Published by The College of Science & Information Technology (COSIT), TASUED, Vol. 18, No. 2, pp. 17-28.

COSIT, TASUED
Journal of Science and Information Technology (JOSIT)

Isolation, Acetylation and Characterization of Cellulose obtained from BeanPod (*Phaseoulus vulgaris*)

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ABSTRACT

Bio-polymers have gained attention as sustainable substitutes for petroleum-based polymers due to the rising need for environmentally friendly materials. Thus, the overall objective of this research was to prepare cellulose acetate from the cellulose obtained from bean pods (Phaseolus vulgaris Linn.), an agricultural waste. Cellulose was extracted through sequential chemical processes to eliminate hemicellulose, lignin, and other non-cellulosic substances. Acetic anhydride was used to acetylate the cellulose under a range of temperature (30–60 °C), reaction time (20–70 min), pH (9.0–13.0), and concentration (0.112–0.520 M) conditions so as to ascertain the degree of substitution (DS). The acetic anhydride concentration of 0.305 M, reaction duration of 50 min, temperature of 60 °C, and pH of 12.0 were the optimum parameters for acetylation, resulting in a maximum degree of substitution of 0.28. FTIR confirmed successful acetylation with the existence of carbonyl (C=O) bands of absorption at 1732–1742 cm^{-1}. The SEM demonstrated notable morphological changes, with cellulose acetate displaying a smooth surface in contrast to the rough morphology of cellulose isolated from bean pods. XRD analysis confirmed cellulose acetate's amorphous nature and cellulose's crystalline structure. These results highlight bean pods' potential as an inexpensive, sustainable feedstock for the production of cellulose and cellulose acetate, enabling a useful route for the value-adding of agricultural waste and the creation of biodegradable materials for a range of industrial uses.

Keywords: Isolation, Acetylation, Cellulose, Cellulose acetate, Bean pods.

INTRODUCTION

The increasing environmental concerns associated with polymers derived from petroleum have accelerated efforts to find biodegradable and renewable substitutes. Among bio-polymers, cellulose, the most prevalent naturally occurring polymer, has drawn a lot of interest because of its sustainability, biodegradability, and versatility in a range of commercial uses (Chen *et al.*, 2023; Aziz *et al.*, 2022). It is derived primarily from plant biomass, with agricultural residues being an increasingly important source (Chen *et al.*, 2023). However, despite the abundance of cellulose, challenges remain in optimizing its extraction and modification for industrial use (Menon *et al.*, 2023). According to Chakraborty et al. (2023) and Li et al. (2024), cellulose-based materials, like cellulose acetate, are especially promising because of their improved solubility, thermoplasticity, and biodegradability, which

Cite as:

Badejo, O.A., Ogunneye, A.L., Ibikunle, A.A., Amosu, A.O., Braimoh, D.S., Ogunmade, T.O., Babarinde, N.A.A. (2024). Isolation, Acetylation and Characterization of Cellulose obtained from BeanPod (Phaseoulus vulgaris), Journal of Science and Information Technology (JOSIT), Vol. 18, No. 2, pp. 17-28.

make them appropriate for use in textiles, medical products, and packaging.

Despite having high cellulose content, agricultural by-products like bean pods (*Phaseolus vulgaris*) are still mainly underutilized (Guo *et al.,* 2024). There is limited study on the extraction and modification of cellulose from bean pod waste, despite the fact that several studies have examined the possibility of different agricultural wastes as sources of cellulose (Debnath *et al.,* 2021). This gap in knowledge presents a significant opportunity for utilizing such underexplored raw materials in bio-polymer production. Previous studies have demonstrated the feasibility of extracting cellulose from different agricultural residues, but these works often focus on crops like cotton, rice, and wheat, leaving a gap in the literature regarding leguminous plants like bean pods (Ahmad Khorairi *et al.,* 2023; Vaidya *et al.,* 2022).

The acetylation of cellulose is a widely studied process for producing cellulose acetate, which has applications in biodegradable plastics, films, and coatings (Bouftou *et al.,* 2024; Yadav *et al.,* 2021). However, the DS and the characteristics of the cellulose acetate are affected by a number of variables that affect the acetylation process itself, including temperature, pH, reaction time, and concentration. Although there have been reports of cellulose acetate synthesis from a variety of plant residues (Das *et al.,* 2014), there are few research that concentrate on bean pods cellulose and the optimization of acetylation conditions. Moreover, the impact of agricultural residue source on the final material properties has not been adequately addressed. This gap in knowledge hinders the broader application of bean pod-derived cellulose acetate in various industries.

This present work aims to bridge these gaps by investigating the extraction of cellulose from bean pods waste (*Phaseolus vulgaris*), followed by its acetylation to produce cellulose acetate. The present research work focuses on optimizing the acetylation conditions, including temperature, pH, reaction time, and concentration of acetylating agent (acetic anhydride), to achieve an optimum DS. Additionally, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) are used to extensively study the structural and morphological characteristics of cellulose and cellulose acetate. By addressing the gaps in both cellulose extraction and acetylation from agricultural waste, this work provides valuable insights into the sustainable production of bio-polymers, contributing to the broader field of material science and waste valorization.

MATERIALS AND METHODS

Materials

A local farm in Ijebu-Ode provided the bean pods (*Phaseolus vulgaris*), which were then extensively cleaned to get rid of any surface contaminants. The analytical quality reagents utilized in this investigation, including sodium hydroxide (NaOH), nitric acid (HNO_3), sodium hypochlorite (NaOCl), acetic anhydride ($C_4H_6O_3$), and others, were acquired from Sigma-Aldrich (USA). All chemicals and solvents were used without any additional purification.

Cellulose Isolation

A multi-step chemical treatment was used to extract cellulose from bean pods, outlined by Ogunneye et al. (2020). The dried bean pods were ground into a fine powder. To remove lignin, 5 g of the powder was treated with 100 mL of 10% (w/v) NaOH solution at 80 - 100 °C for 2 hours with constant stirring. The mixture was filtered, and the residue was washed with distilled water to neutral pH. The residue was then bleached with 100 mL of 2.5% (v/v) NaOCl at 70 °C for 1 hour, following the methodology of Gupta et al. (2023). The purified cellulose was dried at 60 °C until a constant weight was achieved.

Percentage yield

Equation (1) was used to calculate the cellulose percentage yield (%Y) value:

$$\%\ Y = \frac{A}{B} \times 100 \quad (1)$$

where:

A = mass of cellulose (g);

B = mass of bean pods (g).

Acetylation of Cellulose isolated from bean pods

The acetylation of cellulose was carried out via esterification using acetic anhydride, based on the protocol by Chakraborty et al. (2023). Two grams of isolated cellulose were dispersed in a solution containing acetic acid (10 mL) and acetic anhydride (5 mL). Reaction parameters such as temperature (30–100 °C), concentration (0.112 - 0.518 M), pH (9.0–13.0), and reaction time (20–70 min) were optimized to determine the conditions for the highest DS. When the reaction is complete, the cellulose acetate was precipitated in cold water, filtered, washed with distilled water, and dried at 60 °C for 24 hours.

Characterizations

FTIR analysis: An FTIR spectrometer (Nexus 470) was used to examine the functional groups of the isolated cellulose and cellulose acetate. For examination, the samples were compressed into pellets after being crushed with KBr. The spectra were captured at a resolution of 4 cm^{-1} and ranged from 4000 to 400 cm^{-1}.

XRD analysis: A Bruker D8 Advance (Germany) diffractometer fitted with a Cu-Kα radiation source (λ = 1.5406 Å) running at 40 kV and 30 mA was used to measure the crystallinity of the cellulose and cellulose acetate. Data were collected in continuous scan mode with a step size of 0.02° and a scan speed of 1°/min throughout a 2θ range of 10°–80°. A receiving slit of 0.3 mm was maintained, and a divergence slit of 1° was fixed. After being ground into a fine powder, the sample was evenly packed onto a sample holder and examined in an ambient setting.

SEM analysis: A scanning electron microscope (JEOL JSM-6390LV, Japan) was used to examine the surface morphology of the cellulose and cellulose acetate samples. Gold sputter coating was applied to the samples to improve conductivity. Various magnifications of the images were taken in order to observe the morphology and surface structure.

Degree of Substitution (DS): The technique outlined by Li et al. (2023) was used to examine the DS of the acetylated cellulose from bean pod cellulose. A 250 mL flask containing a 5.0 g sample of acetylated cellulose was filled with 50 mL of distilled water. After stirring the liquid, two drops of phenolphthalein indicator were added. A 0.1 M sodium hydroxide solution was then used to titrate the suspension until a persistent pink endpoint was seen. 25 mL of a 0.45 M sodium hydroxide solution was then added, and a rubber stopper was used to firmly seal the flask. The mixture was shaken vigorously for half an hour. The stopper was then carefully taken out and given a good rinse with distilled water. The residual alkali in the saponified mixture was titrated against a 0.2 M HCl solution until the phenolphthalein color disappeared. The unmodified (isolated) cellulose was analysed under similar conditions of acetylated cellulose to get the blank value. Equations 2 and 3 were used to calculate the DS.

$$Percentage\ acetyl\ (dry\ basis) = \frac{(blank\ titre - sample\ titre) \times acid\ molarity \times 0.043}{sample\ weight\ (g)} \times \frac{100}{1} \quad (2)$$

$$Degree\ of\ substitution\ (DS) = \frac{162 \times A}{4300 - 42 \times A} \qquad (3)$$

where, 162: The molecular weight of an anhydroglucose unit (AGU) in cellulose ($C_6H_{10}O_5$), 42: The molecular weight of an acetyl ($-COCH_3$) group, 4300: A constant derived from the molecular weight balance in the equation, while A is the percentage of acetyl on the dry basis

Mechanism of Cellulose Acetylation (Cell-OH): Acetylation of cellulose derived from bean pod agricultural waste enhances its functionality for industrial applications. The acetylation mechanism for cellulose acetate synthesis (Figure 1) involves two pathways: A (intermediate) and B (final). Acetic anhydride reacts with hydroxyl groups via a nucleophilic attack on the acyl carbon of the anhydride (A), followed by the loss of acetic acid to form the ester (B).

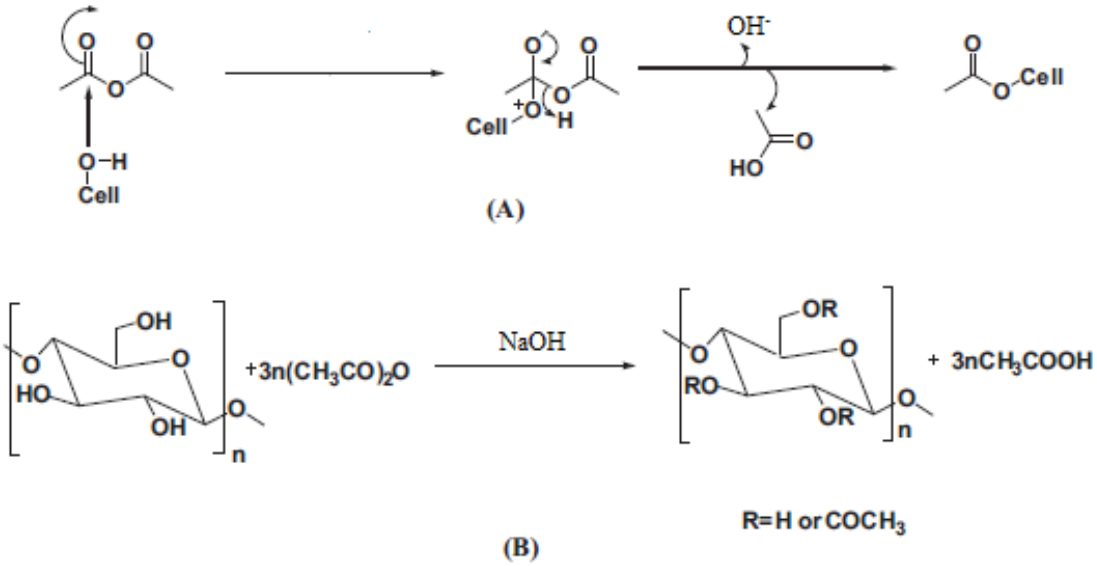

Figure 1. Mechanism of the reaction

RESULTS AND DISCUSSION

Isolation of Cellulose from Bean Pods

This successful cellulose extraction from bean pods shows that agricultural waste can be used as a renewable source of cellulose. The removal of lignin and hemicellulose, which is essential for improving the purity and functioning of cellulose, was accomplished successfully by the chemical treatments (bleach and alkali). The yield of cellulose (45%) is comparable to those found in other studies utilizing agricultural residues for cellulose extraction. For example, Gheribi et al. (2022) reported similar yields when extracting cellulose from cotton stalks.

The isolation process used in this study aligns with recent advancements in cellulose extraction from agricultural wastes, where the goal is to reduce environmental impact while providing a sustainable alternative for industrial applications (El-Sayed *et al.*, 2021; Wang *et al.*, 2023). This approach offers a promising method

for utilizing agricultural waste, such as bean pods, to produce high-quality cellulose for diverse applications in the green chemistry and materials industries.

Acetylation of Cellulose isolated from bean pods

The synthesis of cellulose acetate under different optimization condition from bean pod-derived cellulose was successful, with the process of acetylation effectively modifying the chemical structure of cellulose. The DS was controlled by adjusting the reaction parameters (temperature, acetic anhydride concentration, time, pH and reaction time).

Effect of Temperature on DS

The influence of temperature on the DS of cellulose isolated from bean pods was investigated at a range temperature of 30 to 100°C. The results indicate that DS values increased progressively as the reaction temperature rose from 30 to 60°C, reaching an optimal substitution level at 60°C. However, beyond this temperature, a decline in DS was observed, perhaps as a result of the cellulose backbone's beginning to degrade thermally (Figure. 2).

At the optimum temperature of 60°C, the DS values were recorded as 0.27. This trend suggests that temperature plays a crucial role in facilitating the acetylation process by enhancing molecular interactions and the accessibility of hydroxyl groups for substitution (de Freitas *et al.*, 2017). However, excessive heating leads to structural alterations, which negatively impact the functional integrity of the cellulose acetate (Das *et al.*, 2014). The observed decrease in DS at elevated temperatures indicates possible degradation of the polymeric structure, leading to reduced acetylation efficiency and changes in the physicochemical properties of the modified cellulose. The observed temperature effect on the DS in the acetylation of cellulose from bean pods aligns with findings reported by Maryana et al. (2022) in the acetylation of rice husk cellulose investigated under varying temperatures.

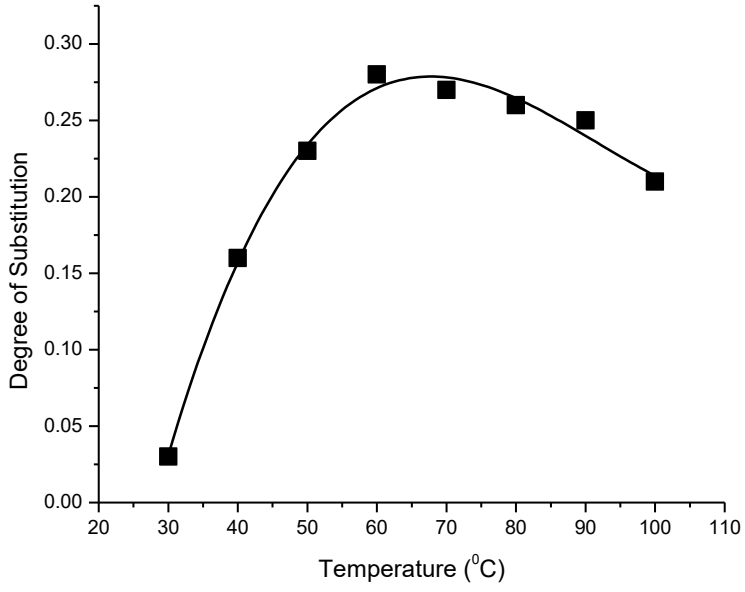

Figure 2. Effect of temperature on DS of Cellulose from bean pod.

Effect of Concentration on DS

The influence of acetic anhydride concentration (ranging from 0.112 to 0.518 M**)** on the DS of cellulose isolated from bean pods was investigated. The results indicate that DS increased with increasing acetic anhydride concentration, reaching an optimum value at 0.305 M (Figure 3). Beyond this concentration, a decline in DS was observed, likely due to excessive acidity in the reaction medium, which inhibits the acetylation process and limits the formation of cellulose acetate.

This trend is consistent with previous studies that reported similar behavior, where an optimal acetic anhydride concentration was necessary to achieve maximum substitution before unfavorable reaction conditions led to reduced efficiency (Biswas *et al.*, 2007). The decline in DS at higher concentrations may be attributed to side reactions, degradation of the cellulose backbone, or reduced availability of reactive hydroxyl groups due to excessive acylation (Candido & Gonçalves, 2016). Thus, optimizing the concentration of acetic anhydride is crucial for achieving high DS while maintaining the structural integrity of cellulose acetate.

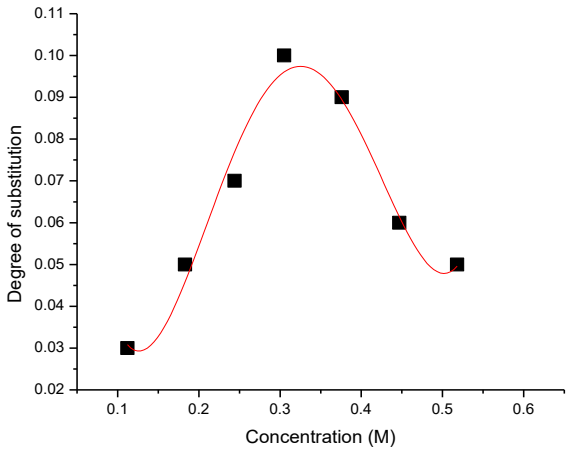

Figure 3. Effect of concentration on DS of bean pods cellulose.

Effect of Time on DS

The influence of reaction time on the DS of cellulose was investigated at 20, 30, 40, 50, 60, and 70 minutes respectively. The findings revealed that DS increased with longer reaction times, reaching an optimum at 50 minutes. Beyond this point, a decline in DS was observed, likely due to degradation effects (Figure 4). The prolonged reaction time resulted in excessive swelling and gel formation rather than maintaining a slurry-like consistency, making filtration challenging due to its rubbery texture.

At the optimal reaction time of 50 minutes, the DS of acetylated cellulose derived from bean pods was 0.06**.** This observation aligns with previous studies, which also reported that prolonged acetylation times could lead to undesirable structural changes, increased viscosity, and difficulty in processing (Li *et al.*, 2014). These findings emphasize the importance of optimizing reaction time to achieve maximum acetylation efficiency while minimizing degradation and processing challenges.

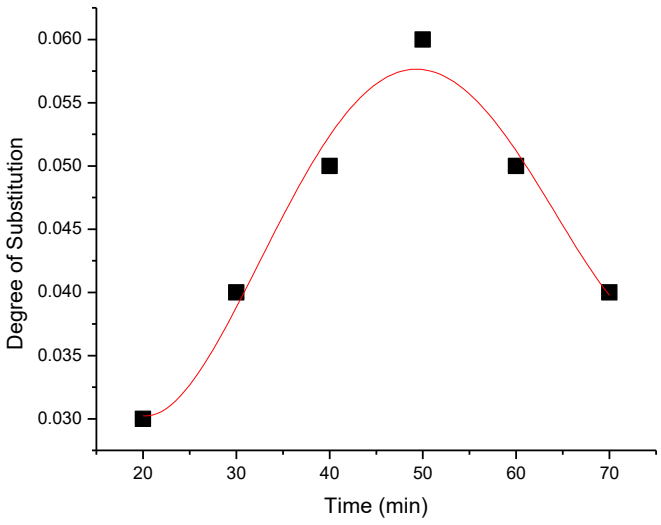

Figure 4. Effect of Time on DS of cellulose isolated from bean pod.

Effect of pH on DS

The influence of pH on the DS of cellulose was examined over a pH range of 9.0 to 13.0. The findings showed that DS rose with rising pH, reaching an optimum at pH 12.0 (Figure 5). Beyond this point, a decline in DS was observed, likely due to degradation effects caused by the highly alkaline reaction medium, which adversely affected the acetylation process. At the optimal pH 12.0, the DS of acetylated cellulose derived from bean pods was 0.25**.** However, at pH values exceeding this threshold, the excessive alkalinity led to cellulose degradation, thereby reducing the efficiency of acetylation. These findings are consistent with previous studies, which reported that while alkaline conditions facilitate acetylation by increasing cellulose reactivity, excessively high pH levels can lead to polymer degradation and reduced substitution efficiency (Ogunneye *et al.,* 2020). Therefore, maintaining an optimal pH balance is crucial to achieving maximum acetylation while preserving the integrity of the cellulose structure.

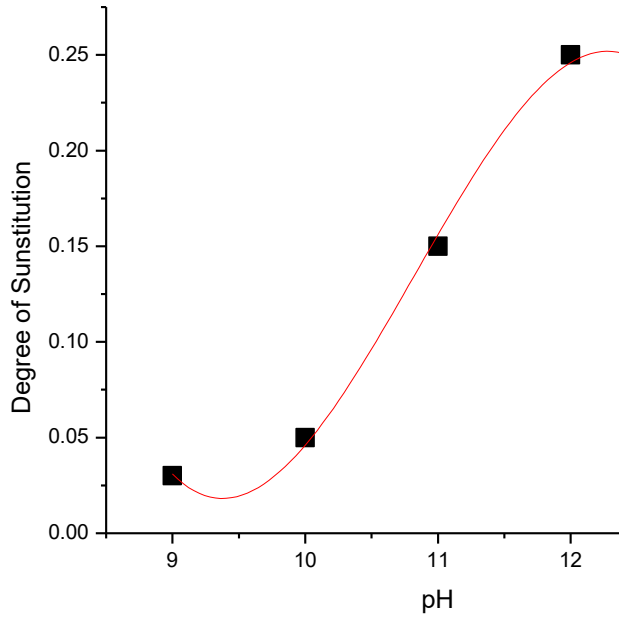

Figure 5. Effect of pH on DS of cellulose from bean pods.

Characterizations

FTIR: The FTIR spectrum of cellulose isolated from bean pods (Figure 6a) displayed characteristic peaks indicative of cellulose and plant biomass, confirming the successful extraction. A broad absorption band at 3336.35 cm^{-1} corresponds to the O-H stretching vibration of -OH groups in cellulose, a feature typical of cellulose and indicative of the availability of hydroxyl groups for further modification, such as acetylation (Tessema *et al.,* 2023). A peak at 2902.28 cm^{-1} is attributed to the C-H stretching vibrations of the cellulose backbone, signaling the presence of methyl and methylene groups (ogunneye *et al.,* 2020). The peak at 1626.42 cm^{-1} is as a results of C-O stretching vibrations in cellulose, further confirming that cellulose is the primary component of the isolated material (Khil *et al.,* 2019). Notably, the absence of peaks corresponding to lignin (around 1600 cm^{-1}) and hemicellulose (around 1740 cm^{-1}) suggests the efficient elimination of non-cellulosic components during the extraction stage (Wang et al., 2023). However, the FTIR spectrum of cellulose acetate (Figure 6b) exhibited distinct changes compared to the bean pods cellulose spectrum, confirming successful acetylation. The most prominent feature is the appearance of a strong band at 1747.20 cm^{-1}, corresponding to the carbonyl stretching vibration (C=O) of the ester group introduced during acetylation, confirming the successful esterification of cellulose (Fe *et al.,* 2017). The O-H band at 3444.50 cm^{-1}, present in the cellulose spectrum, is significantly diminished and broader in the spectrum of cellulose acetate, showing the loss of hydroxyl groups during acetylation (Das *et al.,* 2014). Additional peaks at 1420.51 cm^{-1} and 1374.63 cm^{-1}, corresponding to the C-O stretching and C-H bending vibrations of the ester groups, further

validate the formation of cellulose acetate (Tulos *et al.*, 2019).

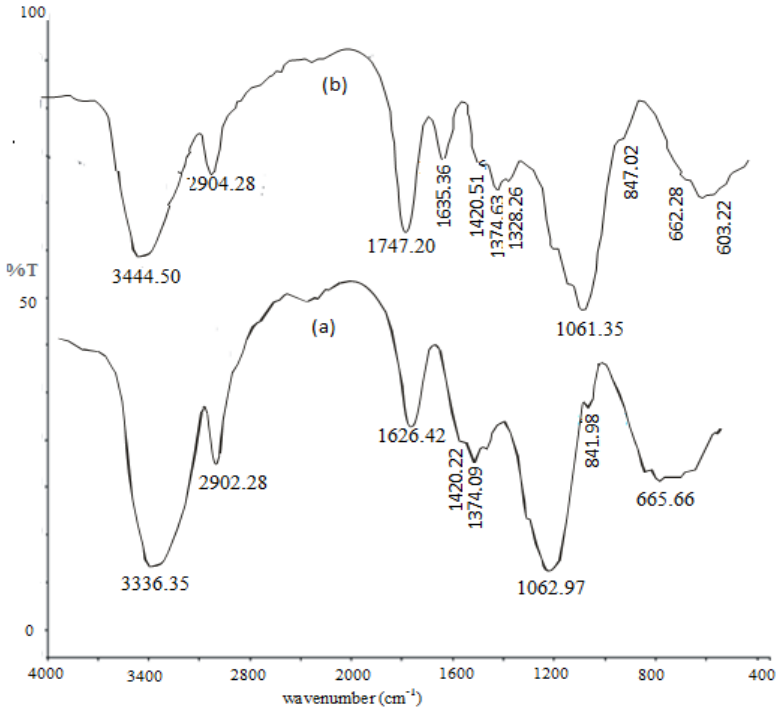

Figure 6. The FTIR spectra of (a) bean pods cellulose and (b) cellulose acetate.

XRD: The XRD patterns of cellulose isolated from bean pods and cellulose acetate prepared from bean pods cellulose are depicted in Figure 7. The X-ray diffractograms of the isolated cellulose showed diffraction peaks at 2θ=17°, 16.1° 22.2° and 34.8° equivalent to the diffraction planes of 101, 101, 002, and 040 respectively, this is a characteristic of cellulose crystal I (Ogunneye *et al.*, 2020). The observation in the present work is similar to previous work by Matsumura (2000). Thus, a shoulder peaks of 16.1°and 34.8°is an indication of° lignin and hemicellulose removal from the samples (Fan *et al.*, 2013). The weak diffraction peaks around 10.6° in the diffraction pattern of cellulose acetate is due to the crystalline nature of cellulose acetate (Das *et al.*, 2014). The new diffraction peak around 2 θ = 19.7°, is due to the amorphous region of the cellulose chains (Battisti *et al.*, 2019; Hu et al., 2011).

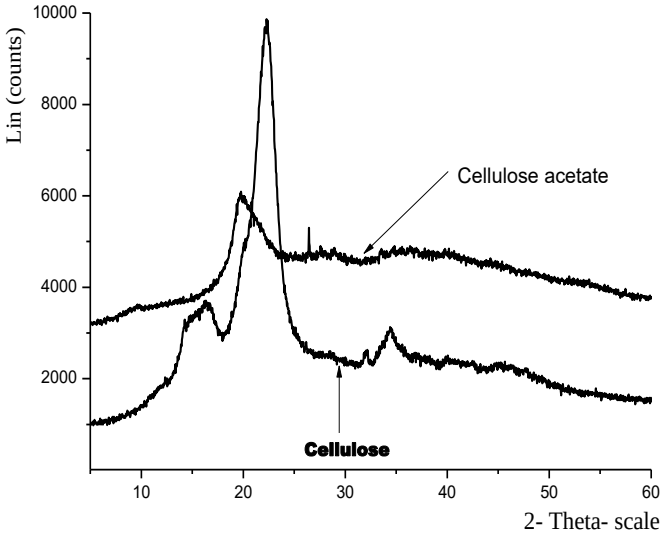

Figure 7. XRD pattern of cellulose acetate and the cellulose isolated from bean pods.

SEM: As revealed in figure 8. The SEM micrograph of cellulose isolated from bean pods exhibited a fibrous and porous structure, which is characteristic of cellulose derived from plant

biomass. The fibers appeared as elongated, fluffy, and curly interconnected bundles with rough surfaces, and the observed porosity is indicative of the elimination of lignin, hemicellulose, and waxes during the extraction process. However, The SEM image of cellulose acetate derived from the isolated cellulose revealed significant morphological changes compared to the bean pods cellulose. The fibrous and porous structure was replaced with **a** smoother and more compact surface morphology, which is typical of acetylated cellulose materials. This observation is due to the loss of the porous network attributed to the disruption of the hydrogen bonding in the cellulose structure and the replacement of hydroxyl groups with acetyl groups during acetylation (Goswami & Das, 2019).

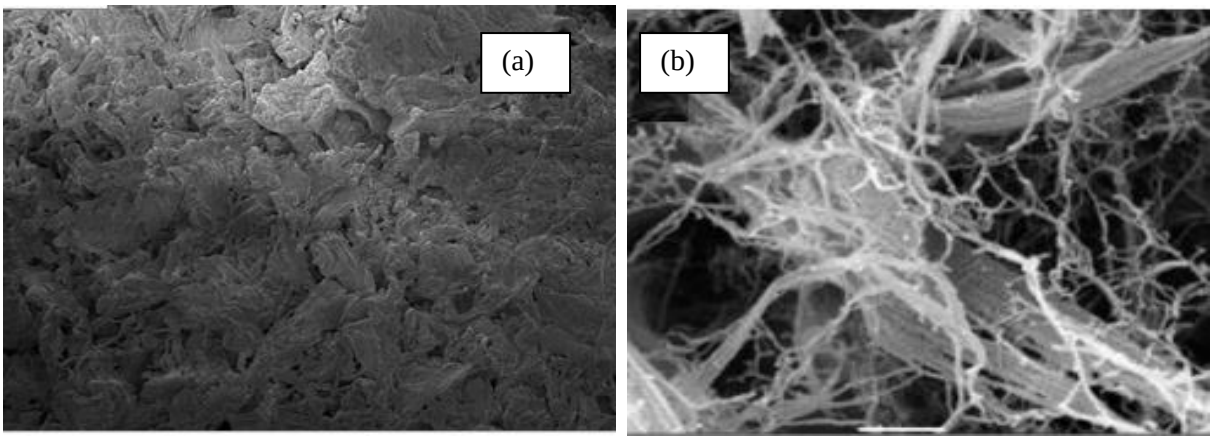

Figure 8. SEM image of (a) bean pods cellulose and (b) cellulose acetate prepared from bean pods cellulose.

CONCLUSION

This study successfully isolated cellulose from bean pods and acetylated it to produce cellulose acetate. FTIR analysis confirmed acetylation through the appearance of the ester carbonyl stretch and the replacement of hydroxyl groups in cellulose by acetate groups ($–COCH_3$), leading to a reduction in hydrogen bonding. XRD patterns showed a decrease in crystallinity, while SEM images revealed changes in surface morphology. The DS was optimized at 60°C**,** 0.305 M acetic anhydride**,** 50 minutes reaction time, and pH 12.0, with higher conditions leading to degradation. These findings highlight the potential of bean pod-derived cellulose for producing cellulose acetate, offering promising applications in biodegradable films and packaging. Further research should focus on optimizing properties and assessing environmental impacts.

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