parameters, researchers can potentially achieve a higher degree of deacetylation that aligns more closely with the SNI standard.

The ramifications of achieving a higher degree of deacetylation extend beyond mere compliance with regulatory standards. A more extensively deacetylated chitosan can exhibit enhanced solubility, increased positive charge density, and improved interaction capabilities (Mima et al, 1983). This, in turn, could broaden the spectrum of potential applications across various industries. Chitosan's properties as a biopolymer, its biocompatibility, and its potential for controlled release mechanisms make it an attractive candidate in fields like bioplastic making.

CONCLUSION

The commercial chitosan that we get, have a quality that not meet the national standard. FTIR Spectra conclude that there is a deacetylation process which indicate a chitosan

forming in our sample but have not meet the national standard on a ≤75%. Water level content measured in our sample on a 13,1% is much more acceptable but slightly higher form standard on ≤12% level. The ash content is on a 8,86% that is lower than standard on ≤5%. All of this measurement is indicated there is still some impurities on a production of chitosan that make the product not meet the required quality. Further research is needed to examine the cause of this impurities.

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