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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.

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 nanofibers" 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

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

Natural fiber	Nanocellulose preparation	Ref.
Cassava bagasse	Hydrolyzed in 6.5 M H$_2$SO$_4$/40 min	[10]
Wheat straw	High pressurize homogenizer/15 min	[12]
Cotton cellulose	Hydrolyzed in 6.5 M 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 fiber	High pressurize homogenizer, 500 bar	[19,21]
Tunicin	55 wt% H$_2$SO$_4$/20 min	[32,33]
Waxy maize starch	H$_2$SO$_4$/5 days	[34,35]
Cottonseed linter	64 wt% H$_{2SO4}$/4 h	[36]
Ramie	64 wt% H$_{2SO4}$/4 h	[37]
Hemp	64 wt% H$_2$SO$_4$/4 h	[38]
Flax	64 wt% H$_2$SO$_4$/4 h	[39]
Bamboo	50 wt% H$_2$SO$_4$/48 h	[40]
Potato peel waste	64 wt% H$_2$SO$_4$/90 min	[41]
Cotton cellulose powders	H$_2$SO$_4$	[42]
Sugarcane bagasse	64 wt% H$_2$SO$_4$/3 h	[43]
Cotton linter	64 wt% H$_2$SO$_4$/1 h	[44]
Sugar palm fiber	60 wt% H$_2$SO$_4$/45 min	[45–47]
Agave gigantea	Ultrafine grider +sonicator	Current study

[25,26], water hyacinth [27], and sugarcane bagasse [28]. Table 1 shows the isolation of nanocellulose using several natural fibers. 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.

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 to 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
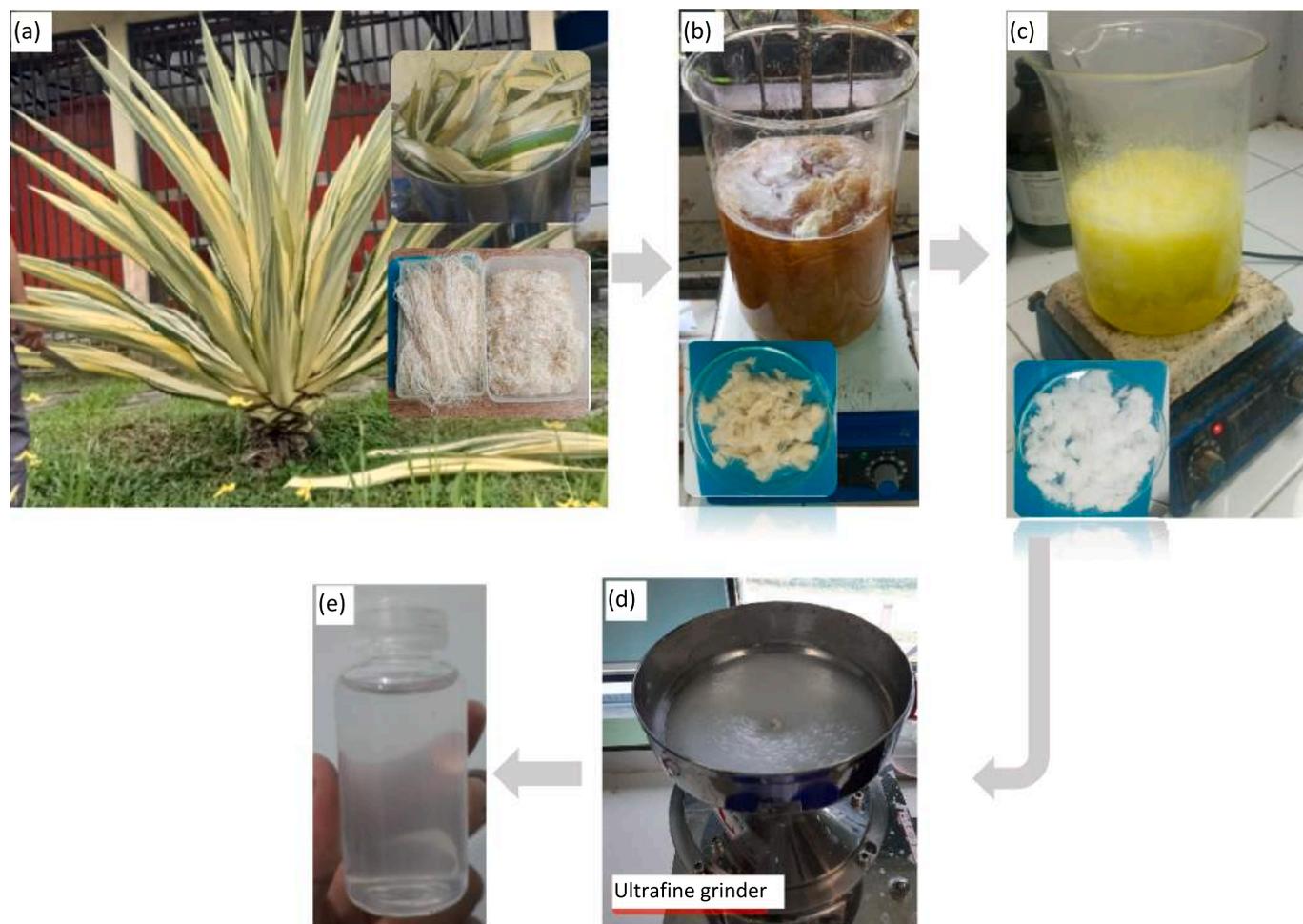

Fig. 1. (a) Leaves of AG fiber and AG fiber, (b) alkalization, (c) bleaching, (d) ultrafine grinding process, (e) CNFs AG.

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$_3$COOH).

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 ultra-sonication 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 Fig. 1.

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 fibers/*NDF), fiber soluble in acid detergent (*acid detergent fibers/*ADF), hemicellulose, cellulose, and lignin. The Van Soest method can determine cellulose,

Table 2
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

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 (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 I$_{002}$ = Intensity for 2θ = 22.3°, which indicates the crystal region. I$_{am}$ is an amorphous region that is at Intensity 2θ = 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 2. This analysis revealed that the cellulose content increased by 20.4%
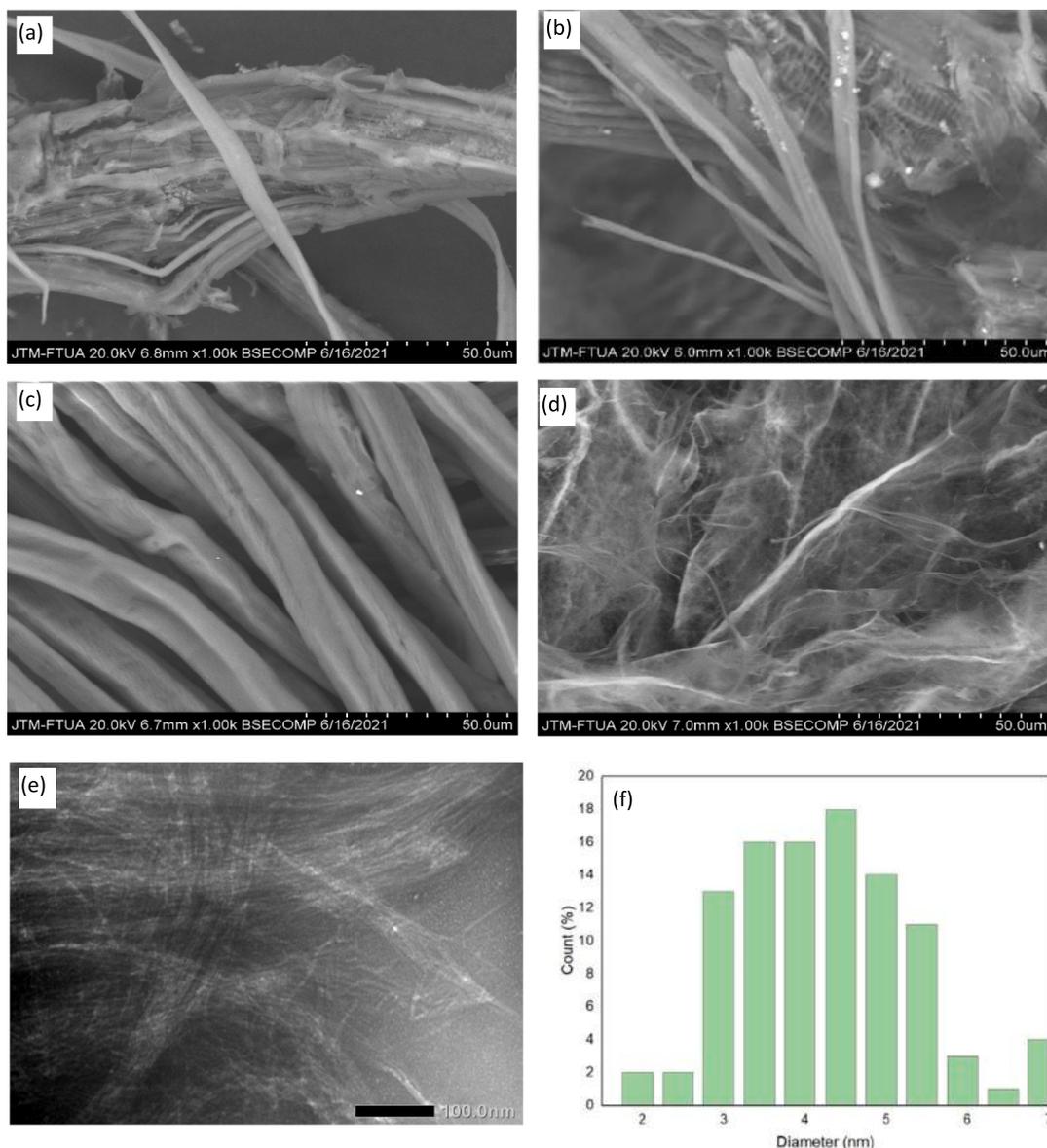
Fig. 2. SEM micrographs of AG fiber raw AG fiber (a); alkalization (b); bleached (c); ultrafine grinding (d); and TEM micrographs of CNFs AG (e); and size of CNFs AG (f).

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 to 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.

3.2. SEM and TEM

Chemical treatments (alkalization and bleaching) and ultrafine grinding yielded cellulose and CNFs from AG fiber. Fig. 2a–d 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 (Fig. 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. Fig. 2b and c shows that the surface morphology of the microfibril bundles was smooth, and the fiber diameter was smaller (10-15 μm) than raw AG fiber due to chemical treatment which successfully removed hemicellulose, lignin, wax, pectin components, and impurities.

On the other hand, Fig. 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
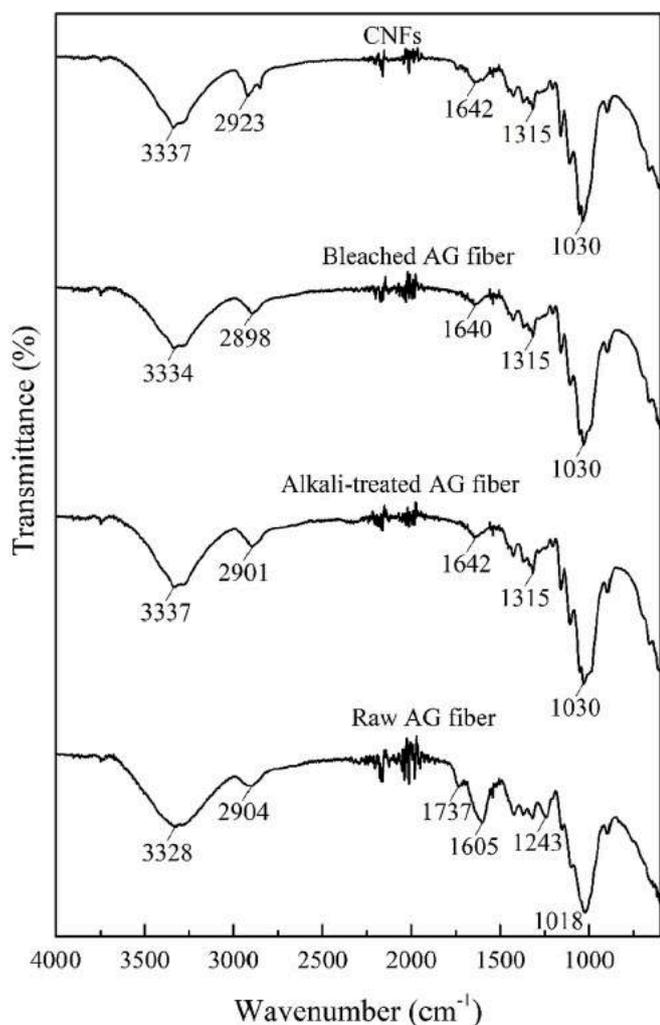
Fig. 3. FTIR spectra of raw AG fiber; alkalization, bleaching, and mechanical treatment.

Fig. 4. XRD curves of raw AG fiber; alkalization, bleaching, and mechanical treatment.

Table 3
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

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 to 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].

Fig. 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, 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 Fig. 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
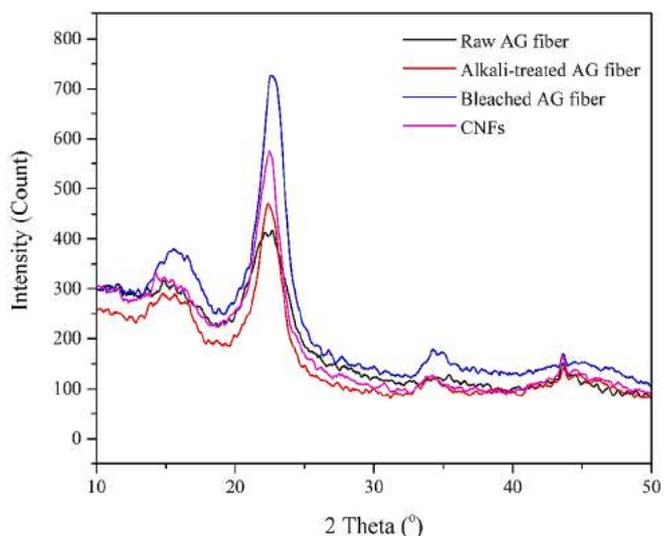
Agave gigantea, intermediates, crystalline cellulose, and cellulose nanofibers (Fig. 3), the peaks at 3328–3337 cm^{-1} corresponded to OH stretching vibrations in cellulose [47,69]. The intensification of these peaks presented an increase in the cellulose content and the removal of amorphous components increased the hydrogen bonds between the cellulose chains. Hernandez et al. obtained cellulose nanocrystals from corn straw using an alkaline treatment, bleaching, and acid hydrolysis [70]. These same authors claimed that peak intensification between 3200 and 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] (Fig. 3). The band at 1737 cm^{-1} was present in the FTIR spectrum of raw AG (Fig. 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].

3.4. Crystallinity index analysis

XRD analysis is an essential parameter in observing the effect of the crystallinity index of AG fiber before and after chemical and mechanical treatment. Fig. 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
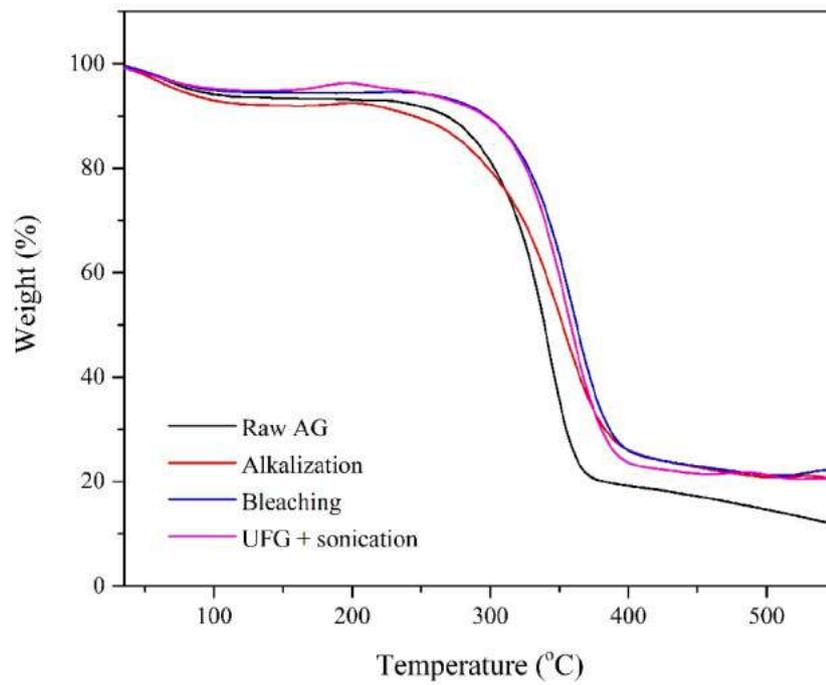
a)
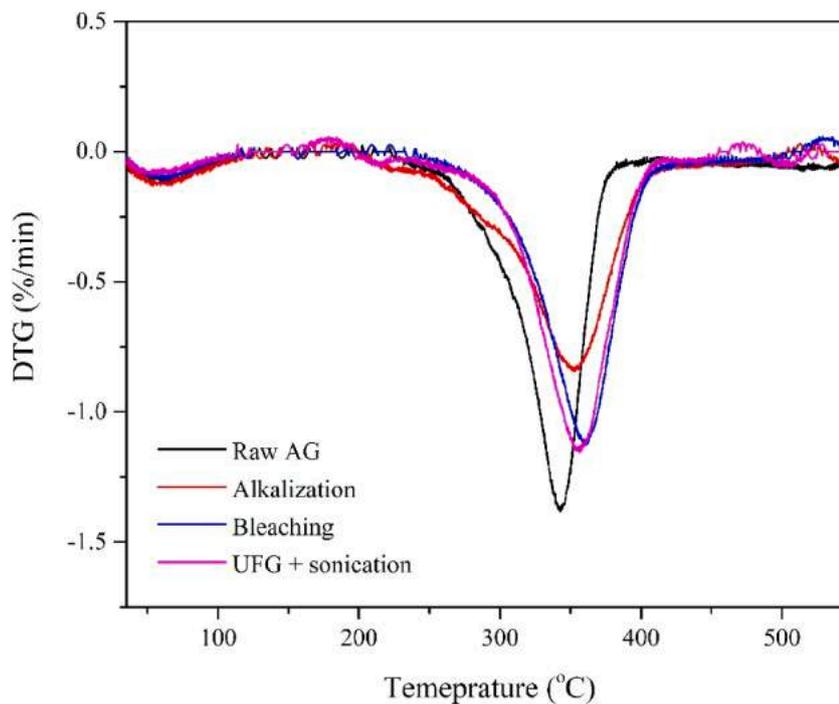
b)

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

are shown in Table 3. The X-ray Diffraction pattern in Fig. 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 3). 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

Thermal degradation analyses of AG fiber and nanocellulose using thermogravimetric (TG) and difference thermogravimetry (DTG) curves are shown in Fig. 5a and b. 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 3. 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 3). 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.

CRediT authorship contribution 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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