Trop J Nat Prod Res, December 2024; 8(12): 9637 - 9642

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Comparison of Extraction Methods and Characterization of Cellulose from Sugarcane Bagasse (*Saccharum officinarum* L.) for Bioplastic Food Packaging

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ARTICLE INFO

Article history:
Received 07 November 2024
Revised 11 November 2024
Accepted 24 November 2024
Published online 01 January 2025

ABSTRACT

Bagasse (*Saccharum officinarum L.*) is a waste generated from the extraction process of sugarcane in the sugar processing industry that is not optimally utilized. However, the high cellulose content of bagasse can be applied in various. This research aims to identify the properties of sugarcane bagasse-derived cellulose. Delignification and bleaching are chemical processes used to perform extraction. Physical characterization parameters and standards used to view bagasse cellulose include cellulose, lignin, hemicellulose, color, and Fourier Transform-Infrared (FTIR) spectrum. Cellulose extraction was carried out using a hotplate stirrer and microwave. The results indicated the amounts of cellulose, hemicellulose, and lignin in sugarcane bagasse cellulose using the microwave method were 85.95%, 3.64%, and 0.26%, respectively, while the amounts using the hotplate stirrer method were 83.25%, 5.01%, and 0.26%. FTIR of sugarcane bagasse cellulose of hotplate stirrer method showed absorption bands at 3335.58 cm^{-1} for O-H, 2893.99 cm^{-1} for C-H, 1022.54 cm^{-1} for C-O, and microwave method showed bands at 3326.43 cm^{-1} for O-H, 2896.87 cm^{-1} for C-H, 1024.49 $cm^{-1}$for C-O. a typical peak of cellulose. The results showed that cellulose isolated from bagasse powder is similar to the standard cellulose. The yields of bagasse cellulose from the hotplate stirrer and microwave methods were 20.07% and 19.60% white powder, respectively. The findings of this study indicate that cellulose from sugarcane bagasse could be an excellent material for producing bioplastics, hence the need for its development as a bioplastics raw material.

Keywords: Bagasse, Cellulose, Characterization, Delignification

Introduction

Indonesia is an archipelago with more than 17,000 islands across the equator[1]. Indonesia's biodiversity is also vibrant, with more than 3,000 endemic species of flora and fauna that only exist in Indonesia[2]. Therefore, it is necessary to produce more valuable materials from domestic natural resources, such as wood, pet food, paper, fibre, clothing, cosmetics, and pharmaceutical products. Sugarcane (*Saccharum officinarum* L.) is one of Indonesia's most widely cultivated plant commodities[3]. Sugarcane (*Saccharum officinarum L.*) is the main ingredient in the sugar industry and contains carbohydrate sources.

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Citation: Rusdin A, Ahmad A, Karim A, Kasim S, Wahid AW, Tahir D, Rohaeti E, Taba P, Awaluddin, Maswati Baharuddin B, Jarre S, Sulastri, Karim H. Comparison of Extraction Methods and Characterization of Cellulose from Sugarcane Bagasse (*Saccharum officinarum* L.) for Bioplastic Food Packaging. Trop J Nat Prod Res. 2024; 8(12): 9637 – 9642 https://doi.org/10.26538/tjnpr/v8i12.42

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

However, by-products of sugar production can produce bagasse[4,5]. Although bagasse is still utilized as a biomass fuel for boilers, the byproduct of burning it is useless. It is typically discarded into rivers, which has a negative effect on the environment, human health, arable land, and water supplies, among other things[6]. Bagasse contains cellulose (37.2%), hemicellulose (22.1%), lignin (26.5%), and ash (8.2%)[7].

Therefore, the use of bagasse waste may be maximized by researching the synthesis of cellulose from bagasse. One of the essential basic elements for the production of biomaterials like bioplastics cosmetics[8], food[9], and other materials in the medical fields[10] is cellulose $(C_6H_{10}O_5)n$ due to its biocompatibility, biodegradability, and lack of toxicity[8]. Biosynthesis of cellulose occurs not just from plants but also from microbes, including bacteria, fungi, and algae[9,10]. Furthermore, cellulose is a polysaccharide comprising glucose unit polymers joined by β-1,4-glycosidic linkages to create long, straight, crystalline, but insoluble chains[11]. Cellulose can be extracted using alkaline solvents[12], enzyme hydrolysis[13], and acid hydrolysis[14]. Enzymatic hydrolysis for cellulose production is costly and requires a long time of about 72 hours[15]. Hydrolysis using concentrated acid is also toxic, producing by-products during cellulose extraction in acetic acid and furfural[16]. Several studies have reported the extraction of cellulose using alkaline solvents. These studies aim to determine the optimal time and concentration of the solvents to convert bagasse into cellulose through the delignification process. The results showed that the optimal yield

was obtained when 2% sodium hydroxide (NaOH) was used at 121°C for 60 minutes[17]. This study compares the hotplate stirrer and microwave methods for cellulose isolation procedures employing alkaline liquids. Cellulose extraction using a microwave involves alkaline extraction with the help of microwaves, while cellulose extraction using a hotplate stirrer uses a direct heating process[18]. Cellulose extraction using a microwave is an alternative method in accelerating chemical reactions employing direct contact between objects subjected to microwave heating and electromagnetic fields, resulting in faster heating[18]. This research aimed to determine the characteristics of cellulose from bagasse (*Saccharum officinarum L.*) by microwave and hotplate stirrer methods. This research aims to provide innovations for the development of cellulose from bagasse, which can be used as a substrate in the manufacture of environmentally friendly and biodegradable bioplastics, reducing the use of synthetic plastics.

Materials and Methods

Materials

Sugarcane bagasse was collected in August 2024 from Makassar, Indonesia, at geographical coordinates 5°8'9" S dan 119°28'58" E. Other materials include glassware (Pyrex®), pH meter (Thermo Fisher Scientific®), FTIR spectrophotometer (Shimadzu FTIR 8400S®), and analytical balance (Ohaus Pioneer PA214®), acid detergent fibre (ADF) (Merck®), acid sulfur (H_2SO_4) (Merck®), alcohol (Merck®), Neutral detergent solution (NDS) (Merck®), aquadest (Onelab®), cellulose (Merck®), H_2O_2 (Merck®), and NaOH (Merck®).

Sample Preparation

The sugarcane bagasse sample utilized to isolate glucose came from Makassar City's Tamalanrea District, Indonesia. The bagasse obtained was washed with water until clean and then cut into small pieces and dried under the sun. The dried samples were then pulverized into powder using a grinding machine at the Hasanuddin University Feed Chemistry Laboratory, and the bagasse powder was sieved using a 100 mesh sieve.

Isolation of cellulose

Bagasse powder (50 g) was added to 800 mL of 10% w/v NaOH solution until the bagasse powder was completely submerged. Then, the solution was heated on a hotplate for 2 hours at 150°C with a stirring speed of 200 rpm and filtered using a filter cloth. The resulting pulp was washed using distilled water until the pH was neutral. Furthermore, the pulp was dissolved in a 15% H_2O_2 solution until completely submerged. After that, the solution was boiled on a hotplate for 120 minutes at 60°C and filtered using a filter. The residue was cleaned and filtered using distilled water. The filtered residue was dried for 24 hours in an oven set at 50°C[19,20]. The same procedure was repeated using a microwave.

Physical characterisation

Basic characterization tests were done to ensure the success of bagasse synthesis. The characterization includes chemical testing, such as lignin, cellulose, and hemicellulose contents determination, as well as functional groups with FTIR (Shimadzu FTIR 8400S®) and physical tests (organoleptic analysis), which includes product form and color. The comparability of characterization results between bagasse-derived cellulose and synthetic cellulose (Merck®) demonstrates the synthesis results[21].

The Van Soest method was used to analyze the amount of lignin, cellulose, hemicellulose, acid detergent Fiber (ADF), and Neutral detergent solution (NDF) in bagasse powder and bagasse cellulose[22].

Determination of Acid Detergent Fiber (ADF) Content

Acid detergent fibre (ADF) solution (40 mL) was added to a test tube containing 0.3 g of bagasse powder (a) and sealed. After that, the mixture was refluxed for an hour in boiling water. It was then filtered with a vacuum pump using a sintered glass funnel of known weight (b). The residue was then cleaned with 50 mL of alcohol (Merck®) and 100 mL of hot water until the foam vanished. The residue was dried in an oven set at 100 °C for eight hours. After cooling for about half an hour in a desiccator, the weight was taken using a weighing balance (c)[23].

The ADF levels were determined from the following equation:

$$ADF\ Content = \frac{c-b}{a}\ x\ 100\% \quad (1)$$

where:

a = weight of a sample
b = weight of empty sintered glass
c = weight of sintered glass + filter residue after baking

Determination of Neutral Detergent Fiber (NDF) Content

A total of 0.2 g of bagasse powder (a) was placed into a test tube, followed by the addition of 25 mL of neutral detergent solution (NDS) (Merck®). The test tube was sealed, and the mixture was refluxed in boiling water for 1 hour. The resulting mixture was filtered through a pre-weighed sintered glass funnel (b) using a vacuum pump. The residue was washed thoroughly with approximately 100 mL of boiling water and 50 mL of alcohol (Merck®) until no foam remained. Subsequently, the residue was dried in an oven at 100 °C for 8 hours, cooled in a desiccator for about 30 minutes, and weighed (c)[23].

The NDF levels were determined from the following equation:

$$NDF\ Content = \frac{c-b}{a}\ x\ 100\% \quad (2)$$

where:

a = weight of a sample
b = weight of empty sintered glass
c = weight of sintered glass + filter residue after baking

Cellulose and Lignin Content Determination

After setting the ADF-containing sintered glass on a Petri dish, 20 mL of 72% H_2SO_4 (Merck®) was added and was rinsed sufficiently with hot water and filtered using a vacuum pump. It was then dried for eight hours at 100˚C in an oven. After cooling in a desiccator, it was weighed (d). After two hours of heating to 500˚C in a furnace, the residue was allowed to cool before being placed in a desiccator for 30 minutes and weighed (e)[23].

The lignin, cellulose, and hemicellulose levels were determined from the following equation:

$$\text{Lignin Content} = \frac{d-e}{a}\ x\ 100\% \quad (3)$$

where:

a = weight of a sample
d = weight of sintered glass + lignin + insoluble ash
e = weight of sintered glass + insoluble ash after the kiln

% Cellulose = % ADF- % Insoluble ash - lignin
% Hemicelluloses = % NDF- % ADF

Fourier-Transform Infrared Spectrometric analysis

The functional groups of sugarcane bagasse celluloses obtained using the hotplate stirrer, microwave methods, and standard cellulose were determined using FTIR spectrometry. In addition, FTIR spectrometry was also used to determine the changes in functional groups that occur during the process of converting waste into cellulose, and the spectra of standard cellulose, hotplate stirrer cellulose, microwave cellulose, and sugarcane bagasse (*Saccharum officinarum L.*) were compared[24].

Data Analysis

Data from the untreated sugarcane bagasse powder cellulose, microwave, and hotplate stirrer extracted cellulose were compared and analyzed statistically with an independent sample test using IBM SPSS ver. 27. A 95% confidence level was considered significant. Analysis of variance (ANOVA) followed by posthoc Tukey's test ($p < 0.05$) was conducted to determine the significant difference between the various methods of cellulose[25].

Results and Discussion

Isolation involves employing certain solvents or techniques to separate particular components from their natural sources[22]. Cellulose is known to interact well with water but is insoluble. Cellulose is a polysaccharide

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

found naturally in various sources: plant cell walls, woody materials, cotton fibers, marine plants, seed hair, and tree bark[26,27]. Sugarcane bagasse is an underutilized waste that contains high amounts of cellulose[14,28,29]. This study isolated cellulose from bagasse powder using 10% NaOH and 15% H_2O. NaOH solution is used for the delignification stage. The crystalline and amorphous forms of lignin components can be broken by this solution, which stops the lignin from splitting. Because the short-chain lignin chains are broken, the residual lignin compounds are removed simultaneously using bleaching, which may alter the color. Following this breakdown, lignin readily dissolves in water or an alkali during washing[21,30]. Calculating cellulose content and amount is crucial since it is needed to ascertain the amount of cellulose obtained during synthesis. The Van Soest method was used to calculate the cellulose content. The yield is determined from the dry weight of cellulose to the amount of bagasse powder used. The total yield obtained in this study was 20.07% and 19.60%. Based on the cellulose content and yield obtained, sugar bagasse has the potential to be further developed as a major source of cellulose, which can be used as raw material for making bioplastics. The yield amount is quite valuable from an economic perspective. This is preliminary research in developing cellulose from pulp powder so that further development in the synthesis methodology for yield optimization can be explored.

Furthermore, the cellulose, hemicellulose, and lignin levels from bagasse cellulose in the hotplate stirrer method were 83.25%, 5.01%, and 0.26%, respectively. Meanwhile, the cellulose, hemicellulose, and lignin contents of sugarcane bagasse cellulose are 85.95%, 3.64%, and 0.26%, respectively (Table 1). The cellulose content of bagasse produced from both methods was higher than that obtained in bagasse powder at 34.10%, possibly because bagasse powder is untreated. Bagasse powder has also not undergone a lignin and hemicellulose removal process. The cellulose content of the bagasse produced in this study is higher than the previous report[26], which shows a cellulose content of bagasse of 70%. Different methods, sampling locations, and solvent concentrations may influence these results.

Table 1: Physical characterization of the cellulose from Sugarcane bagasse and sugarcane bagasse cellulose.

Sample	Composition (%)		
	Cellulose	Hemicellulose	Lignin
Sugarcane bagasse	34.10±0.005[a]	21.27±0.00[a]	7.41±0.005[a]
Cellulose sugarcane bagasse microwave	85.95±0.005[b]	3.64±0.005[b]	0.10±0.005[b]
Cellulose sugarcane bagasse hotplate stirrer	83.25±0.005[c]	5.01±0.005[c]	0.26±0.005[c]

Averages followed by letters are statistically different by Tukey's test. ($p < 0.05$).

Based on the ANOVA statistical test, the significance level was $p=0.000 < 0.05$. This indicates a significant difference in cellulose, lignin, and hemicellulose content in sugarcane bagasse without treatment using the cellulose sugarcane bagasse microwave method and the cellulose sugarcane bagasse hotplate stirrer method. Because the statistical analysis test results showed significant differences, further analysis was done using the post-hoc test. The study of cellulose content with a post-hoc test revealed that significant differences were found between the quantitative data of sugarcane bagasse without treatment with cellulose sugarcane bagasse microwave method and hotplate stirrer method with average values of -51.8500 and -49.15000, respectively. There is a significant difference between the sugarcane bagasse cellulose from the microwave method and that from the hotplate stirrer method data with an average value of 2.70000. This shows that both treatment methods significantly increase the cellulose content compared to sugarcane bagasse without treatment.

The analysis of lignin content with a post-hoc test showed that significant differences were found between the data of sugarcane bagasse without treatment with cellulose sugarcane bagasse microwave method and hotplate stirrer method with an average value of 7.31000 and 7.15000, respectively. There was a significant difference between the sugarcane bagasse cellulose obtained from the microwave method and the hotplate stirrer method data with an average lignin value of 0.16000.

The analysis of hemicellulose content with a posthoc test showed that significant differences were found between the data of sugarcane bagasse without treatment and cellulose sugarcane bagasse microwave method and hotplate stirrer method with an average value of 17.62667 and 16.25667, respectively. There is a significant difference between the data from the sugarcane bagasse cellulose from the microwave method and the hotplate stirrer method, with an average hemicellulose value of 1.37000. Based on the SPSS program output from the cellulose, hemicellulose, and lignin content analysis in sugarcane bagasse and sugarcane bagasse cellulose from the microwave and hotplate stirrer methods, the significant value was $0.000 < 0.05$. The results of ANOVA and post-hoc tests showed that microwave and hotplate stirrer treatments significantly increased cellulose content and changed the composition of lignin and hemicellulose in untreated sugarcane bagasse. These findings show cellulose isolation can increase bagasse powder's cellulose content by reducing lignin and hemicellulose. Organoleptic studies demonstrate that bagasse cellulose using microwave and hotplate stirrer techniques and regular cellulose in the form of white powder then support the above findings. Additionally, the yields of sugarcane bagasse cellulose using microwave and hotplate stirrer techniques were 19.60% and 20.07%, respectively (Table 2).

The results of the isolation, characterization, and FTIR spectra of bagasse powder, bagasse cellulose using microwave and hotplate stirrer techniques, and regular cellulose are presented in Figure 2 and Table 3. After removing hemicellulose and lignin, the functional groups of cellulose were identified by spectroscopic analysis. The vibrational bands of the four test samples are shown in Figure 2. The FTIR spectroscopy results for normal cellulose and sugarcane bagasse cellulose obtained using microwave and hotplate stirrer techniques are comparable, suggesting that their functional groups are similar.

Table 2: Physical characterization of cellulose (organoleptic tests) and yield

Sample	Organoleptic		Yield (%)
	Dose Form	Colour	
Cellulose sugarcane bagasse *hotplate stirrer*	Powder	White	20.07
Cellulose sugarcane bagasse microwave	Powder	White	19.60

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Analysis of functional groups of Sugarcane bagasse, cellulose from Sugarcane bagasse, and standard cellulose.

Wavenumber (cm^{-1})				W.number Range (cm^{-1})	Functional Group Interpretation
Sugarcane bagasse	Cellulose S.bagasse h.stirrer	Cellulose S.bagasse microwave	Cellulose standard		
3342.84	3335.58	3326.43	3329.05	3400-3200	O-H stretching vibration[31]
2903.87	2893.99	2896.87	2897.54	3000-2850	Sp^3 C-H stretching[31]
1724.18	-	-	-	1750-1735	C=O stretching[31]
-	1647.32	1642.58	1641.02	1650-1630	C-O[31]
1604.16	-	-	-	1600-1475	C=C aromatic aromatic ring[31]
1324.48	1321.77	1323.81	1321.13	1440-1000	C-O-H bending[31]
1242.65	-	-	-	1300-1000	C-O stretching vibration[31]
1030.59	1022.54	1024.49	1022.42	1300-1000	C-O stretching vibration[31]
822.86	895.91	895.19	895.19	Around 850	Asymmetric C-O-C[31] stretching vibration

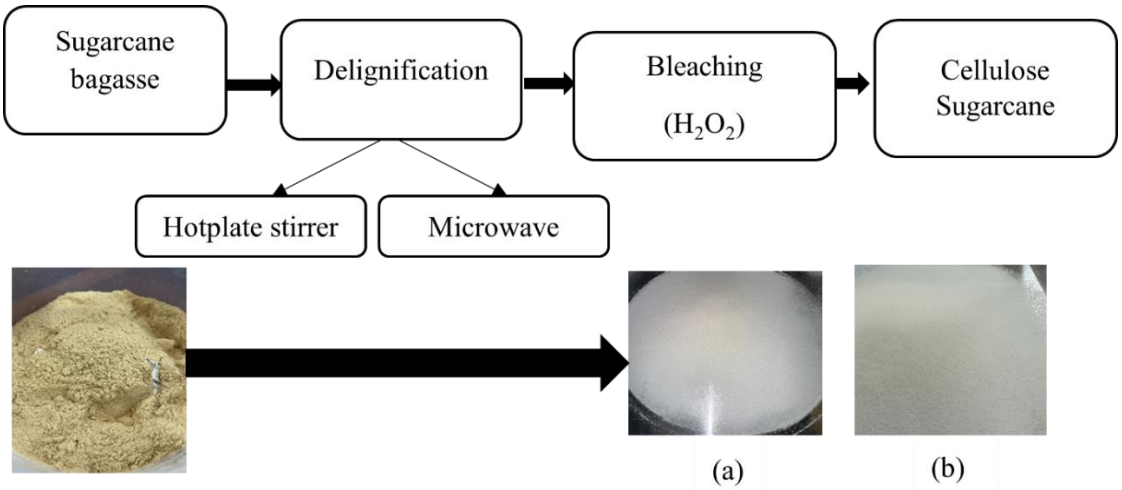

Figure 1: Schematic of the Cellulose extraction from Sugarcane Bagasse (a) cellulose s.bagasse hotplate stirrer. (b) cellulose s.bagasse microwave

The FTIR spectra of bagasse powder, bagasse cellulose from microwave and hotplate stirrer techniques, and regular cellulose are displayed in Figure 2 and Table 3. The absorption bands of 3329.05 cm^{-1} for conventional cellulose, 3335.58 cm^{-1} for bagasse cellulose made using the hotplate stirrer method, and 3326.43 cm^{-1} for bagasse cellulose made using the microwave method all showed hydroxyl peaks. The conventional cellulose's -CH_2- vibrational group was found at 2897.54 cm^{-1}, the hotplate stirrer method's sugarcane bagasse cellulose at 2893.99 cm^{-1}, and the microwave method's sugarcane bagasse cellulose at 2896.87 cm^{-1}. Absorption bands at 1022.42 cm^{-1} for standard cellulose, 1022.54 cm^{-1} for sugarcane bagasse cellulose obtained by the hotplate stirrer method, and 1024.49 cm^{-1} for sugarcane bagasse cellulose obtained by the microwave method showed C-O vibration group. Standard cellulose and sugarcane bagasse cellulose hotplate stirrer and microwave techniques revealed C-O-H bending groups linked to carbon chains at wave numbers 1321.13 cm^{-1}, 1321.77 cm^{-1}, and 1323.81 cm^{-1}, respectively[31–34]. Typical cellulose absorption bands were also visible in the isolated cellulose's FTIR spectrum. The existence of C-O groups was indicated by absorption bands seen at wave numbers 1647.32 cm^{-1} (using the hotplate stirrer method), 1642.58 cm^{-1} (using the microwave method), and 1641.02 cm^{-1} (using standard cellulose). The existence of β-1.4 glycosidic groups and C-O-C stretching vibrations is indicated by the band that appears at 895 cm^{-1}. These findings demonstrate the specificity of the FTIR spectrum for cellulose groups[31–34]. FTIR Spectra of the extraction cellulose also showed typical cellulose absorption bands. Absorption bands were observed at wave numbers 1647 cm^{-1} (sugarcane bagasse cellulose hotplate stirrer method), 1624 cm^{-1} (sugarcane bagasse cellulose microwave method), and 1641 cm^{-1} (cellulose standard), this demonstrated that C-O groups were present.

In the meantime, the band that is 895 cm^{-1} indicates the presence of C-O-C stretching vibrations and β-1.4 glycosidic groups. These results show that the FTIR spectrum is specific for cellulose groups [31–34]. Table 3 and Figure 1 show that bagasse cellulose produced by microwave and hotplate stirrer techniques and regular cellulose have comparable functional groups and absorbance bands at different wavelengths. Bagasse powder has the same wavelength as the conventional spectrum. Furthermore, the shift in functional groups shows that the bagasse powder cellulose synthesis process was completed. Cellulose is an ideal material for various applications: bioplastic[35], pulp[36], bioethanol[37], hydrogel[38], and other medical materials.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

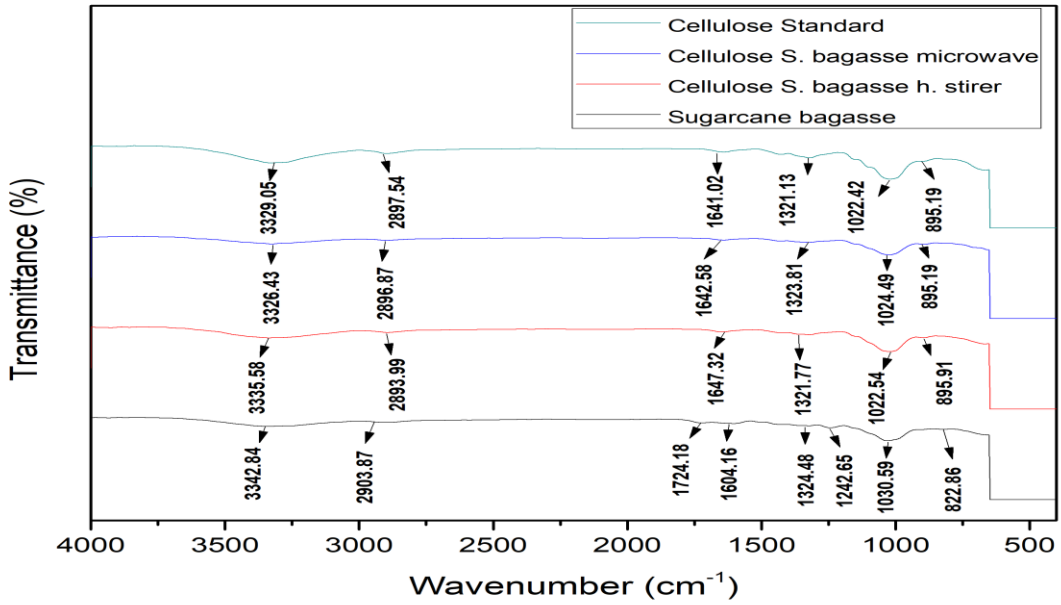

Figure 2: FTIR spectra of s.bagasse, s.bagasse cellulose (hotplate stirrer), s.bagasse cellulose (microwave) and standard cellulose.

Conclusion

The study results showed that the cellulose made from bagasse powder has physical properties and FTIR spectra comparable to regular cellulose. The bagasse cellulose extraction method using a microwave and hotplate stirrer has optimal characteristics and is environmentally friendly. This work demonstrates that bagasse cellulose processed using microwave and hotplate stirring techniques can be used as a raw material for bioplastics, hydrogel, carboxymethyl cellulose, cosmetics, paper, etc. This is because lignocellulose, an alternative source of cellulose, is abundant in bagasse powder.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors affirm that the work presented in this article is original and accept full responsibility for any claims arising from its content.

Acknowledgments

The author would like to thank the feed chemistry and biochemistry laboratories at Hasanuddin University for facilitating and supporting this research.

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