# Bukti Korespondensi & Proses Review

## Paper 1 Syarat Khusus Ajuan Guru Besar 2023

**Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Mechanical Treatment**

**Pengusul:**
**Dr. Edi Syafri, ST, M.Si**

# 1. Proses Submission

Submission to International Journal of Biological Macromolecules - manuscript number Kotak Masuk ×

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Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Ultrafine Grinding Treatment

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Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Ultrafine Grinding Treatment

Dear Dr Syafri,

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I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Dec 01, 2021.

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Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Mechanical Treatment

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Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Mechanical Treatment

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Cover Letter

Dear Editor in Chief,

Thanks for your letter and the thoughtful comments from the referees about our paper entitled "Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Mechanical Treatment" JBIOMAC-D-21-08546 . We carefully analysed all the comments and these comments are very valuable and helpful for perfecting and modifying our manuscript, and also have important guiding significance for our research. Therefore, we carefully checked the manuscript and revised it according to each comment. Consequently, we feel that our manuscript is substantially strengthened. Revised portion are marked using green background in the revised manuscript. The detailed corrections in the paper and the responses to the reviewer's comments are as the following list of revisions.

We also had proofread the manuscript, in hope that this manuscript suit the IJBIOMAC quality.


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CERTIFICATE OF PROOFREADING

ISOLATION AND CHARACTERIZATION OF CELLULOSE NANOFIBERS FROM AGAVE GIGANTEA BY CHEMICAL-MECHANICAL TREATMENT

We look forward to your positive response. If you have any question about this paper, please don't hesitate to let us know. We hope these revisions will make it more acceptable for publication. Thank you.

Sincerely yours,

Edi Syafri

Department of Agricultural Technology, Politeknik Pertanian Negeri Payakumbuh, West Sumatra 26271, Indonesia

# 4. . Decision2 (Accept)

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Manuscript Number: IJBIOMAC-D-21-08546R1

Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Mechanical Treatment

Dear Dr Syafri,

Thank you for submitting your manuscript to International Journal of Biological Macromolecules.

I am pleased to inform you that your manuscript has been accepted for publication.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to International Journal of Biological Macromolecules and hope you will consider us again for future submissions.

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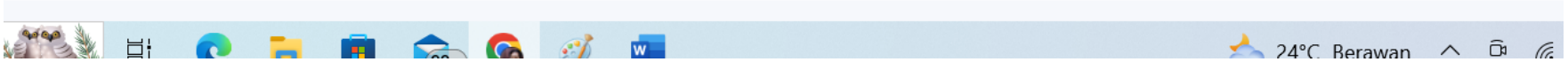

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# Isolation and characterization of cellulose nanofibers from Agave gigantea by chemical-mechanical treatment


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|---|---|
| Journal | International Journal of Biological Macromolecules |
| Our reference | BIOMAC20064 |
| PII | S0141-8130(21)02740-9 |
| DOI | 10.1016/j.ijbiomac.2021.12.111 |


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International Journal of Biological Macromolecules 200 (2022) 25–33

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## Isolation and characterization of cellulose nanofibers from *Agave gigantea* by chemical-mechanical treatment

Edi Syafri [a,*], Jamaluddin [a], Nasmi Herlina Sari [b], Melbi Mahardika [c], Putri Amanda [d], Rushdan Ahmad Ilyas [e,f]

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ARTICLE INFO

*Keywords:*
Nanocellulose
*Agave gigantea*
Chemical-ultrafine grinding
Thermal stability

ABSTRACT

Nanocellulose is a renewable and biocompatible nanomaterial that evokes much interest because of its versatility in various applications. This study reports the production of nanocellulose from *Agave gigantea* (AG) fiber using the chemical-ultrafine grinding treatment. Chemical treatment (alkalization and bleaching) removed non-cellulose components (hemicellulose and lignin), while ultrafine grinding reduced the size of cellulose micro-fibrils into nanocellulose. From the observation of Transmission Electron Microscopy, the average diameter of nanocellulose was 4.07 nm. The effect of chemical-ultrafine grinding on the morphology and properties of AG fiber was identified using chemical composition, Scanning Electron Microscopy, X-ray Diffraction, Fourier Transform Infrared, and Thermogravimetric Analysis. The bleaching treatment increased the crystal index by 48.3% compared to raw AG fiber, along with an increase in the cellulose content of 20.4%. The ultrafine grinding process caused a decrease in the crystal content of the AG fiber. The crystal index affected the thermal stability of the AG fiber. The TGA results showed that AG fiber treated with bleaching showed the highest thermal stability compared to AG fiber without treatment. The FTIR analysis showed that the presence of C—H vibrations from the ether in the fiber. After chemical treatment, the peaks at 1605 and 1243 $cm^{-1}$ disappeared, indicating the loss of lignin and hemicellulose functional groups in AG fiber. As a result, nanocellulose derived from AG fiber can be applied as reinforcement in environmentally friendly polymer biocomposites.

# Proses Revisi terhadap saran reviewer dan editor  IJBIOMAC

International Journal of Biological Macromolecules

# Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Mechanical Treatment

--Manuscript Draft--

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| **Manuscript Number:** | IJBIOMAC-D-21-08546R1 |
| **Article Type:** | Research Paper |
| **Section/Category:** | Carbohydrates, Natural Polyacids and Lignins |
| **Keywords:** | nanocellulose; Agave gigantea; thermal stability |
| **Corresponding Author:** | Edi Syafri, Dr.<br>Politeknik Pertanian Negeri Payakumbuh<br>Lima Puluh Kota, INDONESIA |
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| | Putri Amanda, MSi |
| | Rushdan Ahmad Ilyas, Dr |
| **Abstract:** | Nanocellulose is a renewable and biocompatible nanomaterial that interest because of its various applications. This study reports the production of nanocellulose from Agave gigantea (AG) fiber using the chemical-mechanical treatment. Chemical treatment removed non-cellulose components, while mechanical reduced the size of cellulose microfibrils into nanocellulose. From the observation of Transmission Electron Microscopy, the average diameter of nanocellulose was 4.07 nm. The effect of chemical-mechanical treatment on the morphology and properties of AG fiber was identified using chemical composition, Scanning Electron Microscopy, X-ray Diffraction, Fourier Transform Infrared, and Thermogravimetric analysis. The bleaching treatment increased the crystal index by 48.3% compared to raw AG fiber, along with an increase in the cellulose content of 20.4%. The crystal index affected the thermal stability of the AG fiber. The TGA results showed that AG fiber treated with bleaching showed the highest thermal stability compared to AG fiber without treatment. The FTIR analysis showed that the presence of C-H vibrations from the ether in the fiber. After chemical treatment, the peaks at 1605 and 1243 cm -1 disappeared, indicating the loss of lignin and hemicellulose functional groups in AG fiber. As a result, nanocellulose derived from AG fiber can be applied as reinforcement in biocomposites. |
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| **Response to Reviewers:** | Reviewer 1<br>Reviewer #1: A paper focuses on Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Ultrafine Grinding Treatment. Though the intention of the authors is highly commendable, there is some mistakes throughout the manuscript. Besides, there are some grammatical mistakes throughout the manuscript, particularly in respect of use of singular and plural with the subject or verb. In view of the above comments, whole manuscript should be properly revised.<br>Dear reviewers, thanks for your constructive comments. We really appreciate it. We had proofread the manuscript, in hope that this manuscript suit the IJBIOMAC quality.<br><br>Introduction section is long with a many references based on the literature survey conducted by the authors. This is very good. However, this lacks in proper presentation of literature survey, which should have been systematic whereby existing scientific gaps should have been brought out. This should have given justification for the present study, which should be followed by the objectives of this study.<br>Thanks for your comments. We have revised this section according to your comments. Literature survey as well as the scientific gaps had been improved.<br>Agave gigantea, is the family member of Agavaceae, which contain approximately similar properties like (physical and mechanical) of sisal (Agave sisalana). Agave gigantea is a Central American native non-wood biomass whose leaves have been used as a source of fiber for centuries. Traditionally, Agave gigantea fibers are extracted using the water retting technique and scorching machines, and subsequently used to make ropes and bags [19]. A study conducted by Kumar Singh et al. [30] showed that the cellulosic fiber content of Agave gigantea fiber of 55-70%, which was higher than that of wood, having values ranging from 40–50% [31]. In the same study, it was also demonstrated that the lignin content of Green Agave americana fiber was 3±0.3% [32], which was lower than that of wood (30%) [31]. Besides that, Agave gigantea fiber gives a competitive edge over other types of non-wood biomass like bagasse derived from corn or sugarcane, a crop that demands a certain level of care for adequate growth. Moreover, Agave gigantea can be cultivated in various tropical and warm regions worldwide since it can withstand a quite wide range of temperatures (16 to 34 °C) [33]. Up to the present time, the usage of Agave gigantea fibers has progressed to another successive level, especially to numerous engineering applications. For example, it is being used as reinforcement in polymer matrix composite in material engineering [30,33].<br><br>To the best of our knowledge, no study on Agave gigantea cellulose nanofibers using chemical-ultrafine grinding treatment followed by ultrasonication has been found in the literature. Therefore, the aim of the current study is to extract and characterize cellulose nanofiber from Agave gigantea fibers. Cellulose and cellulose nanofiber were extracted from Agave gigantea fibers by chemical and mechanical methods. The effect of chemical-ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and Thermogravimetric Analysis (TGA).<br><br>Please make a table of isolation of nanocellulose using several natural fibres. It is therefore suggested that 'Introduction Section' should be revised as suggested above because this Section is an important one from the point of view of taking up the present study.<br>Dear reviewer, thanks for your comment. We have revised this section accordingly. We also had add a table of isolation of nanocellulose using several natural fibres.<br>Table 1: Isolation of nanocellulose from natural fiber using various treatments<br>Natural fiberNanocellulose preparationRef.<br>Cassava bagasseHydrolyzed in 6.5 M H2SO4 / 40 min[10]<br><br>Wheat strawHigh Pressurize Homogenizer/ 15 min[12]<br><br>Cotton celluloseHydrolyzed in 6.5M sulfuric acid/ 75 min[13]<br><br>Softwood wood flourSuper masscolloider[14]<br><br>Rice strawUltrasonication[15] |

Kenaf Super masscolloider[30]

KenafSuper masscolloider[31]

Sugar palm fibreHigh Pressurize Homogenizer, 500 bar[19,21]

Tunicin 55 wt % $H_2SO_4$ / 20 mins[32,33]

Waxy maize starch$H_2SO_4$ / 5 days[34,35]

Cottonseed linter 64 wt % $H_2SO_4$ / 4 h[36]

Ramie64 wt % $H_2SO_4$ / 4 h[37]

Hemp 64 wt % $H_2SO_4$ / 4 h[39]

Flax 64 wt % $H_2SO_4$ / 4 h[40]

Bamboo50 wt % $H_2SO_4$ / 48 h[42]

Microcrystalline cellulose (MCC)36.5 wt.% HCl[43]

Potato peel waste64 wt % $H_2SO_4$ / 90 mins[44]

Cotton cellulose powders $H_2SO_4$[45]

Sugarcane bagasse64 wt % $H_2SO_4$/ 3 h[46]

Cotton linter 64 wt % $H_2SO_4$ / 1 h[47]

Sugar palm fibre60 wt% $H_2SO_4$ / 45 min[48–50]

Agave giganteaUltrafine grindingCurrent study

Materials and Methods:
Normally, this section should have two main subsections. The first one is Materials which should give details of all materials used in the study, where from they were procured, known characteristics, if available (for e.g. sodium hydroxide, sodium chlorite, where do you get it, what is the purity of the chemical and etc.).
Here one should also clearly mention the number of samples used, any standards followed for variety of properties, make and model of the instruments used for characterization, their accuracy and experimental conditions used, etc.
Dear reviewer, we had revised this section according to your comment.
The cellulose fiber in this study was sourced from the leaves of the Agave gigantea plant. The leaves (AG) were obtained in the plantation area in Harau District, Limapuluh Kota Regency, West Sumatera Province. Chemicals used in this experiment were sodium hydroxide (NaOH 98% Sigma-Aldrich), sodium chlorite ($NaClO_2$ Sigma-Aldrich), and glacial acetic acid ($CH_3COOH$).

Results & Discussion:
Some of the paragraph should be under Methods and if it is already there then this becomes repetition and hence can be deleted. Secondly, this Section is Results & Discussion and hence only results should be mentioned and then it should be discussed preferably comparing it with earlier reported similar results by other researchers.
Dear reviewer, we had revised this section according to your comment.
Conclusions given here are do not reflect what had been achieved including many speculations. It is too long and should be in 1 paragraph. Hence these need to be suitably modified. It may be remembered that this Section forms a summary of all the major observations/ results obtained. Accordingly, here presentation should consist of the main Results or the observations of the study in short sentences probably with

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bullet points.
Dear reviewer, we had revised this section according to your comment.
This study aims to utilize AG fiber into nanocellulose by chemical and mechanical methods. AG fiber treated with bleaching for 2 h showed the highest cellulose content after removing 56% hemicellulose. Mechanical treatment was successful in the production of nanocellulose with an average diameter of 4.07 nm. A crystallinity index (71%) was observed for bleached AG fibers compared to untreated fibers (49%). The functional group present at 2898 cm−1 in the treated AG fiber increased the load-bearing ability and stiffness when reinforced with a polymer matrix. The bleached AG fiber showed the highest thermal stability (363°C) compared to the untreated fiber (343°C). Based on the findings in this study, it can be concluded that among all parameters, the optimal chemical-mechanical treatment gave excellent properties in terms of cellulose purity and cellulose nanofiber production. Therefore, AG fiber treated with chemical-mechanical treatment can be used as a new fiber reinforcement source for lightweight and environmentally friendly biocomposites.

General Comments:
The paper though contains some interesting results and novelty work, it lacks in its proper presentation in the whole manuscript. Of course there is need for better to check English language throughout the manuscript. It is suggested that the authors should revise the paper in the light of above comments/suggestions.
Thanks for your comment. We have revised the manuscript accordingly.

Reviewer #2: Manuscript ID: IJBIOMAC-D-21-08546
Title: " Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Ultrafine Grinding Treatment"
E. Syafri et all reported the extraction and characterization of CNFs from the leaves of the Agave gigantea plant by using chemical treatment followed by grinding process. the materials were characterized at different stage of treatments and the obtained results in terms of chemical composition, morphology, crystalline and chemical structures and thermal properties were evaluated and discussed in detail. I read the manuscript very carefully, my comments and suggestions are listed below:
- The authors should revise the English thoroughly throughout the manuscript. Generally, I found a lot of errors (grammar, orthograph, punctuation) which indicates that the manuscript is not ready yet to be accepted before a significant English refinement. Attention should be paid also to the units of the parameters (Insert space between the value and the unit, for example 60 °C instead of 60°C).
Dear reviewers, thanks for your constructive comments. We really appreciate it. We had proofread the manuscript, in hope that this manuscript suit the IJBIOMAC quality. The units of the parameters had been revised accordingly.

- More important findings could be added in highlights section (now they are mostly about what was done). Please note that each Highlight can be no more than 85 characters, according to the specifications set out in the Author Guidelines of the Journal IJBIOMAC.
Dear reviewer, thanks for your comment. The highlight section had been revised accordingly to the Author Guidelines of the Journal IJBIOMAC.
Abstract: the authors stated "The FTIR analysis shows that the presence of C-H vibrations from the ether in the fiber will form a strong interaction with the polymer matrix". Which polymer matrix? Please specify
Dear reviewer, we had revised this section.
The FTIR analysis showed that the presence of C-H vibrations from the ether in the fiber.
- The introduction would be improved by explicitly stating the novelty that this research will address. Revisions should also clearly identify the contributions of this paper in terms of the novelty of the approaches and how these relate to previous studies.
Dear reviewer, thanks for your comment. The novelty of this manuscript had been highlighted in this revised manuscript.
In the past decades, many different resources have been used to prepare cellulose nanofiber, such as cassava bagasse [10], wheat straw [11,12], cotton cellulose [13], softwood wood [14], rice straw [15], kenaf [16], bamboo fiber [17], sugar palm fiber [18–24], ginger [25,26], water hyacinth [27], and sugarcane bagasse [28]. Table 1 shows the isolation of nanocellulose using several natural fibres. The purpose of the

isolation of cellulose nanofiber is as reinforcement in the nanocomposite field that has gained tremendous attention since it was first examined by Favier et al. [29]. However, no studies on the production, composition, or properties of natural cellulose nanofibers from Agave gigantea fibers using chemical-ultrafine grinding treatment have been found in the literature.

Table 1: Isolation of nanocellulose from natural fiber using various treatments
Natural fiberNanocellulose preparationRef.
Cassava bagasseHydrolyzed in 6.5 M $H_2SO_4$ / 40 min[10]

Wheat strawHigh Pressurize Homogenizer/ 15 min[12]

Cotton celluloseHydrolyzed in 6.5M sulfuric acid/ 75 min[13]

Softwood wood flourSuper masscolloider[14]

Rice strawUltrasonication[15]

Kenaf Super masscolloider[30]

KenafSuper masscolloider[31]

Sugar palm fibreHigh Pressurize Homogenizer, 500 bar[19,21]

Tunicin 55 wt % $H_2SO_4$ / 20 mins[32,33]

Waxy maize starch$H_2SO_4$ / 5 days[34,35]

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Ramie64 wt % $H_2SO_4$ / 4 h[37]

Hemp 64 wt % $H_2SO_4$ / 4 h[38]

Flax 64 wt % $H_2SO_4$ / 4 h[39]

Bamboo50 wt % $H_2SO_4$ / 48 h[40]

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Cotton linter 64 wt % $H_2SO_4$ / 1 h[45]

Sugar palm fibre60 wt% $H_2SO_4$ / 45 min[46–48]

Agave giganteaUltrafine grindingCurrent study

Agave gigantea, is the family member of Agavaceae, which contain approximately similar properties like (physical and mechanical) of sisal (Agave sisalana). Agave gigantea is a Central American native non-wood biomass whose leaves have been used as a source of fiber for centuries. Traditionally, Agave gigantea fibers are extracted using the water retting technique and scorching machines, and subsequently used to make ropes and bags [19]. A study conducted by Kumar Singh et al. [49] showed that the cellulosic fiber content of Agave gigantea fiber of 55-70%, which was higher than that of wood, having values ranging from 40–50% [50]. In the same study, it was also demonstrated that the lignin content of Green Agave americana fiber was 3±0.3% [51], which was lower than that of wood (30%) [50]. Besides that, Agave gigantea fiber gives a competitive edge over other types of non-wood biomass like bagasse derived from corn or sugarcane, a crop that demands a certain level of care for adequate growth. Moreover, Agave gigantea can be cultivated in various tropical and warm regions worldwide since it can withstand a quite wide range of temperatures

(16 to 34 °C) [52]. Up to the present time, the usage of Agave gigantea fibers has progressed to another successive level, especially to numerous engineering applications. For example, it is being used as reinforcement in polymer matrix composite in material engineering [49,52].

To the best of our knowledge, no study on Agave gigantea cellulose nanofibers using chemical-ultrafine grinding treatment followed by ultrasonication has been found in the literature. Therefore, the aim of the current study is to extract and characterize cellulose nanofiber from Agave gigantea fibers. Cellulose and cellulose nanofiber were extracted from Agave gigantea fibers by chemical and mechanical methods. The effect of chemical-ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and Thermogravimetric Analysis (TGA).
- Experimental section: please add a detail on the sonication treatment, that is performed after mechanical grinding process.
For the next treatment, 1 g of CNFs 1% was added and sonicated at 80% power output for 60 min using a 40 kHz Sonic Ruptor 400 with a tip diameter of 13 mm. The ultra-sonication treatment was conducted at room temperature. At the end of the ultrasonication treatment, the CNF suspension turned from turbid white to transparent.
- Samples codes: I suggest giving a uniform name (code) to all the studied samples, for example: Raw AG fiber, Alkali-treated AG fiber, Bleached AG fiber and CNFs in the manuscript and in the figures and legends.
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- In Figure 4, please mark the 2θ peak that mentioned in the main text.
Dear reviewer, we had marked the 2θ peak that mentioned in the main text.
- The references should be revised according to the journal style.
All the references had been revised accordingly.

Reviewer #3: Comments:
1. In general, it gives a little contribution to isolation process of CNF. Research novelty must be clearly explained. Agave gigantea fiber has been investigated by Singh et al.,2021 with steam explosion method to produce CNF, but this research using different method. Explanation method is also not clear (Sonication process is not explained).
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2. In the introduction, the line 10 found the words fiber and fibers (check the consistency of the words used)
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3. The previous study [30] Singh et al.,2021 the lignin of Agave gigantea fiber composition range is 10-20% Lignin, but chemical composition in this research less than 1% (re-test your chemical composition, because some references are very contradictory)...!
Dear reviewer, thanks for the constructive comment. The results that we got is consistence with other literature review.
4. The cm unit must be changed to mm
We had revised the cm unit to mm unit.
5. Page 9 line 4, How long time to dry until the moisture content is 10%...?
We have revised this paper according to reviewers.
After that, the outer skin of the fiber was removed with a knife. The AG fiber was then dried in the sun for 4 days with a moisture content of about 9 to 10%.
6. Chemical composition test method was developed by Van Soest. by the previous study.....?
The chemical composition of AG fiber was determined using the method developed by Van Soest to determine the cellulose, hemicellulose, and lignin content in AG fiber (Van Soest).
7. POINT 2.8 TEM, The page 12, the result of 4.07 nm, it should be explained to Result and Discussion. Check the unit Voltage is kV nt keV
The result of 4.07 nm, has be explained into Result and Discussion section.

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| | |
|---|---|
| | 8. in the Figure 2 SEM Micrograph add the caption, The Figure 2b and 2c, the diameter of after bleaching bigger than alkalization , explained that...!<br>Dear reviewer, this sentence had been revised.<br>Figures 2b and 2c show that the surface morphology of the microfibril bundles was smooth, and the fiber diameter was smaller (10-15μm) than raw AG fiber due to chemical treatment which successfully removed hemicellulose, lignin, wax, pectin components, and impurities.<br>9. in the Figure 3 FTIR spectra of Ultrafine Grinding + Sonication, ....? The sonication process must be add in the method<br>Dear reviewer, the sonification process had been added in the revised manuscript. Furthermore, the CMF was a mechanically treat using an Ultrafine grinding. For the next treatment, 1 g of CNFs 1% was added and sonicated at 80% power output for 60 min using a 40 kHz Sonic Ruptor 400 with a tip diameter of 13 mm. The ultra-sonication treatment was conducted at room temperature. At the end of the ultrasonication treatment, the CNF suspension turned from turbid white to transparent.<br><br>10. Page 15, line 49 thw word.....individual fibril-fibril is ungrammatically<br>Dear reviewer, we had revised this manuscript accordingly.<br>11. After mechanical treatment the crystallinity index decreases by 8.1% , so is it impacted to decrease the mechanical strength....could you explained that phenomena ...?<br>After mechanical treatment, the CI value decreased by 8.1% compared to AG fiber after bleaching due to the destruction of the cellulose chain resulting from mechanical treatment [64,80]. This result was supported by previous researches [66,81].<br>12. The aspect ratio (L/D) is important factor of CNF, make the Graph of length on the CNF (in the Figure 2)<br>Dear reviewer, thanks for your comments. The graph of length on the CNF cannot be measured due to the limitation by the equipment used.<br>13. The conclusion is not appropriate because this study did not discuss the variation of isolation treatment to produce CNF.<br>Dear reviewer, we had revised this section accordingly. Thanks a lot for all your comment in improvising our manuscript.<br>This study aims to utilize AG fiber into nanocellulose by chemical and mechanical methods. AG fiber treated with bleaching for 2 h showed the highest cellulose content after removing 56% hemicellulose. Mechanical treatment was successful in the production of nanocellulose with an average diameter of 4.07 nm. A crystallinity index (71%) was observed for bleached AG fibers compared to untreated fibers (49%). The functional group present at 2898 cm−1 in the treated AG fiber increased the load-bearing ability and stiffness when reinforced with a polymer matrix. The bleached AG fiber showed the highest thermal stability (363 °C) compared to the untreated fiber (343 °C). Based on the findings in this study, it can be concluded that among all parameters, the optimal chemical-mechanical treatment gave excellent properties in terms of cellulose purity and cellulose nanofiber production. Therefore, AG fiber treated with chemical-mechanical treatment can be used as a new fiber reinforcement source for lightweight and environmentally friendly biocomposites. |

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Dear Editor in Chief,

Thanks for your letter and the thoughtful comments from the referees about our paper entitled “Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Mechanical Treatment” JBIOMAC-D-21-08546 . We carefully analysed all the comments and these comments are very valuable and helpful for perfecting and modifying our manuscript, and also have important guiding significance for our research. Therefore, we carefully checked the manuscript and revised it according to each comment. Consequently, we feel that our manuscript is substantially strengthened. Revised portion are marked using green background in the revised manuscript. The detailed corrections in the paper and the responses to the reviewer’s comments are as the following list of revisions.

We also had proofread the manuscript, in hope that this manuscript suit the IJBIOMAC quality.

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CERTIFICATE OF PROOFREADING

THIS IS TO ACKNOWLEDGE THAT THE JOURNAL PAPER ENTITLED

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WRITTEN BY

**EDI SYAFRI, JAMALUDDIN, NASMI HERLINA SARI, MELBI MAHARDIKA, PUTRI AMANDA AND RUSHDAN AHMAD ILYAS**

HAS BEEN EDITED AND RETURNED TO THE CUSTOMER ON

**27TH NOVEMBER 2021**

SITI NUR ATIKAH MAHAMUD
CHIEF EXECUTIVE OFFICER
EXCELPROOF ENTERPRISE

We look forward to your positive response. If you have any question about this paper, please don’t hesitate to let us know. We hope these revisions will make it more acceptable for publication. Thank you.

Sincerely yours,

Edi Syafri

Department of Agricultural Technology, Politeknik Pertanian Negeri Payakumbuh, West Sumatra 26271, Indonesia

**Reviewer 1**

Reviewer #1: A paper focuses on Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Ultrafine Grinding Treatment. Though the intention of the authors is highly commendable, there is some mistakes throughout the manuscript. Besides, there are some grammatical mistakes throughout the manuscript, particularly in respect of use of singular and plural with the subject or verb. In view of the above comments, whole manuscript should be properly revised.

Dear reviewers, thanks for your constructive comments. We really appreciate it. We had proofread the manuscript, in hope that this manuscript suit the IJBIOMAC quality.

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Introduction section is long with a many references based on the literature survey conducted by the authors. This is very good. However, this lacks in proper presentation of literature survey, which should have been systematic whereby existing scientific gaps should have been brought out. This should have given justification for the present study, which should be followed by the objectives of this study.

Thanks for your comments. We have revised this section according to your comments. Literature survey as well as the scientific gaps had been improved.

*Agave gigantea*, is the family member of Agavaceae, which contain approximately similar properties like (physical and mechanical) of sisal (*Agave sisalana*). *Agave gigantea* is a Central American native non-wood biomass whose leaves have been used as a source of fiber for centuries. Traditionally, *Agave gigantea* fibers are extracted using the water retting technique and scorching machines, and subsequently used to make ropes and bags [19]. A study conducted by Kumar Singh et al. [30] showed that the cellulosic fiber content of *Agave gigantea* fiber of 55-70%, which was higher than that of wood, having values ranging from 40–50% [31]. In the same study, it was also demonstrated that the lignin content of *Green Agave americana* fiber was 3±0.3% [32], which was lower than that of wood (30%) [31]. Besides that, *Agave gigantea* fiber gives a competitive edge over other types of non-wood biomass like bagasse derived from corn or sugarcane, a crop that demands a certain level of care for adequate growth. Moreover, *Agave gigantea* can be cultivated in various tropical and warm regions worldwide since it can withstand a quite wide range of temperatures (16 to 34 °C) [33]. Up to the present time, the usage of *Agave gigantea* fibers has progressed to another successive level, especially to numerous engineering applications. For example, it is being used as reinforcement in polymer matrix composite in material engineering [30,33].

To the best of our knowledge, no study on *Agave gigantea* cellulose nanofibers using chemical-ultrafine grinding treatment followed by ultrasonication has been found in the literature. Therefore, the aim of the current study is to extract and characterize cellulose nanofiber from *Agave gigantea* fibers. Cellulose and cellulose nanofiber were extracted from *Agave gigantea* fibers by chemical and mechanical methods. The effect of chemical-ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and Thermogravimetric Analysis (TGA).

Please make a table of isolation of nanocellulose using several natural fibres. It is therefore suggested that 'Introduction Section' should be revised as suggested above because this Section is an important one from the point of view of taking up the present study.

Dear reviewer, thanks for your comment. We have revised this section accordingly. We also had add a table of isolation of nanocellulose using several natural fibres.

Table 1: Isolation of nanocellulose from natural fiber using various treatments

| Natural fiber | Nanocellulose preparation | Ref. |
|---|---|---|
| Cassava bagasse | Hydrolyzed in 6.5 M $H_2SO_4$ / 40 min | [10] |

| | | |
|---|---|---|
| Wheat straw | High Pressurize Homogenizer/ 15 min | [12] |
| Cotton cellulose | Hydrolyzed in 6.5M sulfuric acid/ 75 min | [13] |
| Softwood wood flour | Super masscolloider | [14] |
| Rice straw | Ultrasonication | [15] |
| Kenaf | Super masscolloider | [30] |
| Kenaf | Super masscolloider | [31] |
| Sugar palm fibre | High Pressurize Homogenizer, 500 bar | [19,21] |
| Tunicin | 55 wt % $H_2SO_4$ / 20 mins | [32,33] |
| Waxy maize starch | $H_2SO_4$ / 5 days | [34,35] |
| Cottonseed linter | 64 wt % H2SO4 / 4 h | [36] |
| Ramie | 64 wt % H2SO4 / 4 h | [37] |
| Hemp | 64 wt % $H_2SO_4$ / 4 h | [39] |
| Flax | 64 wt % $H_2SO_4$ / 4 h | [40] |
| Bamboo | 50 wt % $H_2SO_4$ / 48 h | [42] |
| Microcrystalline cellulose (MCC) | 36.5 wt.% HCl | [43] |
| Potato peel waste | 64 wt % $H_2SO_4$ / 90 mins | [44] |
| Cotton cellulose powders | $H_2SO_4$ | [45] |
| Sugarcane bagasse | 64 wt % $H_2SO_4$/ 3 h | [46] |
| Cotton linter | 64 wt % $H_2SO_4$ / 1 h | [47] |
| Sugar palm fibre | 60 wt% $H_2SO_4$ / 45 min | [48–50] |
| *Agave gigantea* | Ultrafine grinding | Current study |

Materials and Methods:

Normally, this section should have two main subsections. The first one is Materials which should give details of all materials used in the study, where from they were procured, known characteristics, if available (for e.g. sodium hydroxide, sodium chlorite, where do you get it, what is the purity of the chemical and etc.).

Here one should also clearly mention the number of samples used, any standards followed for variety of properties, make and model of the instruments used for characterization, their accuracy and experimental conditions used, etc.

Dear reviewer, we had revised this section according to your comment.

The cellulose fiber in this study was sourced from the leaves of the *Agave gigantea* plant. The leaves (AG) were obtained in the plantation area in Harau District, Limapuluh Kota Regency, West Sumatera Province. Chemicals used in this experiment were sodium hydroxide (NaOH 98% Sigma-Aldrich), sodium chlorite ($NaClO_2$ Sigma-Aldrich), and glacial acetic acid ($CH_3COOH$).

Results & Discussion:

Some of the paragraph should be under Methods and if it is already there then this becomes repetition and hence can be deleted. Secondly, this Section is Results & Discussion and hence only results should be mentioned and then it should be discussed preferably comparing it with earlier reported similar results by other researchers.

Dear reviewer, we had revised this section according to your comment.

Conclusions given here are do not reflect what had been achieved including many speculations. It is too long and should be in 1 paragraph. Hence these need to be suitably modified. It may be remembered that this Section forms a summary of all the major observations/ results obtained. Accordingly, here presentation should consist of the main Results or the observations of the study in short sentences probably with bullet points.

Dear reviewer, we had revised this section according to your comment.

This study aims to utilize AG fiber into nanocellulose by chemical and mechanical methods. AG fiber treated with bleaching for 2 h showed the highest cellulose content after removing 56% hemicellulose. Mechanical treatment was successful in the production of nanocellulose with an average diameter of 4.07 nm. A crystallinity index (71%) was observed for bleached AG fibers compared to untreated fibers (49%). The functional group present at 2898 $cm^{-1}$ in the treated AG fiber increased the load-bearing ability and stiffness when reinforced with a polymer matrix. The bleached AG fiber showed the highest thermal stability (363°C) compared

to the untreated fiber (343°C). Based on the findings in this study, it can be concluded that among all parameters, the optimal chemical-mechanical treatment gave excellent properties in terms of cellulose purity and cellulose nanofiber production. Therefore, AG fiber treated with chemical-mechanical treatment can be used as a new fiber reinforcement source for lightweight and environmentally friendly biocomposites.

General Comments:

The paper though contains some interesting results and novelty work, it lacks in its proper presentation in the whole manuscript. Of course there is need for better to check English language throughout the manuscript. It is suggested that the authors should revise the paper in the light of above comments/suggestions.

Thanks for your comment. We have revised the manuscript accordingly.

Reviewer #2: Manuscript ID: IJBIOMAC-D-21-08546

Title: " Isolation and Characterization of Cellulose Nanofibers from Agave gigantea by Chemical-Ultrafine Grinding Treatment"

E. Syafri et all reported the extraction and characterization of CNFs from the leaves of the Agave gigantea plant by using chemical treatment followed by grinding process. the materials were characterized at different stage of treatments and the obtained results in terms of chemical composition, morphology, crystalline and chemical structures and thermal properties were evaluated and discussed in detail. I read the manuscript very carefully, my comments and suggestions are listed below:

- The authors should revise the English thoroughly throughout the manuscript. Generally, I found a lot of errors (grammar, orthograph, punctuation) which indicates that the manuscript is not ready yet to be accepted before a significant English refinement. Attention should be paid also to the units of the parameters (Insert space between the value and the unit, for example 60 °C instead of 60°C).

Dear reviewers, thanks for your constructive comments. We really appreciate it. We had proofread the manuscript, in hope that this manuscript suit the IJBIOMAC quality. The units of the parameters had been revised accordingly.

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- More important findings could be added in highlights section (now they are mostly about what was done). Please note that each Highlight can be no more than 85 characters, according to the specifications set out in the Author Guidelines of the Journal IJBIOMAC.

Dear reviewer, thanks for your comment. The highlight section had been revised accordingly to the Author Guidelines of the Journal IJBIOMAC.

Abstract: the authors stated "The FTIR analysis shows that the presence of C-H vibrations from the ether in the fiber will form a strong interaction with the polymer matrix". Which polymer matrix? Please specify

Dear reviewer, we had revised this section.

The FTIR analysis showed that the presence of C-H vibrations from the ether in the fiber.

- The introduction would be improved by explicitly stating the novelty that this research will address. Revisions should also clearly identify the contributions of this paper in terms of the novelty of the approaches and how these relate to previous studies.

Dear reviewer, thanks for your comment. The novelty of this manuscript had been highlighted in this revised manuscript.

In the past decades, many different resources have been used to prepare cellulose nanofiber, such as cassava bagasse [10], wheat straw [11,12], cotton cellulose [13], softwood wood [14], rice straw [15], kenaf [16], bamboo fiber [17], sugar palm fiber [18–24], ginger [25,26], water hyacinth [27], and sugarcane bagasse [28]. Table 1 shows the isolation of nanocellulose using several natural fibres. The purpose of the isolation of cellulose nanofiber is as reinforcement in the nanocomposite field that has gained tremendous attention since it was first examined by Favier et al. [29]. However, no studies on the production, composition, or properties of natural cellulose nanofibers from *Agave gigantea* fibers using chemical-ultrafine grinding treatment have been found in the literature.

Table 1: Isolation of nanocellulose from natural fiber using various treatments

| Natural fiber | Nanocellulose preparation | Ref. |
|---|---|---|
| Cassava bagasse | Hydrolyzed in 6.5 M $H_2SO_4$ / 40 min | [10] |
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| Cotton cellulose | Hydrolyzed in 6.5M sulfuric acid/ 75 min | [13] |
| Softwood wood flour | Super masscolloider | [14] |
| Rice straw | Ultrasonication | [15] |
| Kenaf | Super masscolloider | [30] |
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| Sugar palm fibre | High Pressurize Homogenizer, 500 bar | [19,21] |
| Tunicin | 55 wt % $H_2SO_4$ / 20 mins | [32,33] |
| Waxy maize starch | $H_2SO_4$ / 5 days | [34,35] |
| Cottonseed linter | 64 wt % H2SO4 / 4 h | [36] |
| Ramie | 64 wt % H2SO4 / 4 h | [37] |
| Hemp | 64 wt % $H_2SO_4$ / 4 h | [38] |
| Flax | 64 wt % $H_2SO_4$ / 4 h | [39] |
| Bamboo | 50 wt % $H_2SO_4$ / 48 h | [40] |
| Potato peel waste | 64 wt % $H_2SO_4$ / 90 mins | [42] |

| | | |
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| Cotton cellulose powders | $H_2SO_4$ | [43] |
| Sugarcane bagasse | 64 wt % $H_2SO_4$/ 3 h | [44] |
| Cotton linter | 64 wt % $H_2SO_4$ / 1 h | [45] |
| Sugar palm fibre | 60 wt% $H_2SO_4$ / 45 min | [46–48] |
| *Agave gigantea* | Ultrafine grinding | Current study |

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To the best of our knowledge, no study on *Agave gigantea* cellulose nanofibers using chemical-ultrafine grinding treatment followed by ultrasonication has been found in the literature. Therefore, the aim of the current study is to extract and characterize cellulose nanofiber from *Agave gigantea* fibers. Cellulose and cellulose nanofiber were extracted from *Agave gigantea* fibers by chemical and mechanical methods. The effect of chemical-ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and Thermogravimetric Analysis (TGA).

- Experimental section: please add a detail on the sonication treatment, that is performed after mechanical grinding process.

For the next treatment, 1 g of CNFs 1% was added and sonicated at 80% power output for 60 min using a 40 kHz  Sonic Ruptor 400 with a tip diameter of 13 mm. The ultra-sonication treatment was conducted at room temperature. At the end of the ultrasonication treatment, the CNF suspension turned from turbid white to transparent.

- Samples codes: I suggest giving a uniform name (code) to all the studied samples, for example: Raw AG fiber, Alkali-treated AG fiber, Bleached AG fiber and CNFs in the manuscript and in the figures and legends.

Dear reviewers, thanks for your comments. We really appreciate your effort. We had uniformed all the AG fiber name in the figures and legends.

- In Figure 4, please mark the 2θ peak that mentioned in the main text.

Dear reviewer, we had marked the 2θ peak that mentioned in the main text.

- The references should be revised according to the journal style.

All the references had been revised accordingly.

Reviewer #3: Comments:

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Dear reviewer, thanks for your comment. We had revised the manuscript accordingly.

Furthermore, the CMF was a mechanically treat using an Ultrafine grinding. For the next treatment, 1 g of CNFs 1% was added and sonicated at 80% power output for 60 min using a 40 kHz  Sonic Ruptor 400 with a tip diameter of 13 mm. The ultra-sonication treatment was conducted at room temperature. At the end of the ultrasonication treatment, the CNF suspension turned from turbid white to transparent.

2. In the introduction, the line 10 found the words fiber and fibers (check the consistency of the words used)

The words had been revised accordingly. Thank you.

3. The previous study [30] Singh et al.,2021 the lignin of Agave gigantea fiber composition range is 10-20% Lignin, but chemical composition in this research less than 1% (re-test your chemical composition, because some references are very contradictory)...!

Dear reviewer, thanks for the constructive comment. The results that we got is consistence with other literature review.

4. The cm unit must be changed to mm

We had revised the cm unit to mm unit.

5. Page 9 line 4, How long time to dry until the moisture content is 10%...?

We have revised this paper according to reviewers.

After that, the outer skin of the fiber was removed with a knife. The AG fiber was then dried in the sun for 4 days with a moisture content of about 9 to 10%.

6. Chemical composition test method was developed by Van Soest. by the previous study.....?

The chemical composition of AG fiber was determined using the method developed by Van Soest to determine the cellulose, hemicellulose, and lignin content in AG fiber (Van Soest).

7. POINT 2.8 TEM, The page 12, the result of 4.07 nm, it should be explained to Result and Discussion. Check the unit Voltage is kV nt keV

The result of 4.07 nm, has be explained into Result and Discussion section.

8. in the Figure 2 SEM Micrograph add the caption, The Figure 2b and 2c, the diameter of after bleaching bigger than alkalization , explained that...!

Dear reviewer, this sentence had been revised.

Figures 2b and 2c show that the surface morphology of the microfibril bundles was smooth, and the fiber diameter was smaller (10-15µm) than raw AG fiber due to chemical treatment which successfully removed hemicellulose, lignin, wax, pectin components, and impurities.

9. in the Figure 3 FTIR spectra of Ultrafine Grinding + Sonication, ....? The sonication process must be add in the method

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After mechanical treatment, the CI value decreased by 8.1% compared to AG fiber after bleaching due to the destruction of the cellulose chain resulting from mechanical treatment [64,80]. This result was supported by previous researches [66,81].

12. The aspect ratio (L/D) is important factor of CNF, make the Graph of length on the CNF (in the Figure 2)

Dear reviewer, thanks for your comments. The graph of length on the CNF cannot be measured due to the limitation by the equipment used.

13. The conclusion is not appropriate because this study did not discuss the variation of isolation treatment to produce CNF.

Dear reviewer, we had revised this section accordingly. Thanks a lot for all your comment in improvising our manuscript.

This study aims to utilize AG fiber into nanocellulose by chemical and mechanical methods. AG fiber treated with bleaching for 2 h showed the highest cellulose content after removing 56% hemicellulose. Mechanical treatment was successful in the production of nanocellulose with an average diameter of 4.07 nm. A crystallinity index (71%) was observed for bleached AG fibers compared to untreated fibers (49%). The functional group present at 2898 $cm^{-1}$ in the treated AG fiber increased the load-bearing ability and stiffness when reinforced with a polymer matrix. The bleached AG fiber showed the highest thermal stability (363 °C) compared to the untreated fiber (343 °C). Based on the findings in this study, it can be concluded that among all parameters, the optimal chemical-mechanical treatment gave excellent properties in terms of cellulose purity and cellulose nanofiber production. Therefore, AG fiber treated with chemical-mechanical treatment can be used as a new fiber reinforcement source for lightweight and environmentally friendly biocomposites.

**Highlights of this Investigation**:

- The increase in AG cellulose content after the chemical treatment
- Mechanical treatment was successful in the production of nanocellulose with an average diameter of 4.07 nm
- A crystallinity index (71%) was observed for bleached AG fibers compared to untreated fibers (49%)
- Based on the findings in this study, it can conclude that among all parameters, the optimal chemical-mechanical treatment gave excellent properties in terms of cellulose purity and cellulose nanofiber production

**Abstract**

Nanocellulose is a kind of renewable and biocompatible nanomaterials evoke much interest because of its versatility in various applications. This study reports the production of nanocellulose from *Agave gigantea* (AG) fiber using the chemical-ultrafine grinding treatment. Chemical treatment (alkalization and bleaching) removes non-cellulose components (hemicellulose and lignin), while ultrafine grinding reduces the size of cellulose microfibrils into nanocellulose. From the observation of Transmission electron microscopy, the average diameter of nanocellulose is 4.07 nm. The effect of chemical- ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, scanning electron microscope, X-ray diffraction, Fourier transform infrared and Thermal gravimetric analysis. The bleaching treatment increased the crystal index by 48.3% compared to raw AG fiber, along with an increase in the cellulose content of 20.4%. The ultrafine grinding process causes a decrease in the crystal content of the AG fiber. The crystal index affects the thermal stability of the AG fiber. The TGA results showed that AG fiber treated with bleaching showed the highest thermal stability compared to AG fiber without treatment. The FTIR analysis shows that the presence of C-H vibrations from the ether in the fiber will form a strong interaction with the polymer matrix. After chemical treatment, the peaks of 1605 $cm^{-1}$ and 1243 $cm^{-1}$ disappeared, indicating the loss of lignin and hemicellulose groups in AG fiber. As a result, nanocellulose derived from AG fiber can use as reinforcement in environmentally friendly polymer biocomposites.

**Keywords:** Nanocellulose, *Agave gigantea*, Chemical-Ultrafine Grinding, Thermal stability

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# Isolation and Characterization of Cellulose Nanofibers from *Agave gigantea* by Chemical-Ultrafine Grinding Treatment

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**Abstract**

Nanocellulose is a kind of renewable and biocompatible nanomaterials evoke much interest because of its versatility in various applications. This study reports the production of nanocellulose from *Agave gigantea* (AG) fiber using the chemical-ultrafine grinding treatment. Chemical treatment (alkalization and bleaching) removes non-cellulose components (hemicellulose and lignin), while ultrafine grinding reduces the size of cellulose microfibrils into nanocellulose. From the observation of Transmission electron microscopy, the average diameter of nanocellulose is 4.07 nm. The effect of chemical- ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, scanning electron microscope, X-ray diffraction, Fourier transform infrared and Thermal gravimetric analysis. The bleaching treatment increased the crystal index by 48.3% compared to raw AG fiber, along with an increase in the cellulose content of 20.4%. The ultrafine grinding process causes a decrease in the crystal content of the AG fiber. The crystal index affects the thermal stability of the AG fiber. The TGA results showed that AG fiber treated with bleaching showed the highest thermal stability compared to AG fiber without treatment. The FTIR analysis shows that the presence of C-H vibrations from the ether in the fiber will form a strong interaction with the polymer matrix. After chemical treatment, the peaks of 1605 $cm^{-1}$ and 1243 $cm^{-1}$ disappeared, indicating the loss of lignin and hemicellulose groups in AG fiber. As a result, nanocellulose derived from AG fiber can use as reinforcement in environmentally friendly polymer biocomposites.

**Keywords:** Nanocellulose, *Agave gigantea*, Chemical-Ultrafine Grinding, Thermal stability

## 1. Introduction

Cellulose nanofibre isolated from plant fibers attracted a huge interest in material science due to its appealing intrinsic properties including nano-dimension, high surface area (100 $m^2$ $g^{-1}$)[1–3], high aspect ratio of 100 [4,5], high crystallinity [6], low density, high mechanical strength, unique morphology along with availability, renewability and biodegrability [7–9]. Cellulose is the product of biosynthesis from bacteria and plants, whereas the general term ‘’cellulose nanofiber’’ refers to cellulosic isolation or extraction materials, with the outstanding feature of nano-scale structural dimension. The main component of plant fibres is cellulose, semicrystalline polymer, which composed of poly(1,4-β-D-anhydroglucopyranose) units. These units are formed from strong hydrogen bond between hydroxyl groups. Other main components that made up natural fibres structure are lignin and hemicellulose. Lignin is a highly cross-linked phenolic polymer, whereas hemicellulose is a branched multiple polysaccharide polymer composed of different types of sugars comprising xylose, glucose, arabinose, mannose and galactose. However, both lignin and hemicellulose are amorphous polymers.

In the past decades, many different resources have been used to prepare cellulose nanofiber, such as cassava bagasse [10], wheat straw [11,12], cotton cellulose [13], softwood wood [14], rice straw [15], kenaf [16], bamboo fibre [17], sugar palm fibre [18–24], ginger [25,26], water hyacinth [27], and sugarcane bagasse [28]. The purpose of the isolation of cellulose nanofiber is as reinforcements in the field of nanocomposite that has gained tremendous attention since it was first examined by Favier et al. [29]. However, no studies on the production, composition, or

properties of natural cellulose nanofibres from *Agave gigantea* fibres using chemical-ultrafine grinding treatment have been found in the literature.

*Agave gigantea*, is the family members of Agavaceae, which contain approx. similar properties like (physical and mechanical) of sisal (*Agave sisalana*). *Agave gigantea* is a Central American native non-wood biomass whose leaves have been used as a source of fibre for centuries. Traditionally, *Agave gigantea* fibres have been extracted using the water retting technique and scotching machines and subsequently used to make ropes and bags [19]. A study conducted by Kumar Singh et al. [30], has shown that the cellulosic fibre content of *Agave gigantea* fibre is 55-70%, which is higher than that of wood having values ranging from 40–50% [31]. In the same study, it was also demonstrated that the lignin content of *Agave gigantea* fibre is 10-20% [30], which is lower than that of wood (30%) [31]. Besides that, *Agave gigantea* fibre gives it a competitive edge over other types of non-wood biomass like bagasse which is derived from corn or sugarcane, a crop that demands a certain level of care for adequate growth. Moreover, *Agave gigantea* can be cultivated in various tropical and warm regions around the world since it can withstand a quite wide range of temperatures (16 to 34 °C) [32]. Up to the present time, the usage of *Agave gigantea* fibres has progressed to another successive level especially to numerous engineering applications. In example, it is being used as reinforcement in polymer matrix composite in material engineering [30,32].

To the best of our knowledge, no study on *Agave gigantea* cellulose nanofibres using chemical-ultrafine grinding treatment has been found in the literature. Thus the aim of the current study was to extract and characterize cellulose nanofibre from *Agave gigantea* fibres. Cellulose

and cellulose nanofibre were extracted from *Agave gigantea* fibres by chemical and mechanical methods. The effect of chemical- ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, scanning electron microscope, X-ray diffraction, Fourier transform infrared and Thermal gravimetric analysis.

## 2. Materials and Methods

### 2.1. Materials

The cellulose fiber in this study was sourced from the leaves of the *Agave gigantea* plant. The leaves (AG) were obtained in the plantation area in Harau Kabupaten Limapuluh Kota, Sumatera Barat. Chemicals that were used in this experiment are sodium hydroxide (NaOH 98% Sigma-Aldrich), sodium chlorite ($NaClO_2$ from Pubchem), and glacial acetic acid ($CH_3COOH$).

### 2.2. Fiber Extract and Preparation of CNFs

The thorns on the edges of fresh AG leaves were cleaned and cut into 12-15 cm lengths, then soaked in boiling water at 100°C for 3 hours to facilitate fiber release from other extractive substances. After that, the outer skin of the fiber was removed with a knife. The AG fiber is then dried in the sun for four days with a moisture content of about 9 to 10%. Then the AG fiber was cut into 1-2 cm long and decomposed using a blender.

The chemical (alkalization, bleaching), and mechanical treatment (ultrafine grinding) were used to extract and isolate nanocellulose AG fiber. Lignin and hemicellulose AG fibers were removed by alkaline treatment of 5% (w/v) NaOH for 2 hours at 80°C on a hotplate. The brown-colored

fibers were washed until pH 7.0, then dried in an oven at 60°C until the moisture content was about 10%.

After alkalization, 64 grams of AG fiber was bleached using the hotplate. The solution for the bleaching process consisted of equal parts (v:v) acetic buffer (27 g NaOH and 75 mL glacial acetic acid, diluted to 1 L distilled water) and dilute sodium chlorite (1.7 wt% $NaClO_2$). The ratio of the amount of fiber to the solution is 1:25. This treatment was repeated twice for 1 hour at 80°C, producing white AG fibers [33]. The fibers resulting from the bleaching process are Cellulose Microfibers (CMF) AG. Furthermore, the CMF was a mechanical treatment using an Ultrafine grinding.

The fibers were first passed twice through an ultrafine grinding MKCA6-3 (Masuko Sangyo Co, Ltd., Japan) with an open gap (10 μm) for 1 minute to pre-dispersed the material, which make slurry fibers with 1% cellulose and 99% wt% water. Furthermore, the nanofibrillation was conducted in contact mode using rotational Speed at 1500 rpm with the gap of the two discs set to −30 μm for 40 passes. The process of extraction and isolation of cellulose nanofibers (CNFs) AG can be seen in Figure 1.

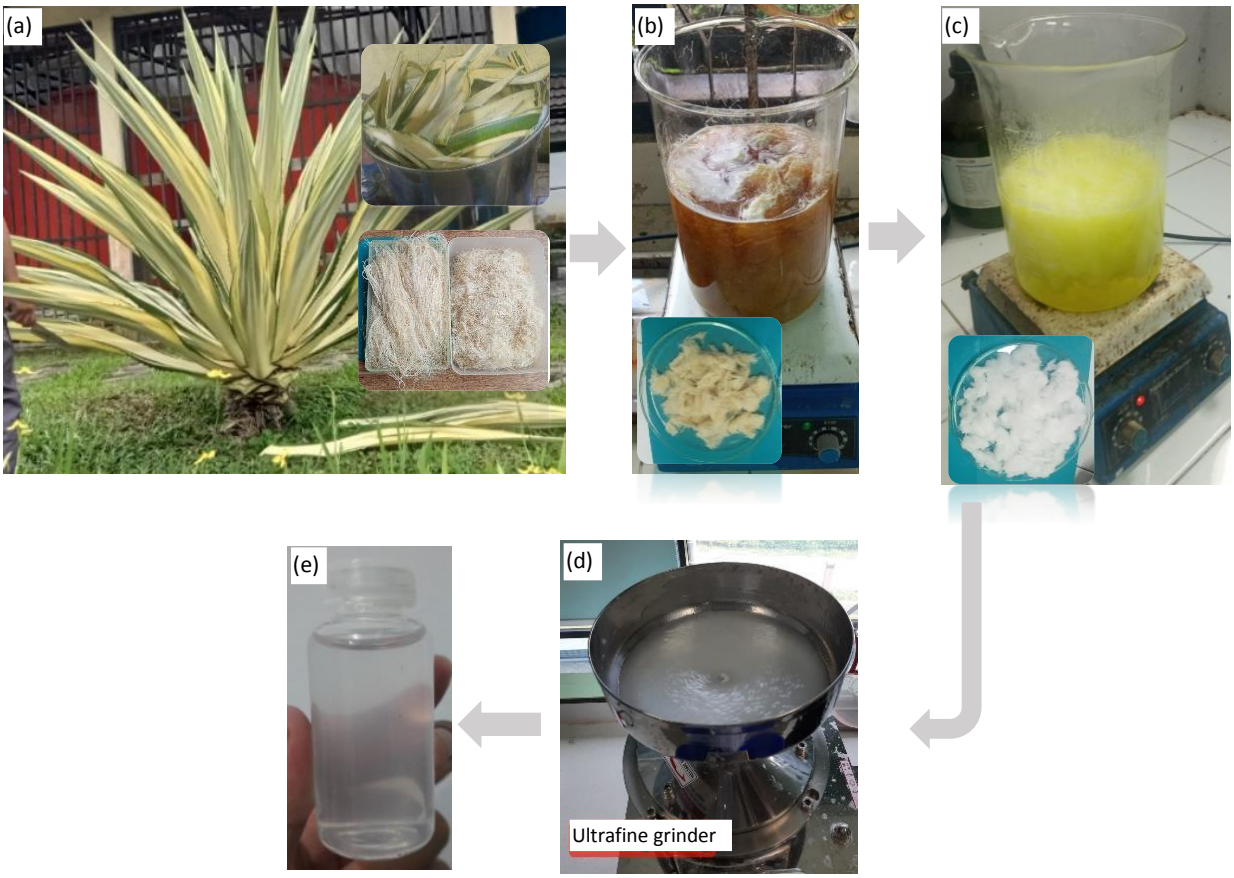



**Figure 1.** Leaves of AG fiber and AG fiber (a), Alkalization (b), bleaching (c), Ultrafine grinding process (d), CNFs AG (e)

## 2.3. Analysis of Chemical Composition

Chemical composition analysis based on the test method was developed by Van Soest. Natural fiber is composed of fiber soluble in neutral detergent (*Neutral Detergent Fiber*/NDF), fiber soluble in acid detergent (*Acid Detergent Fiber*/ADF), hemicellulose, cellulose, and lignin. The Van Soest method can determine cellulose, hemicellulose, and lignin content in the AG fiber.

## 2.4. Scanning Electron Microscopy (SEM)

The surface morphology of AG fiber cellulose was observed using Scanning Electron Microscopy (SEM), Model: S-3400N, Hitachi, Ltd., Japan, with a voltage of 20 kV and a current of 8 mA probe. The test sample is placed on the SEM sample stub. The sample preparation was previously coated with carbon and then further coated with gold to reduce the electron charge and to avoid over charging. SEM photos were enlarged to obtain image clarity.

### 2.5. X-ray Diffraction (XRD)

The crystallinity index of AG fibers before and after chemical treatment was measured using X-ray diffraction (XRD) technique using X'pert PROPANalytical (Model: PW3040/60) with Cu Kα radiation (λ = 0.1542 nm). The X-ray spectrum was recorded between 5° and 50° at 40 kV and 30 mA. The formula used to calculate the crystallinity index ($I_{cr}$) is:

$$CI = [(I_{002} - I_{am})/I_{002}] \times 100$$

Where I002 = Intensity for 2θ=22.3°, which indicates the crystal region. Iam is an amorphous region that is at Intensity 2θ=18° [34].

### 2.6. Fourier Transform Infrared (FTIR)

FTIR characterization was analyzed using PerkinElmer FTIR spectrometer (Frontier instrument, USA). This FTIR test helps identify functional groups from AG fibers before and after chemical treatment. Spectrum scans were recorded with 4 $cm^{-1}$ over a wavenumber range of 4000-600 $cm^{-1}$ [35].

### 2.7. Thermogravimetric Analysis (TGA)

Measurement of the thermal stability of AG fiber without treatment and after chemical treatment was carried out using the DTG-60 SHIMADZU (Kyoto, Japan). Thermal analysis was carried out in a nitrogen atmosphere at a flow rate of 50 mL/min. The heating rate was ten °C/min with a range temperature of 30-550°C.

### 2.8. Transmission Electron Microscopy (TEM)

TEM observations showed that nanocellulose after mechanical treatment (ultrafine grinding) showed a diameter range of 4.07 nm. The surface morphology of CNFs was observed using a JEM-1400 Transmission Electron Microscopy (JEOL Ltd., Japan) at a voltage of 100 keV. The cellulose nanofibers suspension was poured onto a carbon film over a copper network and then dried. Dry samples were observed via TEM at room temperature.

## 3. Results and Discussion

### 3.1. Chemical Composition of AG Fiber

The chemical composition of *Agave gigantea* fiber before and after being given alkalizing and bleaching treatment shows in Table 1. This analysis showed that the cellulose content increased by 20.4% after bleaching compared to raw AG fiber. In addition, the hemicellulose content decreased by 56-58% after being given chemical treatment. Alkalization treatment can modify the chemical content of the fiber by breaking the hydrogen bonds in the lignocellulosic structure, which can remove hemicellulose, pectin, wax, and lignin as the separation of fiber bundles in microfibrils [36–40]. The results also showed that the lignin content ranged from 0.37-0.53%. The lignin content in AG fiber is lower than other fibers such as *Cyrtostachys renda*

(18.77%) [38], *Imperata brasiliensis* (14.3%) [41], walnut shell (27.19%) [40], corncob (15.08%) [40], sugarcane bagasse (20.68%) [40], *Sonchus oleraceus* (17.3%) [39], and *Calotropis gigantea* (21.6%) [39]. The highest cellulose content was produced after the AG fiber was treated with bleaching with 1.7 wt% $NaClO_2$ with a cellulose content of 83.4% because chemical treatment can remove non-cellulosic components and amorphous components from AG fibers. This result was supported by the crystallinity index measurement of the fiber and was also supported by previous studies [38,42]. High cellulose content and low hemicellulose can increase the thermal stability of the fiber.

**Table 1.** Chemical Composition of AG Fiber

| Fiber Treatment | Cellulose (%) | Lignin (%) | Hemicellulose (%) |
|---|---|---|---|
| Raw AG Fiber | 74.22 | 0.37 | 8.47 |
| Alkalized AG Fiber | 88.54 | 0.41 | 3.54 |
| AG Fiber Bleaching | 89.39 | 0.53 | 3.73 |

### 3.2. SEM and TEM

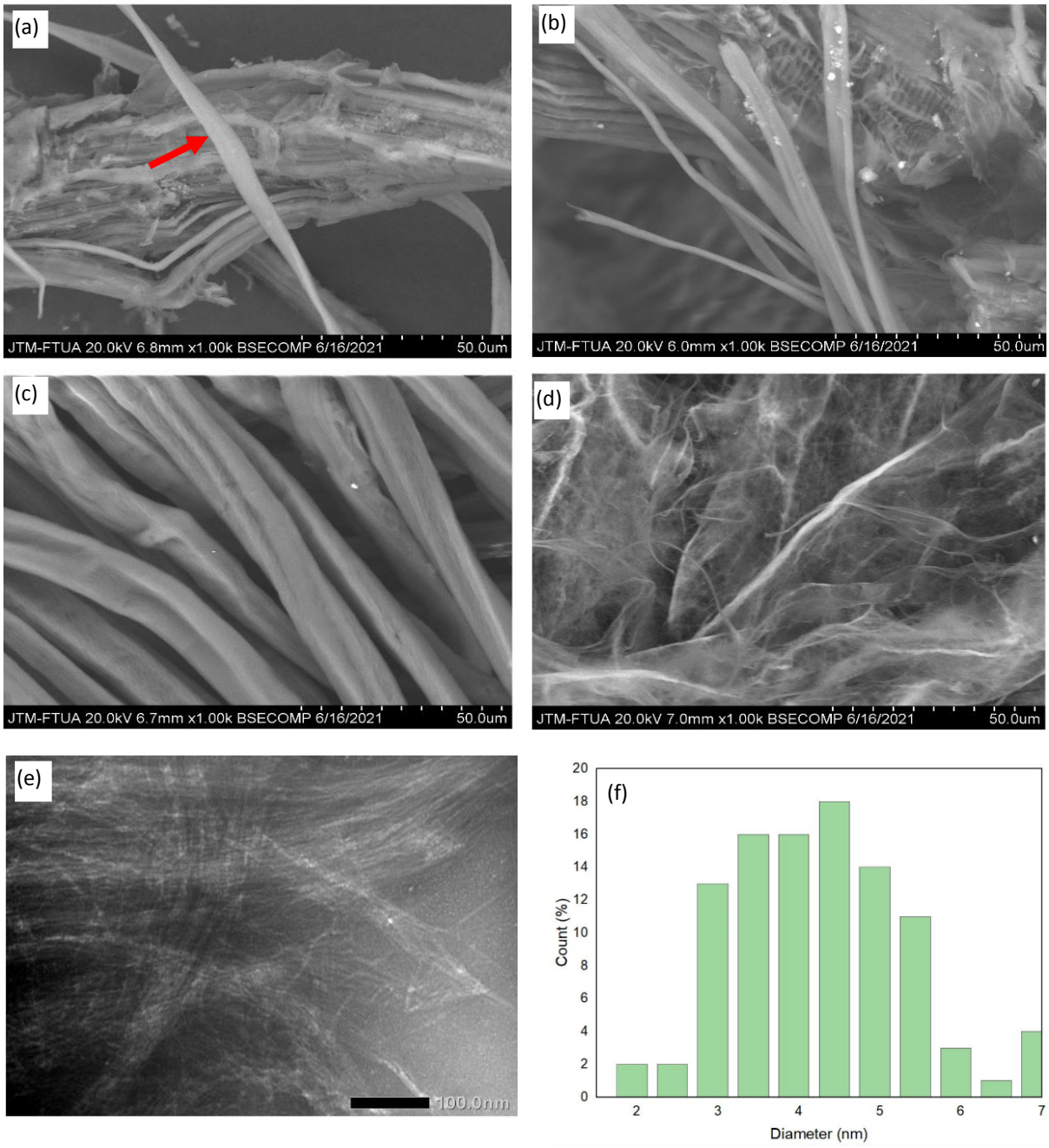



**Figure 2.** SEM micrographs of AG fiber raw AG fiber (a); Alkalization (b); Bleached (c); Ultrafine grinding (d); and TEM micrographs of CNFs AG (e); Size of CNFs AG (f).

Chemical treatment (alkalization and bleaching) and ultrafine grinding obtained cellulose and CNFs from AG fiber. Figure 2a-2d shows the surface morphology of cellulose with magnifications of 1000×. The red arrow indicates the fiber measurement by measuring the average diameter of the fiber. The surface morphology of cellulose from raw AG fiber through SEM micrographs (Figure 2a) shows the structure of long coarse fibril bundles with an average diameter of 50 µm. The rough surface was due to the presence of non-cellulose material. Figures 2b and 2c show that the surface morphology of the microfibril bundles was smooth, and the fiber diameter was smaller (10-15µm) than raw AG fiber due to chemical treatment which successfully removed hemicellulose, lignin, wax, pectin components, and impurities.

On the other hand, Figure 2d showed a smooth surface structure of the fibrils but different sizes. Mechanical treatment (ultrafine grinding) causes a change in the size of the cellulose into nano-dimensional cellulose fibers, which are also known as cellulose nanofibers (CNFs). The high shear force and intensity generated during the ultrafine grinding process cause the cellulose chains to break; the fiber bundles are crushed and split into smaller fibrils [43–45]. The obtained CNFs size proves that nano-dimensional cellulose fibers with diameters ranging from 10–100 nm can produce using the ultrafine grinding treatment. Mechanical treatment with ultrafine grinding significantly affects the fiber's morphology, crystallinity, and thermal stability [46,47].

Figure 2e shows the observations of TEM CNFs AG fibers after the ultrafine grinding treatment. These results indicate that nanocellulose appears as individual fibril-fibril with a diameter of 4.07 nm. This result is similar to the study results reported [47]. The ultrafine grinding treatment for 2.5 hours showed an average nanocellulose diameter of 15−20 nm [46]. In a previous study (Berglund et al., 2016), the ultrafine grinding treatment for 170 min was able to damage the cellulose chain, which resulted in the production of nano-sized cellulose fiber (5–30 nm) [44]. This

study showed significantly cellulose nanofiber production compared to the results reported in previous studies.

### 3.3. Functional Group Analysis

The spectrum of *Agave gigantea* using a chemical-mechanical treatment, intermediate, crystalline cellulose and cellulose nanofibers is shown in Figure 3. The differences during the conversion of macro to nano cellulose are: controlled by changes in the hydroxyl, carboxyl and related regions of the lignin structure [48]. In the FTIR spectrum of *Agave gigantea*, intermediates, crystalline cellulose and cellulose nanofibers (Figure 3), the peaks at 3328-3337 $cm^{-1}$ correspond to OH stretching vibrations in cellulose [49,50]. The intensification of these peaks presents an increase in the cellulose content and the removal of amorphous components increases the hydrogen bonds between the cellulose chains. Hernandez et al. (2018) obtained cellulose nanocrystals from corn straw, using alkaline treatment, bleaching, and acid hydrolysis [51]. These same authors claim that peak intensification between 3200-3500 $cm^{-1}$ is due to the removal of the lignin fraction and results in highly crystalline cellulose nanofibers. Bands at 2898–2923 $cm^{-1}$ are present in the AG spectrum, treated fibers, crystalline cellulose and cellulose nanofibers according to CH stretching vibrations [50] (Figure 3). The band at 1737 $cm^{-1}$ is present in the FTIR spectrum of raw AG (Figure 3), however, in the FTIR spectrum of alkali treatment and bleaching it is no longer present. This peak (1731 $cm^{-1}$) is associated with the C=O bond of unconjugated ketones present in hemicelluloses during chemical extraction [50]. These results can also indicate that alkali treatment is more efficient to remove hemicellulose in the fiber. In the band 1602–1642 $cm^{-1}$ is associated with the stretching structure of the aromatic lignin group [49][52]. Furthermore, in the band 1315 $cm^{-1}$ for bending vibration of $CH_2$ and OH groups, peaks at 1243 $cm^{-1}$, 1018 $cm^{-1}$, and 1030 $cm^{-1}$

for the stretching of C-O, asymmetric stretching of C-O-C, and oscillating vibration of CH of cellulose [53].

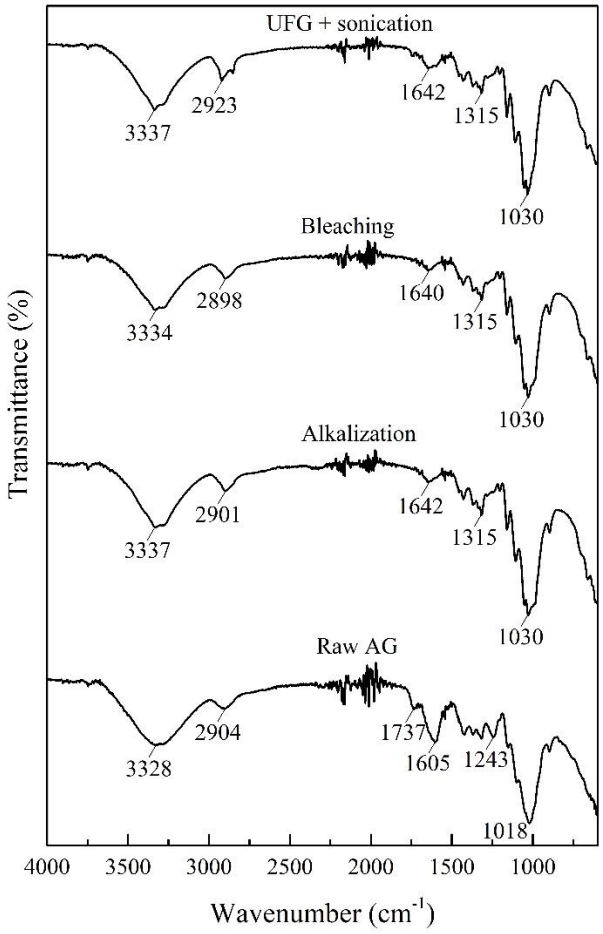



**Figure 3.** FTIR spectra of raw AG fiber; Alkalization, Bleaching, and Ultrafine grinding + sonication

### 3.4. Crystallinity Index Analysis

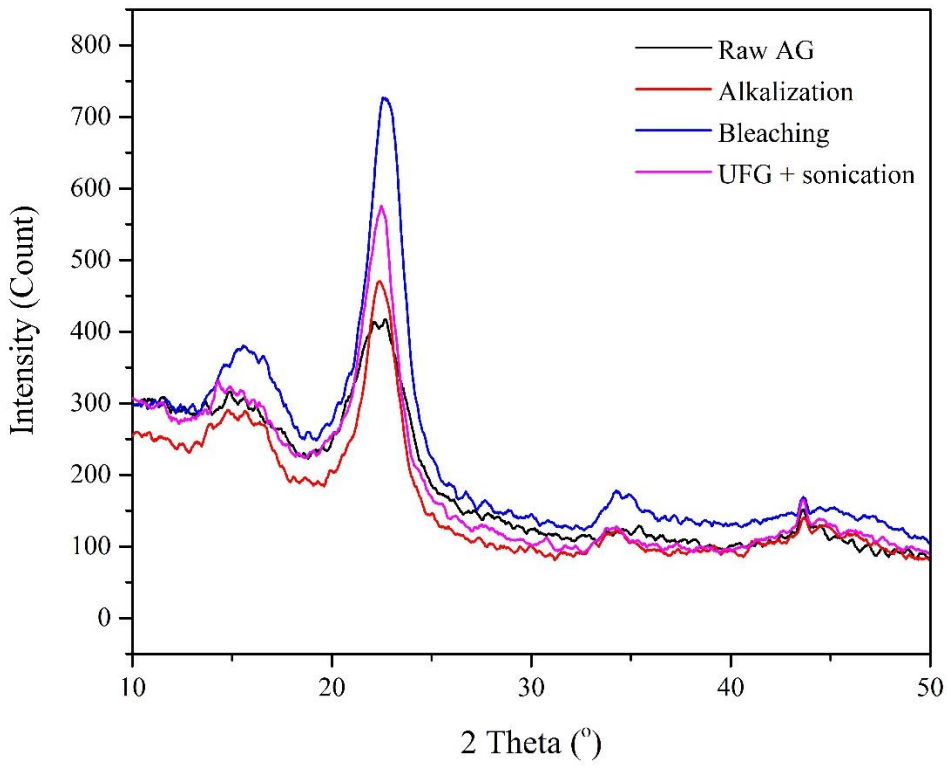


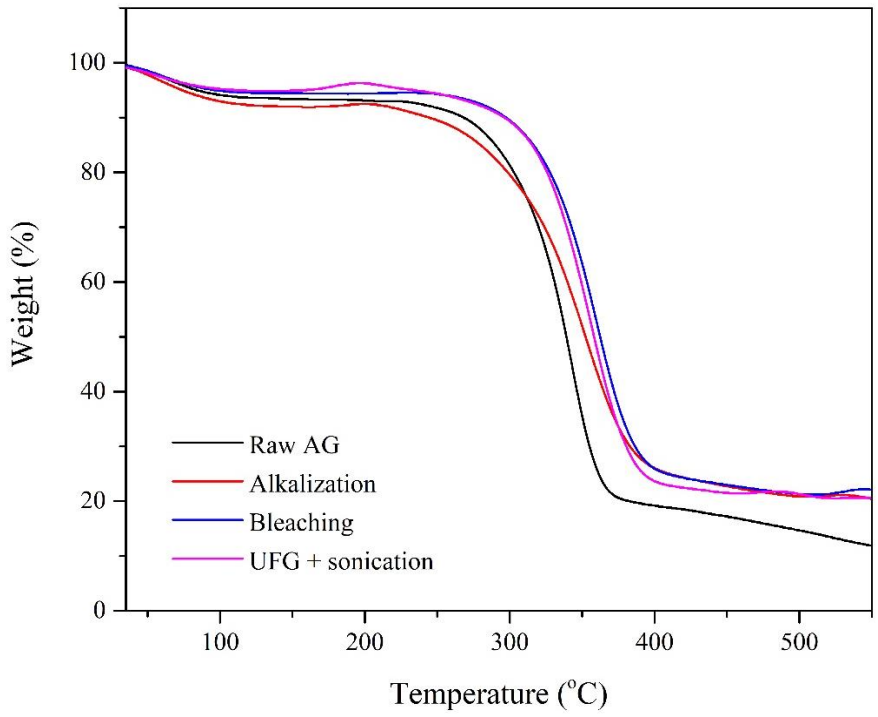

**Figure 4.** XRD curves of raw AG fiber; Alkalization, Bleaching, and Ultrafine grinding + sonication

**Table 2.** Crystallinity Index and $T_m$ of Raw AG Fiber, Alkalized AG Fiber, AG Fiber Bleaching and CNFs AG Fiber

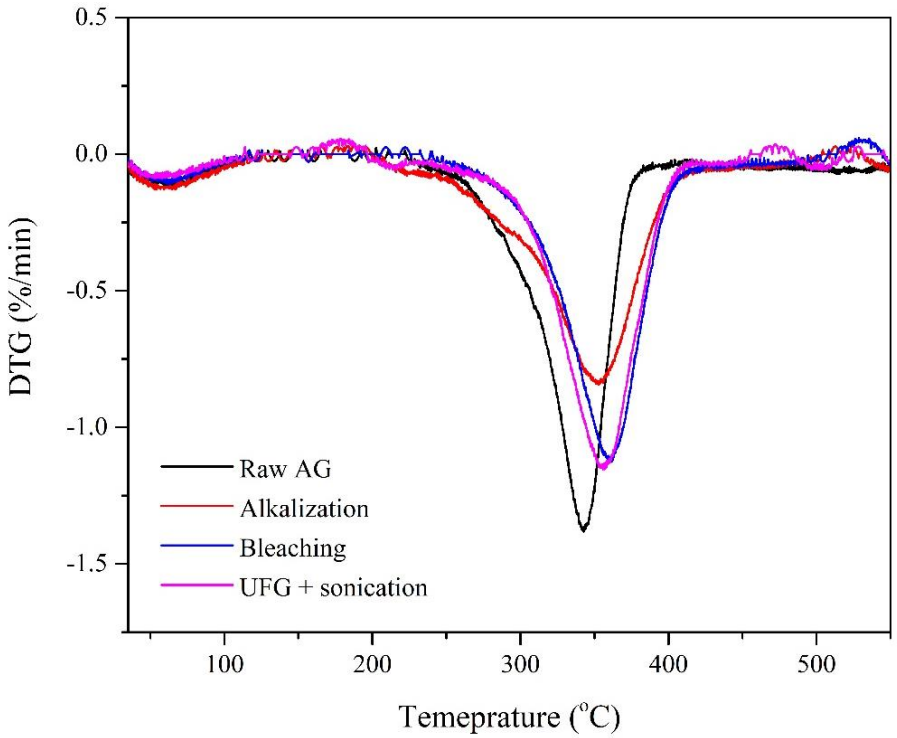
| **Fiber Treatment** | **CI (%)** | **$T_m$ (°C)** |
|---|---|---|
| Raw AG Fiber | 48.29 | 342.50 |
| Alkalized AG Fiber | 62.85 | 352.75 |
| AG Fiber Bleaching | 70.94 | 362.59 |
| CNFs AG Fiber | 65.21 | 355.91 |

XRD analysis is an essential parameter in seeing the effect of the crystallinity index of AG fiber before and after chemical and mechanical treatment. Figure 4 shows the XRD curve of raw AG fiber, fiber after alkalization, and bleaching treatment. Analysis of the XRD curve to determine the crystallinity index of AG fibers using the Segal method [34]. The results of the crystallinity index measurements show in Table 2. The X-ray diffraction pattern in Figure 4 shows the intensity of the diffraction peaks indicated by two theta angles of about 15.6°, 22.6°, and 34.2°, which indicates cellulose I [39,41,42,54,55]. All AG fibers before and after treatment had the same X-ray diffraction pattern, which showed the structure of cellulose fibers persisted after ultrafine grinding treatment. The crystallinity index (CI) for the raw AG sample is 48.29% (see Table 2). This result is higher than other natural fibers such as *Cyperus pangorei* (41%) [56], *Cissus quadrangularis* stem (47.15%) [57], and *Prosopis juliflora* (46%) [58]. After AG fiber received alkalizing treatment, the CI value increased by 30.2% compared to raw fiber. After the bleaching treatment, the maximum CI value was 70.94% because the bleaching process effectively removes amorphous components in AG fibers. Similar results were also shown by previous studies [59,60]. After mechanical treatment, the CI value decreased by 8.1% compared to AG fiber after bleaching due to the destruction of the cellulose chain resulting from mechanical treatment [44,61]. This result was supported by previous research [46,62].

### 3.5. Thermal Stability


Weight (%)
100
80
60
40
20
0
100
200
300
400
500
Temperature (°C)
Raw AG
Alkalization
Bleaching
UFG + sonication

a)


DTG (%/min)
0.5
0.0
-0.5
-1.0
-1.5
100
200
300
400
500
Temeprature (°C)
Raw AG
Alkalization
Bleaching
UFG + sonication

b)

**Figure 5.** TGA curve (a), DTG curve (b) of raw fibers, alkalized, bleaching, and hydrolyzed.

Thermal degradation analysis of AG fiber and nanocellulose using TGA and Difference Thermogravimetry (DTG) curves shows Figures 5a and 5b. The way to calculate thermal degradation is to measure the weight loss with temperature changes. The TGA curve shows three regions of fiber degradation temperature starting from the evaporation of moisture in the fiber at a temperature of 100-150°C, region two at a temperature of 250-350°C shows thermal degradation of cellulose and region three at a temperature of 400-450°C which shows the residual substance in the form of ash [33,37,63,64]. The maximum temperature ($T_m$) of each sample before and after chemical and mechanical treatment shows in Table 2. Raw AG fiber has a maximum temperature of 342.5°C. After AG fiber was given alkalizing treatment, the Tm of the fiber increased by 3% compared to raw AG fiber indicates an increase in the thermal stability of the fiber due to the increase in the crystal structure. This result is supported by the measurement of the crystallinity index (Table 2). The Tm of AG fiber that has undergone bleaching treatment is 362.7°C. This result is higher than previous studies such as Cyperus pangorei (324°C) [56], Thespesia populnea barks (323°C) [60], and Cardiospermum Halicababum (336°C) [55]. After mechanical treatment of ultrafine grinding, the Tm of nanocellulose was reduced by 1.8% due to the destruction of the crystalline structure of cellulose [61,62]. This result is supported by previous research [44,46].

## 4. Conclusion

This study aims to utilize AG fiber into nanocellulose by chemical and mechanical methods. AG fiber treated with bleaching for 2 hours showed the highest cellulose content by removing 56% hemicellulose. Mechanical treatment was successful in the production of nanocellulose with an average diameter of 4.07 nm. A crystallinity index (71%) was observed for

bleached AG fibers compared to untreated fibers (49%). The functional group present at 2898 $cm^{-1}$ in the treated AG fiber will increase the load-bearing ability and stiffness when reinforced with a polymer matrix. The bleached AG fiber showed the highest thermal stability (363°C) compared to the untreated fiber (343°C). Based on the findings in this study, it can conclude that among all parameters, the optimal chemical-mechanical treatment gave excellent properties in terms of cellulose purity and cellulose nanofiber production. Therefore, AG fiber treated with chemical-mechanical treatment can use as a new fiber reinforcement source for lightweight and environmentally friendly biocomposites.

**Acknowledgment**

The authors thank to Atmi Wirasti Colleagues from Department of Agricultural Technology, Agricultural Polytechnic, Payakumbuh, West Sumatra for for their contributions towards the successful completion of this research. This work was financially supported by the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia (PDUPT grant number: 102/E4.1/AK.PT/2021).

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# Author statement

All the authors have contribution in preparation of the manuscript. The first, second and third authors have original idea, conceptualization and methodology. The fourth and fifth authors did the data analysis and validation. The sixth author did organization of the manuscript including improvement language corrections and formal analysis to contributed in substantial revision, editing, review and improvement of the first draft of the manuscript.

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# Isolation and Characterization of Cellulose Nanofibers from *Agave gigantea* by Chemical-Mechanical Treatment

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**Abstract**

Nanocellulose is a renewable and biocompatible nanomaterial that evokes much interest because of its versatility in various applications. This study reports the production of nanocellulose from *Agave gigantea* (AG) fiber using the chemical-ultrafine grinding treatment. Chemical treatment (alkalization and bleaching) removed non-cellulose components (hemicellulose and lignin), while ultrafine grinding reduced the size of cellulose microfibrils into nanocellulose. From the observation of Transmission Electron Microscopy, the average diameter of nanocellulose was 4.07 nm. The effect of chemical-ultrafine grinding on the morphology and properties of AG fiber was identified using chemical composition, Scanning Electron Microscopy, X-ray Diffraction, Fourier Transform Infrared, and Thermogravimetric analysis. The bleaching treatment increased the crystal index by 48.3% compared to raw AG fiber, along with an increase in the cellulose content of 20.4%. The ultrafine grinding process caused a decrease in the crystal content of the AG fiber. The crystal index affected the thermal stability of the AG fiber. The TGA results showed that AG fiber treated with bleaching showed the highest thermal stability compared to AG fiber without treatment. The FTIR analysis showed that the presence of C-H vibrations from the ether in the fiber. After chemical treatment, the peaks at 1605 and 1243 $cm^{-1}$ disappeared, indicating the loss of lignin and hemicellulose functional groups in AG fiber. As a result, nanocellulose derived from AG fiber can be applied as reinforcement in environmentally friendly polymer biocomposites.

**Keywords:** Nanocellulose, *Agave gigantea*, Chemical-Ultrafine Grinding, Thermal stability

## 1. Introduction

Cellulose nanofibers isolated from plant fibers have attracted huge interest in material science due to their appealing intrinsic properties, including nano-dimension, high surface area (100 $m^2$ $g^{-1}$) [1–3], high aspect ratio of 100 [4,5], high crystallinity [6], low density, high mechanical strength, unique morphology along with availability, renewability, and biodegradability [7–9]. Cellulose is the product of biosynthesis from bacteria and plants, whereas the general term "cellulose nanofibres" refers to cellulosic isolation or extraction materials, with the outstanding feature of nano-scale structural dimension. The main component of plant fibers is cellulose, a semicrystalline polymer composed of poly(1,4-β-D-anhydroglucopyranose) units. These units are formed from a strong hydrogen bond between hydroxyl groups. Other main components that make up natural fibers' structure are lignin and hemicellulose. Lignin is a highly cross-linked phenolic polymer, whereas hemicellulose is a branched multiple polysaccharide polymer composed of different types of sugars comprising xylose, glucose, arabinose, mannose, and galactose. However, both lignin and hemicellulose are amorphous polymers.

In the past decades, many different resources have been used to prepare cellulose nanofiber, such as cassava bagasse [10], wheat straw [11,12], cotton cellulose [13], softwood wood [14], rice straw [15], kenaf [16], bamboo fiber [17], sugar palm fiber [18–24], ginger [25,26], water hyacinth [27], and sugarcane bagasse [28]. Table 1 shows the isolation of nanocellulose using several natural fibres. The purpose of the isolation of cellulose nanofiber is as reinforcement in the nanocomposite field that has gained tremendous attention since it was first examined by Favier et al. [29]. However, no studies on the production, composition, or properties of natural cellulose

nanofibers from *Agave gigantea* fibers using chemical-ultrafine grinding treatment have been found in the literature.

Table 1: Isolation of nanocellulose from natural fiber using various treatments

| Natural fiber | Nanocellulose preparation | Ref. |
|---|---|---|
| Cassava bagasse | Hydrolyzed in 6.5 M $H_2SO_4$ / 40 min | [10] |
| Wheat straw | High Pressurize Homogenizer/ 15 min | [12] |
| Cotton cellulose | Hydrolyzed in 6.5M sulfuric acid/ 75 min | [13] |
| Softwood wood flour | Super masscolloider | [14] |
| Rice straw | Ultrasonication | [15] |
| Kenaf | Super masscolloider | [30] |
| Kenaf | Super masscolloider | [31] |
| Sugar palm fibre | High Pressurize Homogenizer, 500 bar | [19,21] |
| Tunicin | 55 wt % $H_2SO_4$ / 20 mins | [32,33] |
| Waxy maize starch | $H_2SO_4$ / 5 days | [34,35] |
| Cottonseed linter | 64 wt % H2SO4 / 4 h | [36] |
| Ramie | 64 wt % H2SO4 / 4 h | [37] |
| Hemp | 64 wt % $H_2SO_4$ / 4 h | [38] |
| Flax | 64 wt % $H_2SO_4$ / 4 h | [39] |
| Bamboo | 50 wt % $H_2SO_4$ / 48 h | [40] |
| Potato peel waste | 64 wt % $H_2SO_4$ / 90 mins | [41] |
| Cotton cellulose powders | $H_2SO_4$ | [42] |
| Sugarcane bagasse | 64 wt % $H_2SO_4$/ 3 h | [43] |
| Cotton linter | 64 wt % $H_2SO_4$ / 1 h | [44] |
| Sugar palm fibre | 60 wt% $H_2SO_4$ / 45 min | [45–47] |
| *Agave gigantea* | Ultrafine grinding | Current study |

*Agave gigantea*, is the family member of Agavaceae, which contain approximately similar properties like (physical and mechanical) of sisal (*Agave sisalana*). *Agave gigantea* is a Central American native non-wood biomass whose leaves have been used as a source of fiber for centuries. Traditionally, *Agave gigantea* fibers are extracted using the water retting technique and scorching machines, and subsequently used to make ropes and bags [19]. A study conducted by Kumar Singh et al. [48] showed that the cellulosic fiber content of *Agave gigantea* fiber of 55-70%, which was higher than that of wood, having values ranging from 40–50% [49]. In the same study, it was also

demonstrated that the lignin content of *Green Agave americana* fiber was 3±0.3% [50], which was lower than that of wood (30%) [49]. Besides that, *Agave gigantea* fiber gives a competitive edge over other types of non-wood biomass like bagasse derived from corn or sugarcane, a crop that demands a certain level of care for adequate growth. Moreover, *Agave gigantea* can be cultivated in various tropical and warm regions worldwide since it can withstand a quite wide range of temperatures (16 to 34 °C) [51]. Up to the present time, the usage of *Agave gigantea* fibers has progressed to another successive level, especially to numerous engineering applications. For example, it is being used as reinforcement in polymer matrix composite in material engineering [48,51].

To the best of our knowledge, no study on *Agave gigantea* cellulose nanofibers using chemical-ultrafine grinding treatment followed by ultrasonication has been found in the literature. Therefore, the aim of the current study is to extract and characterize cellulose nanofiber from *Agave gigantea* fibers. Cellulose and cellulose nanofiber were extracted from *Agave gigantea* fibers by chemical and mechanical methods. The effect of chemical-ultrafine grinding on the morphology and properties of AG fibers was identified using chemical composition, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and Thermogravimetric Analysis (TGA).

## 2. Materials and Methods

### 2.1. Materials

The cellulose fiber in this study was sourced from the leaves of the *Agave gigantea* plant. The leaves (AG) were obtained in the plantation area in Harau District, Limapuluh Kota Regency,

West Sumatera Province. Chemicals used in this experiment were sodium hydroxide (NaOH 98% Sigma-Aldrich), sodium chlorite ($NaClO_2$ Sigma-Aldrich), and glacial acetic acid ($CH_3COOH$).

### 2.2. Fiber Extract and Preparation of CNFs

The thorns on the edges of fresh AG leaves were cleaned and cut into 120-150 mm lengths, then soaked in boiling water at 100°C for 3 h to facilitate fiber release from other extractive substances. After that, the outer skin of the fiber was removed with a knife. The AG fiber was then dried in the sun for 4 days with a moisture content of about 9 to 10%. Then, the AG fiber was cut into 10-20 mm long and crushed using a blender.

The chemical (alkalization, bleaching) and mechanical treatment (ultrafine grinding) were used to extract and isolate nanocellulose AG fiber. Lignin and hemicellulose of AG fibers were removed by alkaline treatment of 5% (w/v) NaOH for 2 h at 80°C on a hotplate. The brown-colored fibers were washed until pH 7.0, then dried in an oven at 60°C for 14 h until the moisture content was about 10%.

After alkalization, 64 g of AG fiber was bleached using the hotplate. The solution for the bleaching process consisted of equal parts (v:v) acetic buffer (27 g NaOH and 75 mL glacial acetic acid, diluted to 1 L distilled water) and dilute sodium chlorite (1.7 wt% $NaClO_2$). The ratio of the amount of fiber to the solution was 1:25. This treatment was repeated twice for 1 h at 80 °C, producing white AG fibers [52]. The fibers resulting from the bleaching process were Cellulose Microfibers (CMF) AG. Furthermore, the CMF was a mechanically treat using an Ultrafine grinding. For the next treatment, 1 g of CNFs 1% was added and sonicated at 80% power output for 60 min using a 40 kHz  Sonic Ruptor 400 with a tip diameter of 13 mm. The ultra-sonication treatment was

conducted at room temperature. At the end of the ultrasonication treatment, the CNF suspension turned from turbid white to transparent.

The fibers were first passed twice through an ultrafine grinding MKCA6-3 (Masuko Sangyo Co, Ltd., Japan) with an open gap (10 µm) for 1 min to pre-dispersed the material, which make slurry fibers with 1% cellulose and 99% wt% water. Furthermore, the nanofibrillation was conducted in contact mode using rotational speed at 1500 rpm with the gap of the two discs set to −30 µm for 40 passes. The process of extraction and isolation of cellulose nanofibers (CNFs) AG can be observed in Figure 1.

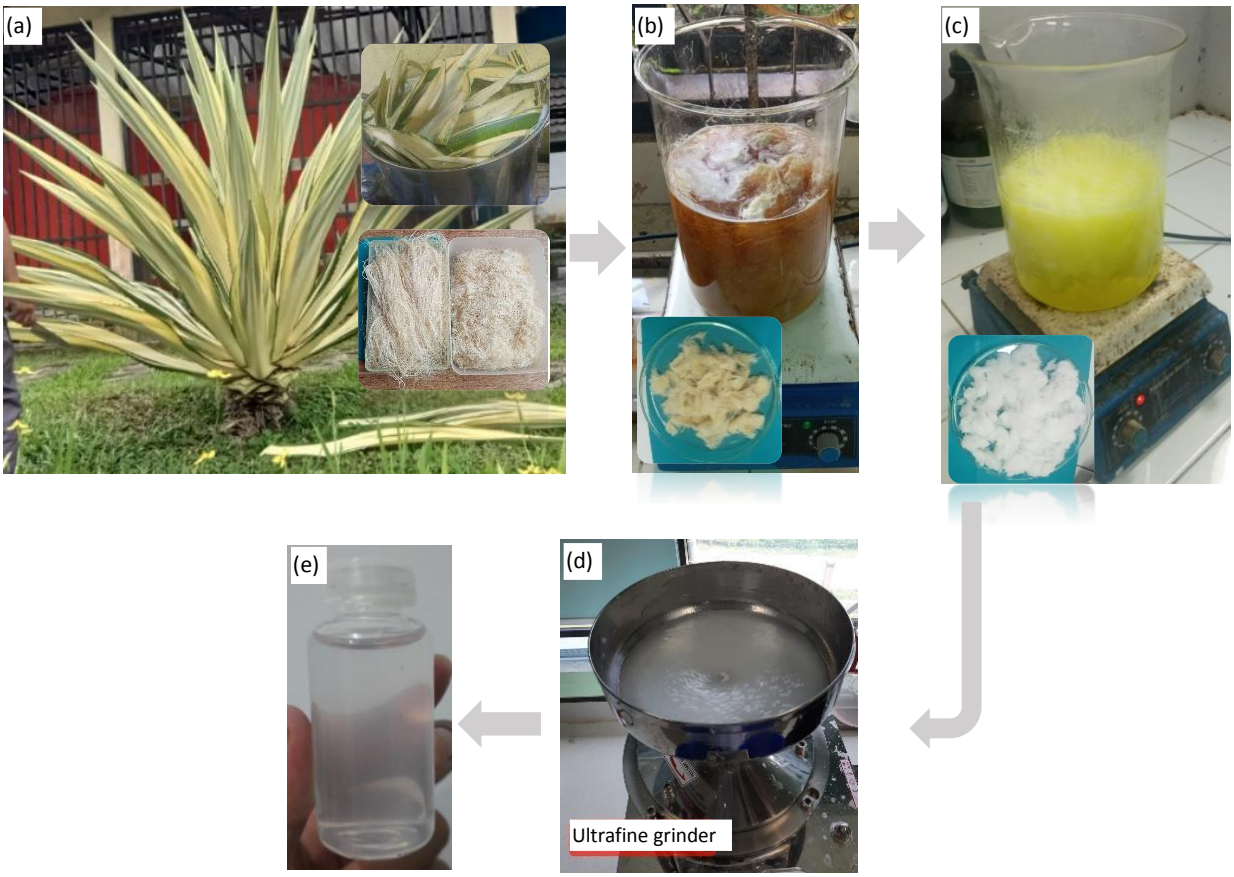


**Figure 1.** (a) Leaves of AG fiber and AG fiber, (b) Alkalization, (c)Bleaching, (d)Ultrafine grinding process, (e) CNFs AG

### 2.3. Analysis of Chemical Composition

The chemical composition of AG fiber was determined using the method developed by Van Soest to determine the cellulose, hemicellulose, and lignin content in AG fiber [53]. The natural fiber is composed of fiber soluble in neutral detergent (*Neutral Detergent Fibres* /NDF), fiber soluble in acid detergent (*Acid Detergent Fibres*/ADF), hemicellulose, cellulose, and lignin. The Van Soest method can determine cellulose, hemicellulose, and lignin content in the AG fiber.

### 2.4. Scanning Electron Microscopy (SEM)

The surface morphology of AG fiber cellulose was observed using Scanning Electron Microscopy (SEM), Model: S-3400N, Hitachi, Ltd., Japan, with a voltage of 20 kV and a current of 8 mA probe. The test sample was placed on the SEM sample stub. The prepared sample was previously coated with carbon and then further coated with gold to reduce the electron charge and to avoid overcharging. SEM images were enlarged to obtain image clarity.

### 2.5. X-ray Diffraction (XRD)

The crystallinity index of AG fibers before and after chemical treatment was measured using X-ray Diffraction (XRD) technique using X'pert PROPANalytical (Model: PW3040/60) with Cu Kα radiation ($\lambda = 0.1542$ nm). The X-ray spectrum was recorded between 5 and 50° at 40 kV and 30 mA. The formula used to calculate the crystallinity index ($I_{cr}$) is:

$CI = [(I_{002} - I_{am})/I_{002}] \times 100$

Where $I_{002}$ = Intensity for $2\theta=22.3°$, which indicates the crystal region. $I_{am}$ is an amorphous region that is at Intensity $2\theta=18°$ [54].

### 2.6. Fourier Transform Infrared (FTIR)

FTIR characterization was analyzed using a PerkinElmer FTIR spectrometer (Frontier instrument, USA). This FTIR test helped to identify functional groups from AG fibers before and after chemical treatment. Spectrum scans were recorded with 4 $cm^{-1}$ over a wavenumber range of 4000-600 $cm^{-1}$ [55].

### 2.7. Thermogravimetric Analysis (TGA)

Measurement of the thermal stability of AG fiber without treatment and after chemical treatment was carried out using the DTG-60 SHIMADZU (Kyoto, Japan) in a nitrogen atmosphere at a flow rate of 50 mL/min. The heating rate was 10 °C/min with a range temperature of 30-550 °C.

### 2.8. Transmission Electron Microscopy (TEM)

TEM observation was performed to nanocellulose after mechanical treatment (ultrafine grinding and ultrasonication). The surface morphology of CNFs was observed using a JEM-1400 Transmission Electron Microscopy (JEOL Ltd., Japan) at a voltage of 100 kV. The cellulose

nanofibers suspension was poured onto a carbon film over a copper network and then dried. Dry samples were observed under TEM at room temperature.

## 3. Results and Discussion

### 3.1. Chemical Composition of AG Fiber

The chemical compositions of *Agave gigantea* fiber before and after being given alkalizing and bleaching treatment are shown in Table 1. This analysis revealed that the cellulose content increased by 20.4% after bleaching compared to raw AG fiber. In addition, the hemicellulose content decreased by 56-58% after being given chemical treatment. Alkalization treatment can modify the chemical content of the fiber by breaking the hydrogen bonds in the lignocellulosic structure, which can remove hemicellulose, pectin, wax, and lignin as the separation of fiber bundles in microfibrils takes place [56–60]. The results also showed that the lignin content ranged from 0.37-0.53%, which was lower than other fibers such as *Cyrtostachys renda* (18.77%) [58], *Imperata brasiliensis* (14.3%) [61], walnut shell (27.19%) [60], corncob (15.08%) [60], sugarcane bagasse (20.68%) [60], *Sonchus oleraceus* (17.3%) [59], and *Calotropis gigantea* (21.6%) [59]. The highest cellulose content was produced after the AG fiber was bleached with 1.7 wt% $NaClO_2$ with a cellulose content of 83.4% because chemical treatment can remove non-cellulosic and amorphous components from AG fibers. This result was supported by the crystallinity index measurement of the fiber and was also supported by previous studies [58,62]. High cellulose content and low hemicellulose could increase the thermal stability of the fiber.

**Table 1.** Chemical Composition of AG Fiber.

| Fiber Treatment | Cellulose (%) | Lignin (%) | Hemicellulose (%) |
|---|---|---|---|
| Raw AG Fiber | 74.22 | 0.37 | 8.47 |
| Alkalized AG Fiber | 88.54 | 0.41 | 3.54 |
| AG Fiber Bleaching | 89.39 | 0.53 | 3.73 |

### 3.2. SEM and TEM

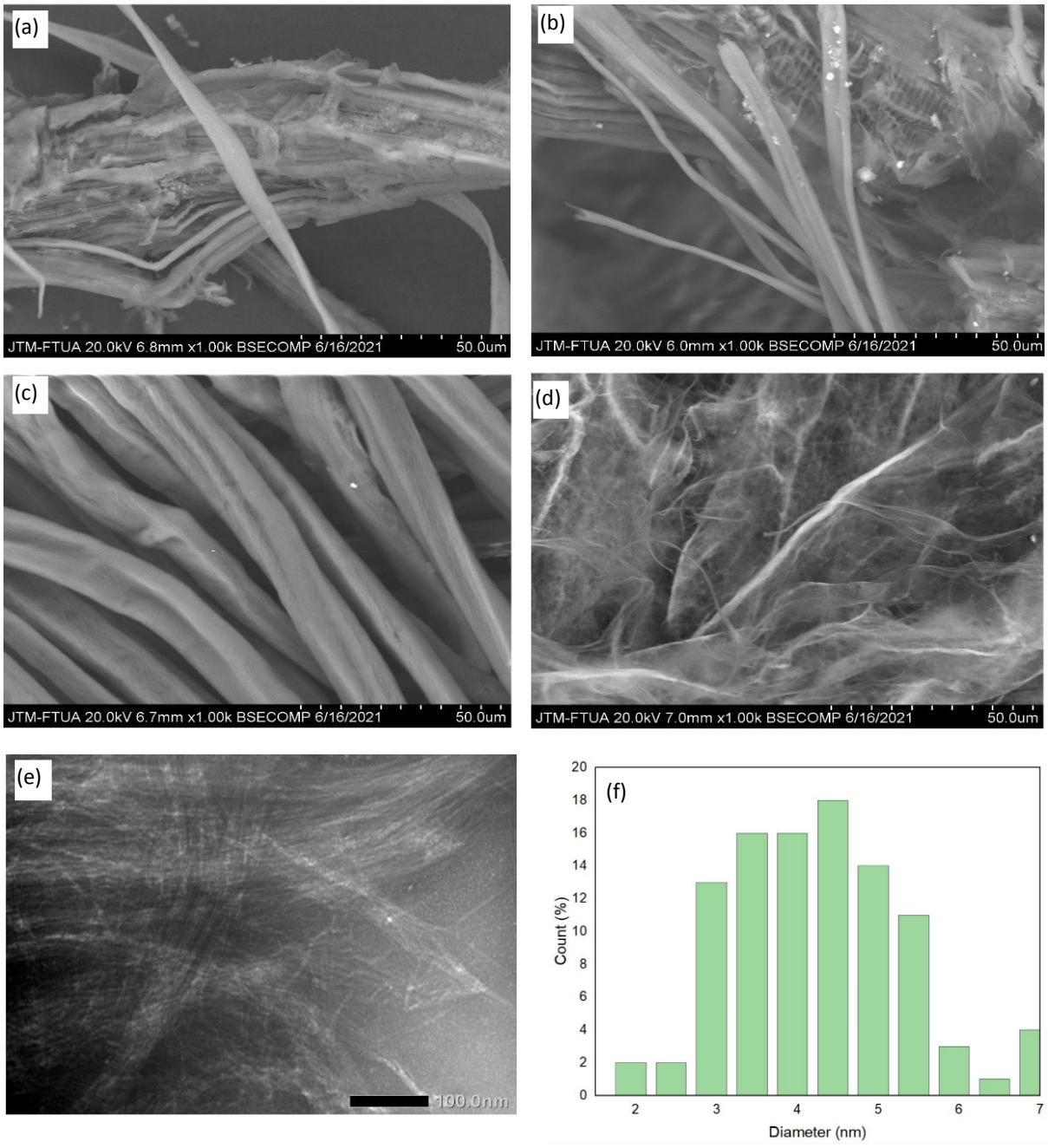



**Figure 2.** SEM micrographs of AG fiber raw AG fiber (a); Alkalization (b); Bleached (c); Ultrafine grinding (d); and TEM micrographs of CNFs AG (e); and Size of CNFs AG (f).

Chemical treatments (alkalization and bleaching) and ultrafine grinding yielded cellulose and CNFs from AG fiber. Figure 2a-2d presents the surface morphology of cellulose with the magnification of 1000×. The red arrow indicates the fiber measurement by measuring the average diameter of the fiber. The surface morphology of cellulose from raw AG fiber through SEM micrographs (Figure 2a) demonstrated the structure of long coarse fibril bundles with an average diameter of 50 µm. The rough surface was due to the presence of non-cellulose material. Figures 2b and 2c show that the surface morphology of the microfibril bundles was smooth, and the fiber diameter was smaller (10-15µm) than raw AG fiber due to chemical treatment which successfully removed hemicellulose, lignin, wax, pectin components, and impurities.

On the other hand, Figure 2d showed a smooth surface structure of the fibrils but different sizes. Mechanical treatment (ultrafine grinding) caused a change in the size of the cellulose into nano-dimensional cellulose fibers, which are also known as cellulose nanofibers (CNFs). The high shear force and intensity generated during the ultrafine grinding process caused the cellulose chains to break; the fiber bundles were crushed and split into smaller fibrils [63–65]. The obtained CNFs size proved that nano-dimensional cellulose fibers with diameters ranging from 10–100 nm could be produced using the ultrafine grinding treatment. Mechanical treatment with ultrafine grinding significantly affected the fiber's morphology, crystallinity, and thermal stability [66,67].

Figure 2e displays the TEM observations of CNFs' AG fibers after the ultrafine grinding treatment. These results indicated that nanocellulose appeared as individual fibril-fibril with a diameter of 4.07 nm. This result was similar to the findings reported in [67]. The ultrafine grinding treatment for 2.5 h yielded an average nanocellulose diameter of 15−20 nm [66]. In a previous study (Berglund et al., 2016), the ultrafine grinding treatment for 170 min was able to damage the cellulose chain, which resulted in the production of nano-sized cellulose fiber (5–30 nm) [64]. This

study showed significant cellulose nanofiber production compared to the results reported in other previous studies.

### 3.3. Functional Group Analysis

The spectra of *Agave gigantea* using a chemical-mechanical treatment, intermediate, crystalline cellulose, and cellulose nanofibers are shown in Figure 3. The differences during the conversion of macro to nano cellulose are: controlled by changes in the hydroxyl, carboxyl, and related regions of the lignin structure [68]. In the FTIR spectrum of *Agave gigantea*, intermediates, crystalline cellulose, and cellulose nanofibers (Figure 3), the peaks at 3328-3337 $cm^{-1}$ corresponded to OH stretching vibrations in cellulose [47,69]. The intensification of these peaks presented an increase in the cellulose content and the removal of amorphous components increased the hydrogen bonds between the cellulose chains. Hernandez et al. (2018) obtained cellulose nanocrystals from corn straw using an alkaline treatment, bleaching, and acid hydrolysis [70]. These same authors claimed that peak intensification between 3200-3500 $cm^{-1}$ was due to the removal of the lignin fraction and resulted in highly crystalline cellulose nanofibers. Bands at 2898–2923 $cm^{-1}$ were present in the AG, treated fibers, crystalline cellulose, and cellulose nanofibers spectra according to CH stretching vibrations [69] (Figure 3). The band at 1737 $cm^{-1}$ was present in the FTIR spectrum of raw AG (Figure 3), however, in the FTIR spectrum of alkali treatment and bleaching, it was no longer present. This peak (1731 $cm^{-1}$) was associated with the C=O bond of unconjugated ketones present in hemicellulose during chemical extraction [69]. These results could also indicate that alkali treatment was more efficient in removing hemicellulose in the fiber. The band at 1602–1642 $cm^{-1}$ was associated with the stretching structure of the aromatic lignin group [47,71]. Furthermore, the band at 1315 $cm^{-1}$ was ascribed

to the bending vibration of $CH_2$ and OH groups. Meanwhile, the peaks at 1243 $cm^{-1}$, 1018 $cm^{-1}$, and 1030 $cm^{-1}$ were associated with C-O stretching, asymmetric stretching of C-O-C, and oscillating vibration of C-H in cellulose [72].

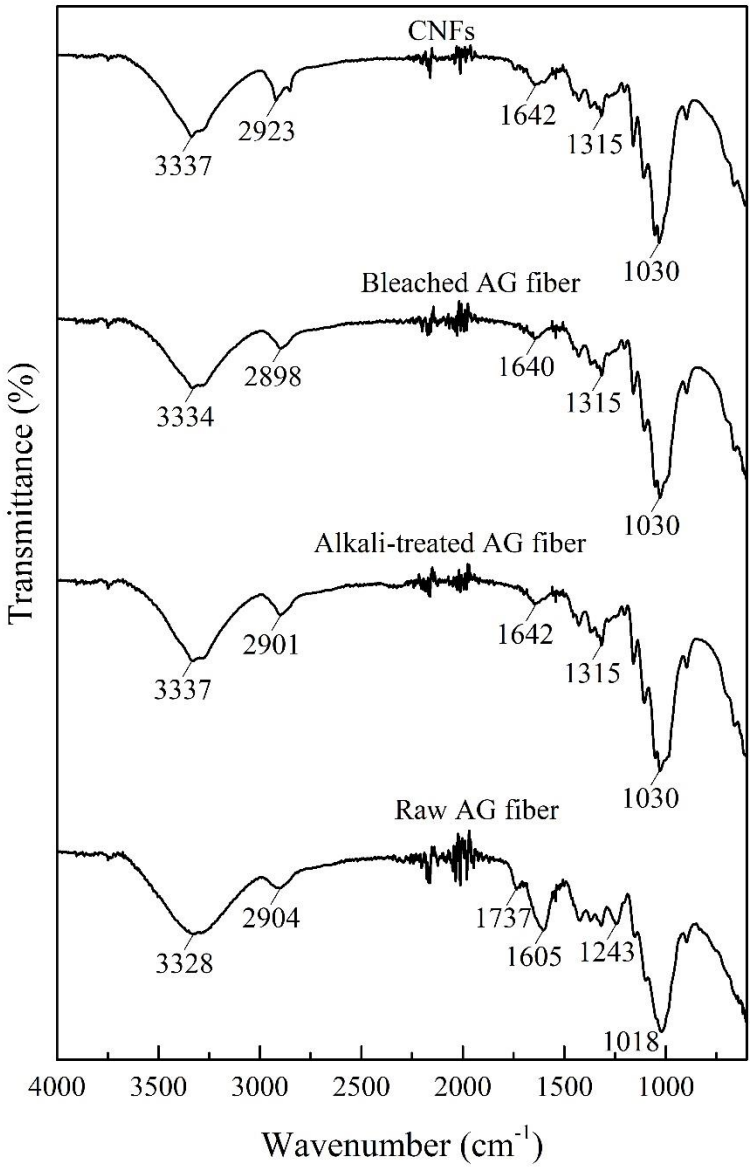



**Figure 3.** FTIR spectra of raw AG fiber; Alkalization, Bleaching, and Mechanical treatment.

### 3.4. Crystallinity Index Analysis

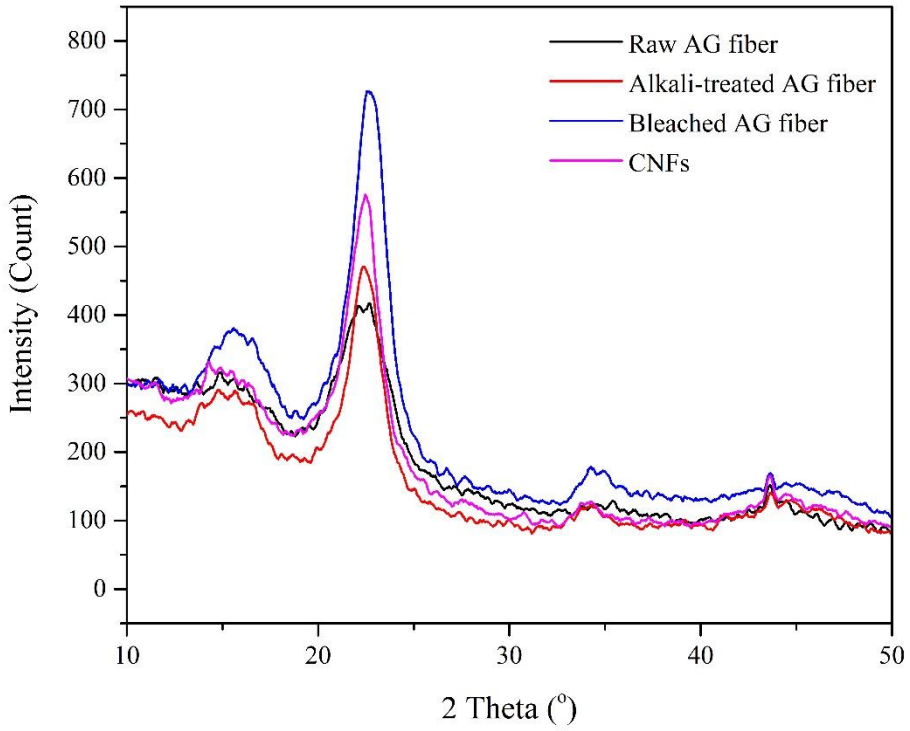



**Figure 4.** XRD curves of raw AG fiber; Alkalization, Bleaching, and Mechanical treatment.

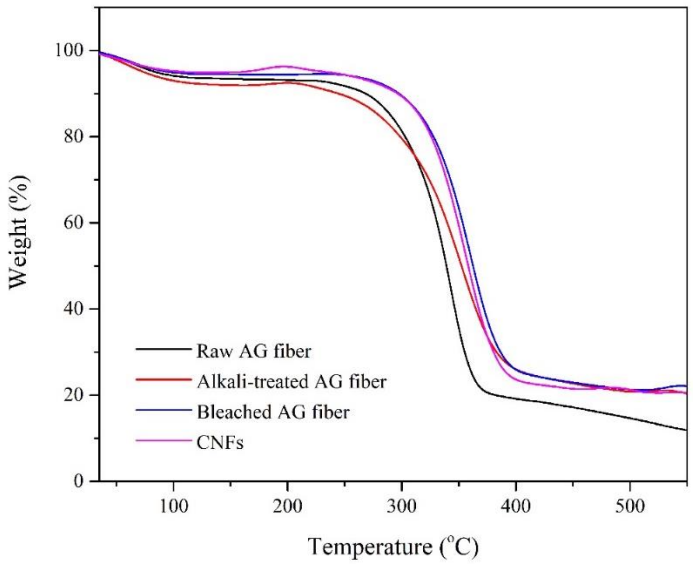
**Table 2.** Crystallinity Index and $T_m$ of Raw AG Fiber, Alkalized AG Fiber, AG Fiber Bleaching and CNFs AG Fiber.

| Fiber Treatment | CI (%) | $T_m$ (°C) |
|---|---|---|
| Raw AG Fiber | 48.29 | 342.50 |
| Alkalized AG Fiber | 62.85 | 352.75 |

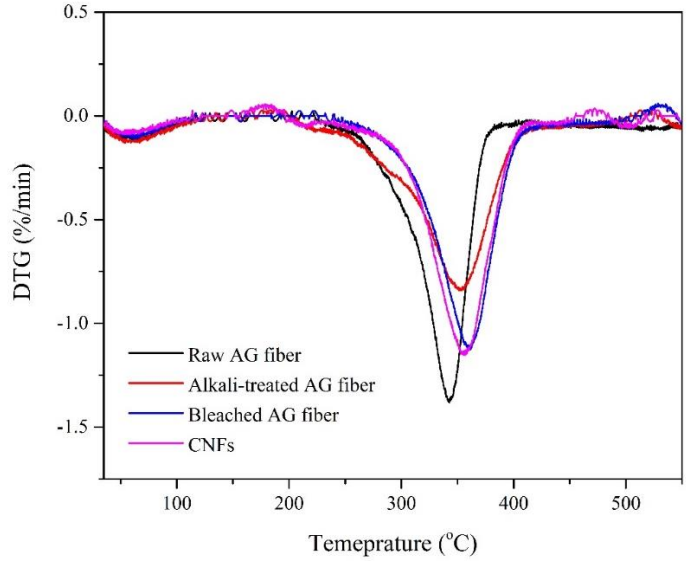
| | | |
|---|---|---|
| AG Fiber Bleaching | 70.94 | 362.59 |
| CNFs AG Fiber | 65.21 | 355.91 |

XRD analysis is an essential parameter in observing the effect of the crystallinity index of AG fiber before and after chemical and mechanical treatment. Figure 4 shows the XRD curve of raw AG fiber and fiber after alkalization and bleaching treatment. Analysis of the XRD curve to determine the crystallinity index of AG fibers was conducted using the Segal method [54]. The results of the crystallinity index measurements are shown in Table 2. The X-ray diffraction pattern in Figure 4 shows the intensity of the diffraction peaks indicated by two theta angles of about 15.6, 22.6, and 34.2°, indicating cellulose I [59,61,62,73,74]. All AG fibers before and after treatment demonstrated the same X-ray diffraction pattern, which showed the structure of cellulose fibers persisted after ultrafine grinding treatment. The crystallinity index (CI) for the raw AG sample was 48.29% (see Table 2). This result was higher than other natural fibers such as *Cyperus pangorei* (41%) [75], *Cissus quadrangularis* stem (47.15%) [76], and *Prosopis juliflora* (46%) [77]. After AG fiber received alkalizing treatment, the CI value increased by 30.2% compared to raw fiber. After the bleaching treatment, the maximum CI value was 70.94% because the bleaching process effectively removed amorphous components in AG fibers. Similar results were also shown by previous studies [78,79]. After mechanical treatment, the CI value decreased by 8.1% compared to AG fiber after bleaching due to the destruction of the cellulose chain resulting from mechanical treatment [64,80]. This result was supported by previous researches [66,81].

### 3.5. Thermal Stability




a)




b)

**Figure 5.** TGA curve (a), DTG curve (b) of raw fibers, alkalized, bleaching, and mechanical treatment.

Thermal degradation analyses of AG fiber and nanocellulose using thermogravimetric (TG) and difference thermogravimetry (DTG) curves are shown in Figures 5a and 5b. The thermal degradation was calculated to measure the weight loss with temperature changes. The TG curve shows three regions of fiber degradation temperatures starting from the evaporation of moisture in the fiber at a temperature range of 100-150 °C, cellulose degradation at region two at a temperature range of 250-350 °C, and region three at 400-450 °C temperature range showing the residual substance in the form of ash [52,57,82,83]. The maximum temperature ($T_m$) of each sample before and after chemical and mechanical treatment is shown in Table 2. Raw AG fiber demonstrated a maximum temperature of 342.5 °C. After AG fiber underwent alkalizing treatment, the $T_m$ of the fiber increased by 3% compared to raw AG fiber, indicating an increase in the thermal stability of the fiber due to the increase in the crystal structure. This result was supported by the measurement of the crystallinity index (Table 2). The $T_m$ of AG fiber after bleaching treatment was 362.7 °C. This result was higher than previous studies such as *Cyperus pangorei* (324 °C) [75], *Thespesia populnea* barks (323°C) [79], and *Cardiospermum halicababum* (336 °C) [74]. After mechanical treatment of ultrafine grinding, the $T_m$ of nanocellulose was reduced by 1.8% due to the destruction of the cellulose crystalline structure [80,81]. This result was in good agreement with previous works [64,66].

## 4. Conclusion

This study aims to utilize AG fiber into nanocellulose by chemical and mechanical methods. AG fiber treated with bleaching for 2 h showed the highest cellulose content after

removing 56% hemicellulose. Mechanical treatment was successful in the production of nanocellulose with an average diameter of 4.07 nm. A crystallinity index (71%) was observed for bleached AG fibers compared to untreated fibers (49%). The functional group present at 2898 $cm^{-1}$ in the treated AG fiber increased the load-bearing ability and stiffness when reinforced with a polymer matrix. The bleached AG fiber showed the highest thermal stability (363 °C) compared to the untreated fiber (343 °C). Based on the findings in this study, it can be concluded that among all parameters, the optimal chemical-mechanical treatment gave excellent properties in terms of cellulose purity and cellulose nanofiber production. Therefore, AG fiber treated with chemical-mechanical treatment can be used as a new fiber reinforcement source for lightweight and environmentally friendly biocomposites.

**Acknowledgment**

The authors thank Atmi Wirasti Colleagues from the Department of Agricultural Technology, Agricultural Polytechnic, Payakumbuh, West Sumatra, for their contributions towards the successful completion of this research. This work was financially supported by the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia (PDUPT grant number: 3056/PL.25/PG/2021).

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