

Characteristics of Chitosan from Black Soldier Fly Pupa Shells as a Crosslinking Agent in the Manufacture of Slow-Release Fertilizer Hydrogels

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Abstract: Chitosan, a natural polysaccharide obtained through the deacetylation of chitin, exhibits unique properties that make it a potential material for various applications, including agriculture. This study aims to examine the characteristics of chitosan derived from Black Soldier Fly (BSF) pupa shells and its role as a crosslinking agent in polymer-based hydrogel synthesis for slow-release fertilizers. Chitosan was isolated through chemical processes, including demineralization, deproteinization, and deacetylation. Characterization was conducted using FTIR, XRD, SEM, TEM, and TGA. The results revealed that chitosan from BSF pupa shells has a degree of deacetylation of 83%, a semi-crystalline and slightly amorphous structure, pores that enhance water absorption capacity, and high thermal stability. These properties make chitosan an effective crosslinking agent, improving hydrogel stability and extending nutrient release duration. These findings demonstrate the potential of chitosan as an innovative material for hydrogel applications in sustainable agriculture.

Keywords: Black soldier fly; Chitosan; Crosslink agent; Hydrogel; Slow-release fertilizer

Introduction

Chitosan is a natural biopolymer obtained through chitin deacetylation and is widely found in the exoskeleton of insects, crustaceans, and some types of fungi. Chitosan has attracted attention for its properties which include biocompatibility, biodegradability, as well as the ability to form gels and films. These characteristics of chitosan makes it suitable for application in various fields such as biomedical, environmental, agricultural and food industry (Wang et al., 2020). One potential application of chitosan is as a crosslinking agent in the manufacture of hydrogels (Deng et al., 2021; Saragih et al., 2020) particularly for slow-release fertilizers (SRF).

Hydrogel is a cross-linked polyelectrolyte polymer material with high water absorption and retention capabilities as well as the ability to release water gradually under osmotic pressure (Lestari et al., 2022; Liu et al., 2019). Hydrogels are manufactured from natural or synthetic sources and exhibit unique biocompatibility properties that contribute to excellent mechanical properties (Gun'ko et al., 2017; Jelita et al., 2024). Other properties of hydrogels such as insoluble in water (Miratsi et al., 2021; Saragih et al., 2021), able to maintain their original shape, soft, elastic, and flexible, make hydrogels often used for various applications in the food, agriculture, medicine, and cosmetic industries (Lubis et al., 2023; Susanto et al., 2024). Hydrogels, with their exceptional water absorption and retention

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capacities, play a crucial role in improving water and nutrient management in agriculture. By gradually releasing water or nutrients, hydrogels enhance fertilizer efficiency and mitigate environmental pollution (Thombare et al., 2021; Zinge & Kandasubramanian, 2020). The effectiveness of hydrogels, however, depends heavily on the stability of their three-dimensional structure, which is determined by the choice of crosslinking agents.

Traditionally, chitosan is sourced from crustacean shells, such as shrimp and crab. However, reliance on crustacean sources poses sustainability challenges due to seasonal availability, geographic limitations, and competition with other industries. To address this, Black Soldier Fly (BSF) pupa shells have emerged as a promising alternative source. BSF larvae are known for their efficiency in bioconverting organic waste into valuable biomass, with their pupa shells rich in chitin (Budikania et al., 2021; Mirwandhono et al., 2024; Sulistyawati et al., 2022). Utilizing BSF pupa shells for chitosan production not only offers a sustainable and abundant raw material but also contributes to reducing organic waste, aligning with circular economy principles (Soetemans et al., 2020; Zlotko et al., 2021).

This study is novel in its focus on utilizing chitosan derived from BSF pupa shells as a crosslinking agent for hydrogel synthesis. While previous studies have explored crustacean-sourced chitosan, research on insect-based chitosan, particularly from BSF pupa shells, remains limited. Furthermore, this research examines the potential of BSF-sourced chitosan to improve the stability, mechanical properties, and nutrient release efficiency of hydrogels for SRF applications, addressing key challenges in sustainable agriculture.

The importance of this research lies in its ability to utilize insect-derived chitosan, this study reduces dependency on traditional crustacean sources and supports waste valorization. BSF-derived chitosan offers a biodegradable and renewable alternative to synthetic crosslinkers, which is in line with the goal of developing environmentally friendly solutions. Improving the performance of hydrogels in SRF applications can improve nutrient use efficiency, reduce environmental pollution, and support the improvement of sustainable agricultural efficiency (Hapsari & Suparno, 2023; Nguyen et al., 2019). Investigating the characteristics and applications of BSF-derived chitosan contributes to a broader understanding of insect biopolymers, their industrial potential and expands knowledge in materials science.

This study aims to characterize chitosan from black soldier fly pupa shells and evaluate its potential as a crosslink agent in the manufacture of hydrogels for slow release fertilizer applications. It is hoped that this

research can contribute to the development of sustainable fertilizer technology and utilize insect waste as value-added materials.

Method

Materials

The main ingredient used in this study is dried black soldier fly (BSF) pupa shells. The chemicals used include sodium hydroxide (NaOH), hydrochloric acid (HCl), potassium permanganate (KMnO₄), oxalic acid (H₂C₂O₄), ethanol, and distillate water. All chemicals used have a pro analysis (p.a) purity level.

Preparation of BSF Pupa Shells

BSF pupa shells are washed with clean water to remove any dirt that sticks to them. After that, the shells are dried in the oven at 60°C for 24 hours until completely dry. The dried shells are then crushed using a grinder until they become a fine powder

Chitosan Extraction Process

The extraction process of chitosan from BSF pupa shells was carried out through several stages, namely deproteinization, demineralization, and deacetylation (Hisham et al., 2024) can be shown in figure 1. At the demineralization stage, bubbles and large bubbles are formed, which indicate the breakage of mineral bonds in the shell of the BSF pupa. The Deproteinization stage aims to remove the protein content using a NaOH solution based on the number of protein components present in the sample used. In the deproteinization stage, bubbles are formed and a brownish-red color changes. Handayani et al. (2018), stated that the appearance of a few bubbles, and the solution turned reddish-brown and thickened which occurred during the deproteinization process due to the release of protein content and binding with Na⁺ ions to form Na-proteinate. The deacetylation stage aims to break down the acetamide group (NHCOCH₃) in chitin into an amine group (NH₂) by using a NaOH solution, this process usually uses a 50% NaOH concentration and at this stage is very important in the success rate of chitosan production. Lin et al. (2021) states that the chitosan obtained is highly dependent on the effectiveness of the deacetylation step and the source of the chitin used.

Characterization

The resulting chitosan was analyzed using the Fourier Transform Infrared (FTIR) method to examine the functional group and degree of deacetylation (DD). X-ray Diffraction (XRD) characterization is performed to identify the crystal structure and degree of crystallinity of chitosan. The XRD pattern of chitosan generally shows typical peaks at 2θ around 10° and 20°, the

presence of a semi-crystalline structure. Surface morphological characterization was carried out using Scanning Electron Microscopy (SEM) and EDX (Energy Dispersive X-ray) to see high purity of chitosan and the structural dimensions of chitosan was determined using JEOL JEOL JEM 1400 at voltage of 80 kV. The thermal stability of chitosan was determined by thermogravimetric analysis using DTG-60 Shimadzu at a temperature range of 30-600°C with a heating rate of 10°C/min.

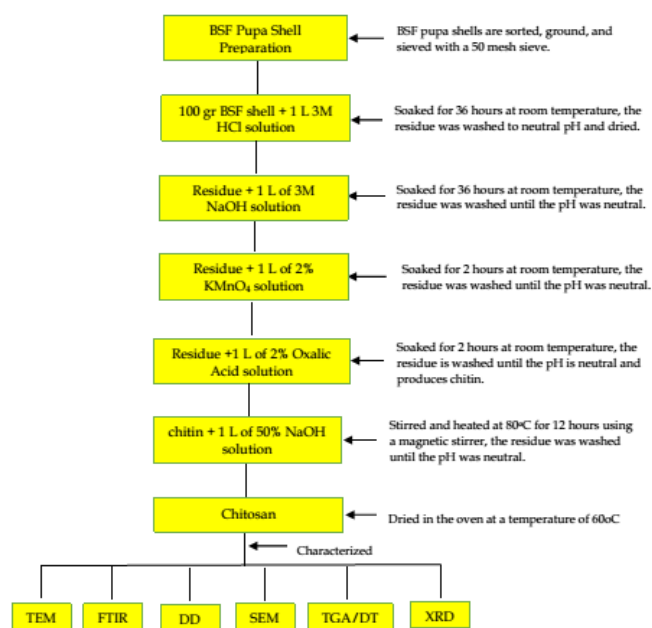


Figure 1. Flow diagram of chitosan production from BSF pupa shells

Result and Discussion

From the research that has been carried out as shown in figure 2, from 100 g of BSF pupa shells, 8.5 g of chitosan was obtained, so that a percentage of yield of 8.5% was obtained. The mass reduction that occurred by 91.5% was due to the demineralization and deproteinization process, namely the removal of minerals and proteins dissolved in HCl and KMnO₄ reagents which were then lost through washing, as well as the deacetylation process, namely the presence of broken and dissolved acetyl groups in NaOH.

FTIR

FTIR spectrum can be used to characterize compounds through the analysis of peaks in the spectrum that correspond to the typical functional groups in chitosan. The characteristic characteristics of chitosan are in the amide group and hydroxyl group (Yadav et al., 2019). FTIR spectrum of chitosan synthesis results were presented in Figure 3, it can be seen that

there is an absorption band in the wave number region of 3295 cm⁻¹ which indicates the existence of overlapping hydroxyl (-OH) stretching and amine (-NH₂) group. Absorption in area of 2922 cm⁻¹ represents the C-H stretching vibration of the methyl (-CH₃) and methylene (-CH₂) groups. The presence of these peaks indicates the structure of hydrocarbons in chitosan molecules. At the absorption area of 1640 cm⁻¹ which indicates the presence of a peak amidal cluster related to the C=O vibration of amide (carbonyl group), which indicates the degree of chitosan deacetylation. The smaller these peaks, the higher of deacetylation degree level, as the deacetylation process removes of acetyl group (-COCH₃) in chitosan, converting chitin to chitosan (Natalia et al., 2021; Oktavia et al., 2024). The wave number region of 1148 cm⁻¹ indicates the presence of a C-O-C group, which is characteristic of glycosidic bonds on the structure of polysaccharides such as chitosan in this uptake is important because it shown the basic structure of the chitosan polymer. FTIR spectrum obtained confirms the presence of amine group that allows chitosan to interact with other hydrogel-forming agents. These groups play an important role in the crosslinking process, where ionic and covalent bonds can be formed with other agents such as aldehydes or polyaspartic acid (Hakim S et al., 2023; Oktavia et al., 2024).



Figure 2. Chitosan from BSF pupa shell

The degree of deacetylation (DD) is a description of the success of the process of deacetylating chitin into chitosan by calculating the acetyl group released from amide. DD chitosan can be calculated based on the FTIR spectrum obtained shown in Figure 3. The DD produced from this study was 83%, the DD produced was greater than the DD produced by Rachmawaty et al. (2023) which is 81.5%, and is smaller than the DD produced by Triunfo et al. (2022) is 90%. The DD produced from this study meets the SNI quality standard No.7949-2013, which is ≥75%. High DD will

result in greater ability of chitosan as a crosslinking agent (Rachmawaty et al., 2023).

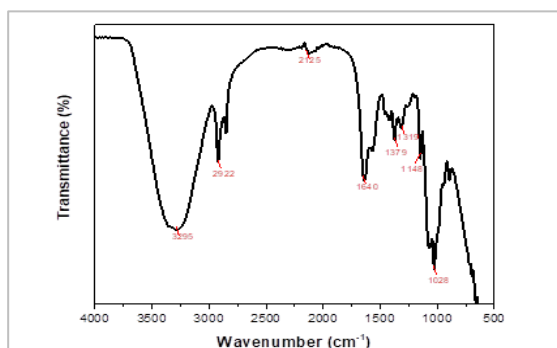


Figure 3. FTIR spectra of chitosan from BSF pupa shell

XRD

X-ray Diffraction (XRD) is used to characteritics the crystal structure and degree of crystallinity of the extracted chitosan. The XRD pattern of chitosan usually shows three main peaks at about $2\theta \approx 10^\circ$, $2\theta \approx 30^\circ$ and $2\theta \approx 40^\circ$, which indicates the presence of a semi-crystalline structure. This degree of crystallinity depends on the conditions of the extraction process, such as temperature and concentration of solution used during in deacetylation process.

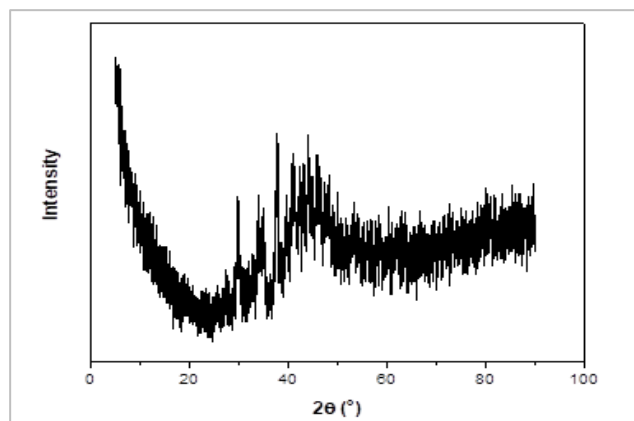


Figure 4. XRD spectra of chitosan from BSF pupa shells

In chitosan extracted from BSF pupa shells, a distinctive diffraction pattern is also expected to appear at about $2\theta \approx 20^\circ$. This suggests that chitosan has semi-crystalline properties, with interactions between molecular chains less regular when compared to fully crystalline materials.

The XRD pattern obtained in figure 4, it also provides information about the success of the deacetylation process, where certain diffraction peaks can indicate the extent to which the chitin structure has been transformed into chitosan. The XRD results in Figure 4 shown that the chitosan from the BSF pupa shell at a peak of major diffraction about $2\theta \approx 10^\circ$, $2\theta \approx 20^\circ$ and $2\theta \approx 30^\circ$, which indicates that this material has a semi-crystalline structure. The level of crystallinity of chitosan is affected by various factors, such as extraction method and conditions used. Lower levels of crystallinity, for example, may increase the solubility of chitosan in water, which makes it more applicable in certain fields, such as sewage treatment applications, agriculture and pharmaceuticals (Azmi et al., 2024; Ihsan & Ratnawulan, 2023). The level of crystallinity of this chitosan also greatly influences the physical and chemical properties of the material, especially in its application as a crosslink agent in hydrogel systems.

TEM

TEM analysis was carried out to study the morphology and particle size distribution of extracted chitosan. TEM provides detailed information regarding the structure of the nanoparticles formed. Based on the results of TEM analysis (Figure 5) using image software, chitosan particles show a porous structure with a relatively uniform particle size, ranging from 25 to 100 nm. This particle size is considered ideal for slow-release fertilizer applications, as it allows for the gradual release of nutrients through an efficient diffusion mechanism. The pore structure observed in TEM also favors better adsorption and nutrient release processes, especially when chitosan is used as a crosslinking agent in hydrogels.

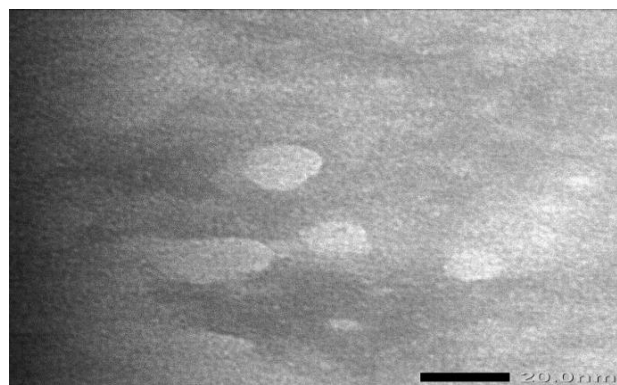
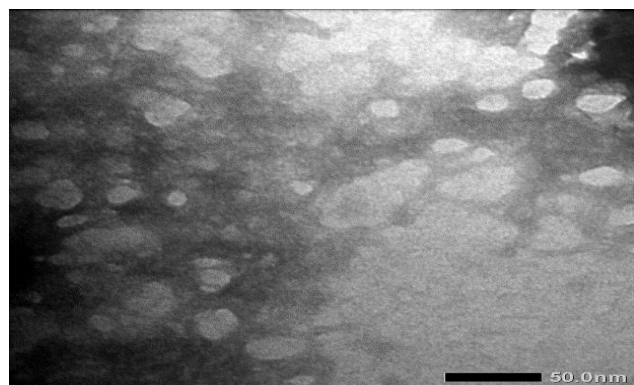


Figure 5. TEM images of chitosan from BSF pupa shells at 20 nm and 50 nm scale

TEM results also shown that the chitosan from the BSF pupa shells had a low level of crystallinity, characterized by a more dispersed electron distribution and a lack of a regular diffraction pattern. This characteristic is important in the application of SRF hydrogels because the amorphous structure of chitosan facilitates the formation of more flexible crosslinking, so the formed hydrogels have a higher swelling capacity, which is directly related to the hydrogel's ability to regulate the release of water and nutrients (Mahendra et al., 2019; Niu et al., 2018).

SEM- EDX

The results of imaging using electron microscopy (SEM) at magnifications of 250 $\times$ and 500 $\times$ in figures 6a and 6b shown that the chitosan obtained from the BSF pupa shell has a porous surface structure. This pore structure is important because it affects chitosan's ability to absorb and release nutrients gradually. The porous surface also shows the potential for chitosan to act as an efficient crosslink agent in hydrogels, as these structures can enhance the physical interaction between chitosan

molecules and hydrogel polymers. At a higher magnification level of 2500 $\times$ in Figure 6c, it can be seen that chitosan has a non-homogeneous surface, which may result from variations in degrees of deacetylation in the material. This also has implications for variations in mechanical properties and chemical interactions in formed hydrogel systems. The EDX analysis in Figure 6d shown in the presence of key elements, such as carbon (C), oxygen (O), and nitrogen (N), which are the basic components of chitosan. Nitrogen comes from the amine group, which is a characterization of chitosan. The existence of these elements confirms that the resulting material is indeed chitosan and not chitin, which only has a smaller amount of nitrogen. In addition, the detection of traces of minerals such as calcium (Ca) and phosphorus (P) derived from pupa remains was absent, which shows that the process of purification of chitosan from BSF pupa shells in the demineralization process successfully removed the mineral components from the pupa shells.

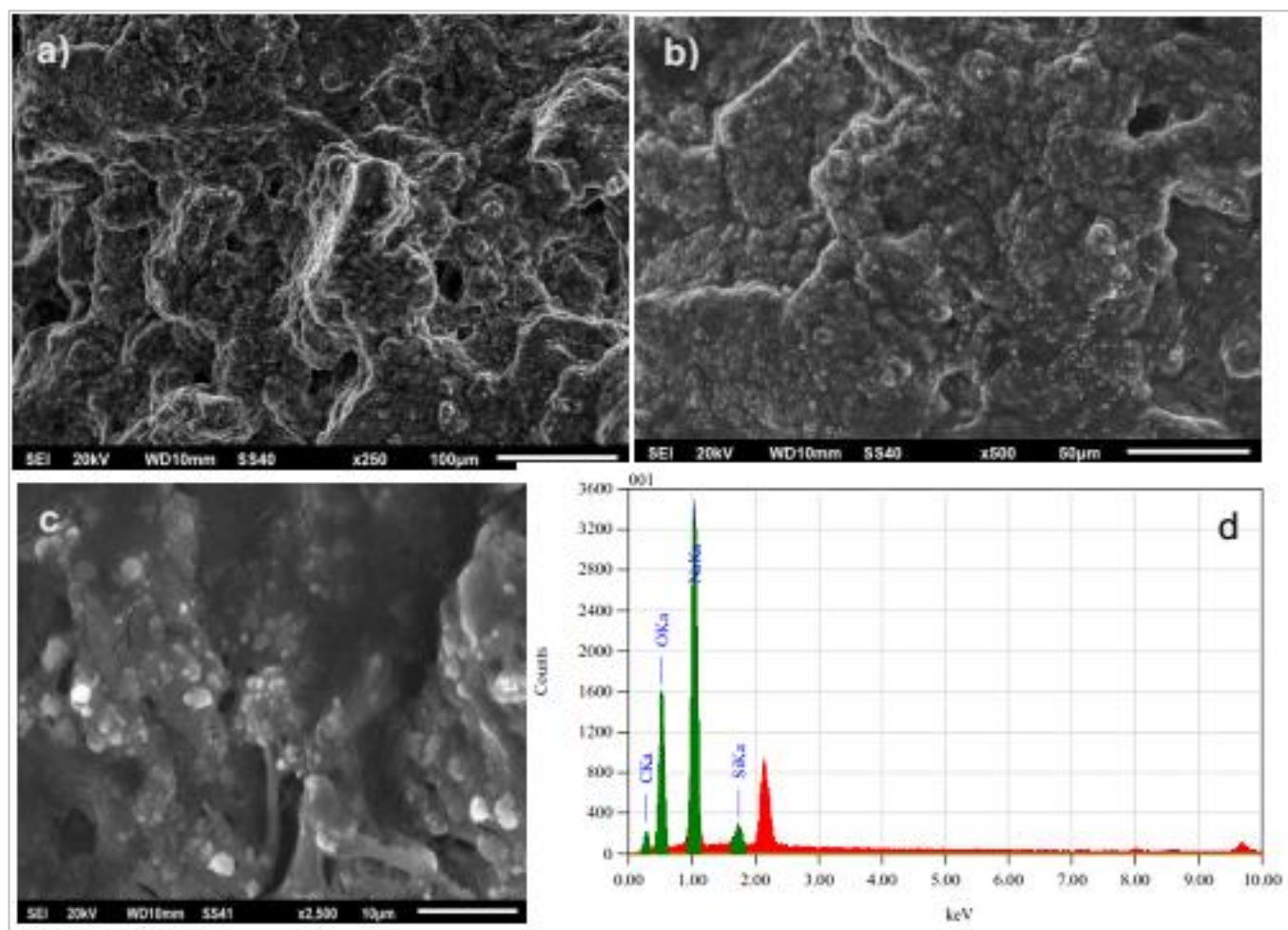


Figure 6. SEM images of chitosan, a) 250 $\times$ magnification, b) 500 $\times$ magnification, c) 2500 $\times$ magnification, d) EDX analysis

TGA-DTA

Thermal analysis through Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA) is a technique that is widely used to evaluate the thermal stability and physicochemical changes of materials in relation to emperature. In this context, the analysis of TGA-DTA chitosan obtained from Black Soldier Fly (BSF) pupa shells has an important role in understanding the thermal characteristics as well as the potential of this material as a hydrogel crosslink agent for slow release fertilizer (SRF) applications.

The TGA results in figure 7 shown that the chitosan from the BSF pupa shells undergoes several stages of thermal degradation. At low temperatures (113°C), there is a mass loss of 7.2% due to the evaporation of water absorbed in the chitosan material. The second stage, which occurs at temperatures between 163-314°C, is the main stage of chitosan degradation associated with the breaking of polymer chains and the decomposition of glucosamine groups, the main component of chitosan. Previous studies have shown that chitosan generally degrades in the temperature range of 250–350 °C, with significant mass loss (Hamsina et al., 2024; Mohan et al., 2020; Subhan et al., 2024).

From the results of the DTA (figure 7), an endothermic peak was obtained at an ambient temperature of 329 °C, which is consistent with the stage of chitosan decomposition. This is in accordance with other references indicating that the active group in chitosan begins to degrade at that ambient temperature (Hahn et al., 2020; Ulfa et al., 2023). This endothermic profile also shows good thermal stability to be applied as an agent crosslink, as chitosan can maintain its structure before reaching higher temperatures.

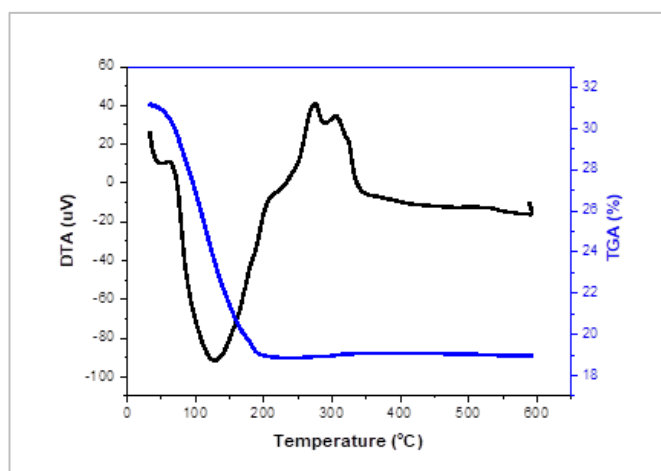


Figure 7. TGA-DTA analysis of chitosan from BSF pupa shells

As a hydrogel crosslinking agent, the thermal stability of chitosan from BSF is important in the formulation of slow release fertilizers. Hydrogels made

from chitosan with stable ability at various temperatures can facilitate the slow and controlled release of nutrients (Ramli, 2019; Siddiqui et al., 2022), thereby prolonging the availability of nutrients for plants. In addition, the gradual thermal degradation of chitosan can support agricultural applications in varied environmental conditions, especially in areas with significant temperature changes.

Thus, the TGA-DTA results that show the characteristics of gradual thermal degradation support the application of chitosan from BSF pupa shells as a crosslinking agent in hydrogels for slow-release fertilizers. This confirms the potential for the use of environmentally friendly natural materials and supports sustainable agricultural practices.

Conclusion

This study successfully characterized chitosan from Black Soldier Fly (BSF) pupa shells and evaluated its potential as a crosslinking agent in hydrogel production for slow-release fertilizer applications. The resulting chitosan exhibited a degree of deacetylation of 83%, a porous morphology with fine pores ideal for water absorption, and good thermal stability, making it an effective crosslinking agent. SEM-EDX analysis demonstrated the successful demineralization process, while TEM and TGA confirmed homogeneous particle distribution and supportive thermal properties. This chitosan enhanced the hydrogel's capacity to absorb and gradually release water and nutrients, making it a promising material for developing of environmentally friendly fertilizers and sustainable agricultural practices.

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Author Contribution

Conceptualization; SWS: designed the research idea and experimental methodology. He also collected and analyzed the data, and interpreted the results; WHI; IOY; contributed jointly to the writing of the manuscript, reviewing, and editing the manuscript for intellectual content. MF; BY; AF; assisted in the overall research process. All authors have read and approved the published version of the manuscript.

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Conflicts of Interest

The author declares that there is no conflict of interest related to the publication of this article.

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