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Research Paper

Isolation and Characterization of Cellulose Microfibril (MFC) from *Gracilaria sp.* with Different Quality Grades

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Abstract

The cellulose found in *Gracilaria sp.* has not been utilized optimally. This study investigated the characteristics of cellulose and cellulose microfibril (MFC) isolated from three grades of *Gracilaria sp*. Descriptive tests were performed to determine the quality of each grade, including observations on moisture content, ash content, CAW, and impurities. The extraction process involved separating agar from *Gracilaria sp.*, isolating cellulose using 10% NaOH, and bleaching cellulose with 3% NaOCl. The bleached cellulose was then ultrasonicated to produce MFC. Characterization was performed using FTIR, XRD, PSA, STA, DSC, and py-GC/MS. FTIR analysis indicated similar peaks for both cellulose forms but only differed in transmittance intensity. The crystallinity index from XRD analysis was 22–39% for raw *Gracilaria sp.*, 25–46% for cellulose, and 68–89% for MFC. The particle size distribution of MFC mostly ranged between 200–500 nm, with 63.16% frequency. TG analysis showed cellulose decomposition with a T_{onset} of 231–260°C and a T_{max} of 318–326°C. DSC analysis revealed that sonication enhances the polymer structure's crystallization compared to pre-sonicated cellulose and raw material. The py-GC/MS analysis showed that D-allose and n-Hexadecanoic acid were the major components.

Keywords

Gracilaria sp., Cellulose Extraction, Cellulose Microfibril (MFC), Ultrasonication

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1. INTRODUCTION

Cellulose sources generally come from land plants. However, previous studies reported that the highest cellulose potential from the sea is from seaweed. Siddhanta et al. (2011) stated that of the 23 seaweed species studied, red and brown seaweed groups contained the highest cellulose content. The cellulose content of brown seaweed is 9.0-10.0% dry weight, red seaweed 1.2-13.65% dry weight, and green seaweed 1.5-11.6% dry weight. Among these types of seaweed that have high cellulose content, the one cultivated is Gracilariales (Baghel et al., 2021). Indonesia's dried seaweed production reached the 2nd highest in 2019 after China, of which 30% was *Gracilaria sp*. Kaur et al. (2023), which has been used for various purposes, including direct consumption as human food Mouedden et al. (2024) and medicine (Costa et al., 2016; Da Costa et al., 2017; Insani et al., 2022; Makkar and Chakraborty, 2017). *Gracilaria sp.* is also used in agriculture as a biostimulant or fertilizer Torres et al. (2018), in aquaculture as immune enhancer Lin et al. (2011), and feed Al-Asgah et al. (2016)). In addition, *Gracilaria sp* has been reported to be used as an insecticide Govindarajan and Benelli (2017) and acaricide (Ruangsomboon and Pumnuan, 2016).

The use of *Gracilaria sp.* in various industrial activities has the potential to produce solid waste containing around 26.12% cellulose. Cellulose is found in the cell walls of seaweed associated with other polysaccharides such as lignin, pectin, hemicellulose, xylan, mannan, galactan, hydrocolloids (alginate, agar, carrageenan), rhamnose glucuronate, depending on the type of seaweed (Ciancia et al., 2020). *Gracilaria sp.* was reported to contain total carbohydrates of 61.38%, protein of 15.38%, fat of 1.82% Yudiati et al. (2020), and crude fibre of 9.72% (Nafiqoh et al., 2021). Additionally, *Gracilaria sp.* has a tissue carbon content ranging from 33.7% to 35.9%, depending on salinity levels (Wu et al., 2018). Cellulose contained in *Gracilaria sp.* can also be utilized for the chemical industry, including the packaging industry. The extraction of cellulose from seaweed is known to be easier and more effective than extraction from terrestrial plants because seaweed has a low lignin content 0.5–4.5% compared to land plants 22.66–35.20% (Chen et al., 2016). Further synthesis can produce MFC (Zhang et al., 2021). Microcellulose is cellulose with a tiny size (micro), renewable, easily degraded, and non-toxic material (Nechyporchuk et al., 2016). MFC is produced using several methodologies, including electrospinning, bacterial formation, acid digestion, and mechanical defibrillation techniques. The morphology of the resulting micromaterial is specific to the production process. Modifying cellulose into MFC needs to be explored to reveal the characteristics of MFC isolated from *Gracilaria sp.* with various qualities of raw materials used.

2. EXPERIMENTAL SECTION

2.1 Materials

Gracilaria sp. used in this study consisted of three different qualities, grade 2 (G2), grade 3 (G3), and grade 4 (G4), obtained from cultivators in Karawang, West Java, Indonesia. Dried *Gracilaria sp.* was washed until the washing water was clear, and the remaining physical contaminants, such as snails and other seaweed types, were removed. The washed *Gracilaria sp.* was dried in an oven at 60°C for 48–72 hours, then ground using a blender. The chemicals used included HCl, NaOH, NaOCl, and H_2O_2. The equipment used is ultrasonicator (Shandong Biomaisen UCD-250) for microfibrilizing the cellulose, Attenuated Total Reflectance Infrared (ATR-IR) Bruker Optics, Germany for spectroscopy analysis, particle size analyzer Horiba SZ-100, Simultaneous Thermal Analyzer (STA) 6000 for thermal stability analysis, Perkin-Elmer DSC–4000 for thermal analysis, py-GCMS Shimadzu (GCMS-QP2020 NX) for chemical composition analysis.

2.2 Quality Grading of Cultivated *Gracilaria sp.*

Gracilaria sp. with three different quality grades (G2, G3, and G4) were determined for their characteristics, i.e. appearance through descriptive observation, moisture content, ash content, CAW, and impurities. Moisture content and ash content followed the AOAC International (2005). Moisture content was calculated as the per cent difference in weight between samples that have not been evaporated and samples that have been evaporated. Ash content was a mixture of inorganic or mineral components found in a material. CAW (Clean Anhydrous Weed) followed the SNI 2690:2015 about Dried Seaweed by determining the percentage of seaweed that is clean and free from any impurities compared to the initial dry material. Temperature, pH and visual depth of water were measured on-site using EZ-9909 Meter Backlight Waterproof. The water depth was measured using a specially-made PVC-based local such disk of 20 cm in diameter and 1-2 cm in thickness.

2.3 Cellulose Preparation from *Gracilaria sp.*

Cellulose isolation using dried *Gracilaria sp.* started with agar separation. To remove agar, dried *Gracilaria sp.* (G2, G3, and G4) flour was soaked in HCl solution (pH 4) at 95°C for 2 hours with a seaweed and solution ratio of 1:20 w/v. The fibre obtained was filtered while hot. The filtrate is liquid agar. The precipitate was neutralized and dried in a 60°C oven until dried fibre was obtained and used for cellulose isolation. Dry fibre was soaked in 10% NaOH at 80°C for 2 hours with a ratio of fibre to solution 1:20 (w/v). The fibre was then bleached with 3% NaOCl for 2 hours at ambient temperature with a fibre and solution ratio of 1:20 w/v. The bleached fibres were washed until neutral, then dried in an oven at 60°C until cellulose fibres were obtained. The cellulose fibres were then ultrasonicated. Approximately 0.5% cellulose fibre was added and homogenized in 250 mL of water with a stirrer for 1 hour, then sonicated with a Shandong Biomaisen UCD-250 ultrasonicator for 90 minutes to obtain MFC.

2.4 MFC Characterization

The characterization was carried out on cellulose extracted from *Gracilaria sp.* grade 2 (CG2), grade 3 (CG3), and grade 4 (CG4), as well as MFC from cellulose extracted from *Gracilaria sp.* grade 2 (UCG2), grade 3 (UCG3), and grade 4 (UCG4). The MFC quality analysis included spectroscopy, particle size, thermal profile and stability, and py-GC/MS. Those measurement were also carried out on raw *Gracilaria sp.* grade 2 (G2), grade 3 (G3), and grade 4 (G4) for comparison.

2.4.1 Spectroscopy Analysis

Functional group analysis was performed with Attenuated Total Reflectance Infrared (ATR-IR) equipment (Bruker Optics, Germany). The spectrum of functional groups is determined at waves 4000–400 cm^{-1}. The resulting data is in the form of spectrum peaks with certain specific transmittances. The crystallinity of the 2×2 cm dried sample was assessed using Shimadzu XRD-7000 MaximaX (Japan), utilizing monochromatic radiation form CuKα (λ = 0.1542 nm) generated at a 40 kV power, 30 mA current, speed of 2°/min, and diffraction intensity at the 2θ angle ranging from 10–50°.

2.4.2 Particle Size Analysis

The cellulose samples from the ultrasonication process were analyzed for particle size and size distribution using a particle size analyzer (Horiba SZ-100) with the DLS/Dynamic Light Scattering method.

2.4.3 Thermal Stability

Thermal stability was assessed utilizing a Simultaneous Thermal Analyzer (STA 6000). Approximately 10 mg of the dried sample was placed in an STA container and heated at 25–500°C under a nitrogen flow rate of 20 mL/min, with a heating rate of 10°C/min. The TGA results were analyzed using the Origin 2019 software tool to observe and compute alterations in sample weight throughout the analysis.

2.4.4 Thermal Analysis

Thermal analysis was measured using the Perkin-Elmer DSC–4000 test, using 1 g of 100 mesh sample with in a temperature range of 20–350°C and a flow rate of increasing temperature during heating of 2°C/min in nitrogen air.

Figure 1. Digital Photo of Three Quality Grades of Cultivated Dried *Gracilaria sp*

Table 1. Quality Description of *Gracilaria sp.* with Different Quality Grades

Parameter	Quality			(Badan Standardisasi Nasional, 2015)
	G2	**G3**	**G4**	
Appearance	Clean, no dirt attached, no mud or dust, and no shells attached. The thallus measuring ±40 cm, still has wrinkles, clean. Harvest age 60 days.	Dirty, difficult to wash. Thallus short. Harvest age 60 days.	Very dirty, lots of shells still attached, difficult to wash. Short thallus, with many white spots. Harvest age 60 days.	Clean, bright color and large branches (thallus) according to type specifications.
Odour	Fresh type specifications	Not fresh	Not fresh	Fresh type specifications
Texture	Not easy to break between stems and branches	Not easy to break between stems and branches	Not easy to break between stems and branches	Not easy to break between stems and branches

Note: Dried *Gracilaria sp.* grade 2 (G2), grade 3 (G3), and grade 4 (G4).

2.4.5 py-GC/MS Analysis

py-GC/MS was used about 0.3 g of cellulose and put in a pyrolysis tube in Shimadzu 221-48600. Next, the sample was pyrolysed. MS was set on the ion source with a temperature of 250°C and an interface temperature of 280°C with a covalent cut time of 1 minute. The detector voltage was selected relative to the running result. The column used was SH-Rtx-Wax Cap column type 30m, 0.25mm to 0.25 μm. The start time was 1.01 min and ended after 54 min. Mass spectra were obtained in the m/z 40–600 range. For Single-Shot analysis, the Pyrolyzer was selected at 500°C with a time of 0.10 minutes, and the interface was set at a temperature above 280°C.

3. RESULTS AND DISCUSSION

3.1 Quality Description of Cultivated *Gracilaria sp.*

Determination of seaweed grade is generally based on scientific studies, market needs, and industry considerations to ensure that the product meets specific applicable criteria. Dried *Gracilaria sp.* from cultivation is divided into four grades: grades 1, 2, 3, and 4. However, the harvest from the waters of Karawang, West Java, Indonesia, can only be included in grades 2, 3, and 4 (Figure 1), carried out through direct descriptive observation by local seaweed collectors. The description of the *Gracilaria sp.* grade is presented in Table 1, based on the parameters of appearance, odour, and texture. The ideal harvest age of *Gracilaria sp.* is 45 days. If harvesting occurs before the ideal time, the seaweed produced will be low-quality (Syahrul et al., 2023). Table 1 shows that the harvest age of *Gracillaria sp.* for grades 2, 3 and 4 is 60 days, and this condition has met the harvest age.

Katili et al. (2019)) stated that the CAW content in seaweed dried using the para-para method is influenced by the distance or gap between the dried seaweed. If the seaweed overlaps, crystal formation can occur due to low evaporation, allowing dirt to stick easily. *Gracilaria sp.* from ponds in Karawang is generally dried conventionally on the ponds edge with a tarpaulin and given a certain distance, so it is prone to binding dirt, which causes its quality to be lower. Other parameters also considered in determining quality are appearance, moisture content, ash content, impurities, CAW and agar yield, as presented in Table 2.

The moisture content of *Gracilaria sp.* for the study was in the range of 12.74–15.74%. This value is below the Indonesian national quality standard, 16%. The moisture content of *Gracilaria sp.* in this study depends on the drying method used. The ash content of *Gracilaria sp.* is 8.30–9.50%, much lower than other observations, 45.93–48.20% Syahrul et al. (2023) and 15.08% (Munandar et al., 2019). The quality of seaweed decreases, accompanied by ash content that is too high. Ash content is correlated with the minerals contained in seaweed. The condition of the waters of origin of the cultivated seaweed can influence the mineral content. The longer the seaweed is cultivated, more minerals are absorbed and the ash content increase (Wenno and Loppies, 2019).

Measuring impurities or impurity residues aimed to determine the amount of residues in *Gracilaria sp.* with different qualities. The lowest impurity value was found in grade 2, 1.52%, which was significantly different from grades 3 and 4. The CAW value of grade 2 showed the highest value, 36.02%, inversely proportional to the low impurity value. A high CAW value indicates good post-harvest handling of seaweed immediately after harvest (Al Wazzan et al., 2021). Environmental conditions and growth period are the main factors determining seaweed's cellulose and agar yields. Three analysis parameters were observed in the cultivation waters, i.e. temperature, acidity, and depth. The light factor plays a role in the photosynthesis process for the growth of *Gracilaria sp.* Seaweed grows at a reasonable brightness level in pond waters at a water depth of 0.6–1.0 meters. Water turbidity also dramatically affects the brightness and cleanliness of the seaweed produced because it can cause thin and short thallus growth, such as in Grades 2 and 3. The pH value of the *Gracilaria sp.* cultivation pond waters was 7 (Table 3). The pH value of the pond is in the ideal range because the water is filled naturally following the movement of the ebb and flow of the tide (Yulistiana et al., 2020).

3.2 Cellulose Yield

Cellulose extraction using non-economic raw materials or by-products of industrial activities has been widely studied, as in Table 4. Yield determines a business's economic feasibility and effectiveness in producing materials or products. The raw materials studied include date by-products Aisy et al. (2024), kraft pulp Filipova et al. (2018), and rice straws Dilamian and Noroozi (2019). Cellulose extraction is generally done by chemical treatment through delignification and bleaching treatments followed by physical treatment to maximize the desired cellulose quality. Seaweed raw materials are studied because of their abundant availability in nature, such as *Sargassum sp.* and *Turbinaria sp.*, as well as the development of cultivation of several species of seaweed, including *Kappaphycus sp.* and *Gracilaria sp.* Muthukumar and Chidambaram (2023) reported that the cellulose content of this seaweed raw material was in the range of 0.38-74.58%. In research, the cellulose yield was between 11.65 and 19.97%, with the highest yield obtained from grade 4 as shown in Table 2.

3.3 Functional Groups of *Gracilaria sp.* Cellulose

The typical absorption at a wavelength between 4000–400 cm^{-1} through FTIR spectroscopy indicates the vibration between organic compound molecules. The spectrum of raw *Gracilaria sp.*, cellulose extracted from *Gracilaria sp.*, and MFC are presented in Figure 2. The characteristic peaks at these wavelengths were compared with the publication of Bhutiya et al. (2018), Chen et al. (2016), Doh et al. (2020), Kallappa et al. (2023)), and Mariia et al. (2023) to show identical functional groups in the research results. Hydrogen bonds showed by −OH stretching vibration at 3345 cm^{-1}, lignocellulose identified by C−H bond vibration at 2916 cm^{-1}, hemicellulose identified by C=O stretching and C=C stretching aromatic hydrocarbons at 1734 cm^{-1}, lignin identified by C−O skeletal vibration at 1538 cm^{-1}, alkyl-ester identified by C−O stretching vibration 1059 cm^{-1}, Glycosidic ring to cellulose structure identified by C−H bond at 896 cm^{-1}, and asymmetrical bond trough C−H stretching at 558 cm^{-1}.

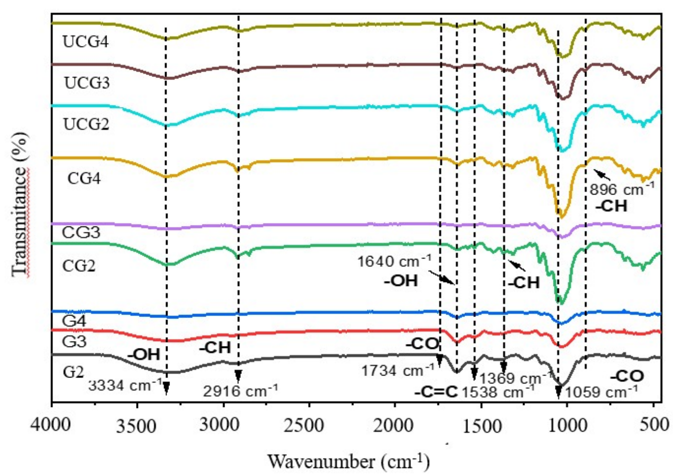

Figure 2. FTIR Spektra of Three Different Grade Dried *Gracilaria sp.* (G2, G3, G4), Cellulose Extracted (CG2, CG3, CG4), and MFC Sonicated Cellulose (UCG2, UCG3, UCG4)

The peak observed at 3400 cm^{-1} is associated with O–H vibrations caused by hydrogen bonds in cellulose. The absorption area at 2916 cm^{-1} is the C−H stretching of CH_2 from the CH_2−OH group of cellulose. The aliphatic C−H group in the FTIR test is indicated by the presence of peaks in all samples except for samples G4 and CG3, where no such absorption was found, and the peak at 1640 cm^{-1} is associated with absorbed water. The peak at 1375 cm^{-1} corresponds to the asymmetric vibration of the C–H band, and the sharp peak at 1060 cm^{-1} corresponds to the stretching vibration of C–O.

Both cellulose and MFC show similar peaks, differing only in the intensity of transmittance, while in *Gracilaria sp.*, the peak

Table 2. Quality Characteristics of *Gracilaria sp.* with Different Quality Grades

Parameter	Quality			(Badan Standardisasi Nasional, 2015)
	G2	G3	G4	
Moisture content (%)	15.74 ± 1.74	12.74 ± 1.77	14.09 ± 1.75	Max. 16%
Ash content (%)	8.30 ± 0.42	9.50 ± 0.71	8.50 ± 0.71	-
Impurities (%)	1.52 ± 0.05	2.86 ± 0.03	2.94 ± 0.02	Max. 3%
CAW (%)	36.02 ± 0.92	31.45 ± 0.96	35.51 ± 0.84	Min. 10%
Agar yield (%)	13.83 ± 1.35	15.27 ± 3.52	11.73 ± 0.46	-
Cellulose yield (%)	16.18 ± 0.14	11.65 ± 0.46	19.97 ± 0.47	-

Note: Dried *Gracilaria sp.* grade 2 (G2), grade 3 (G3), and grade 1 (G4).

Table 3. Cultivation Waters Quality of *Gracilaria sp.* Samples

Parameter	Value	Ideal*	Reference (Yulistiana et al., 2020)
Temperature (°C)	33.2–33.9	20–28	33–35
Depth (cm)	60–100	50–80	40–80
Acidity degree (pH)	7	6–9	8

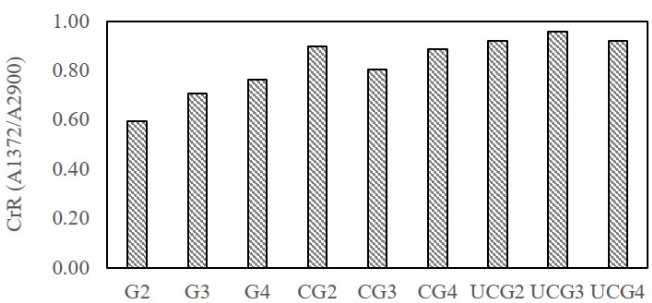

Figure 3. Crystallinity Ratio of Three Different Grade Dried *Gracilaria sp.* (G2, G3, G4), Cellulose Extracted (CG2, CG3, CG4), and MFC Sonicated Cellulose (UCG2, UCG3, UCG4)

is broader. At 1740 cm^{-1}, which is related to the stretching vibration of C=O, these peaks do not appear. Active oxygen produced from the breakdown of NaOCl reacts with the hydroxyl groups in cellulose, changing the hydroxyl groups into ketone groups (C=O) or aldehyde groups, which can increase the brightness and reduce the dye content in cellulose, thereby increasing the purity and whiteness of the fibre The crystallinity ratio (CrR) in samples were calculated by comparing the intensities of the absorption peaks form FTIR spectrum at 1372 cm^{-1} (A1372) and 2900 cm^{-1} (A2900) (Nelson and O'Connor, 1964). The CrR profile gradually increased along with the process, indicating that the crystallinity index also increased. From these results, it can be concluded that chemical and mechanical treatments are effective in removing the amorphous area. Figure 3 shows the CrR for each sample.

3.4 XRD (X−ray Diffraction)

Figure 4 shows the XRD spectra of raw *Gracilaria sp.*, cellulose extracted from *Gracilaria sp.*, and MFC. Each treatment used *Gracilaria sp.* with three different quality levels. All three levels of *Gracilaria sp.* showed one characteristic peak in the range of 16–17°. The extracted cellulose and MFC showed two specific peaks at 15 and 22°, which represent the crystallographic planes (110) and (200) of the monoclinic cellulose lattice, similar to what Nishiyama (2009) has reported. The difference in the number of peaks may be due to the removal of non-crystalline components involved in the extraction process. Table 5 shows that raw *Gracilaria sp.* has a 22–39% crystallinity index, lower than extracted cellulose (25–46%) and MFC (68–89%). A similar correlation was also observed by Filipova et al. (2018), who used Kraft pulp as a raw material to obtain nanofibril cellulose. The cellulose had a CI of 74.3%, higher than the CI of bleached original Kraft pulp (69.6%). Another study revealed that the crystallinity value of extracted fibres showed an increase of 50.6 to 65.78% compared to whole straw (37.1%) (Dilamian and Noroozi, 2019).

Cellulose has a higher CI value than raw seaweed, indicating that some amorphous areas in the raw material have been removed during the extraction treatment. Raw seaweed contains several non-cellulose components, such as hemicellulose, lignin, and other components with an amorphous matrix, so they have low crystallinity. Removal of non-crystalline components through sequential chemical treatments can increase crystallinity due to the formation of intra- and intermolecular hydrogen bonds in the hydroxyl groups of the cellulose chain. These bonds limit the free movement of the cellulose chain, thus forming regular bonds and resulting in increased crystallinity (Chirayil et al., 2014). In addition to the extraction

Table 4. Cellulose Extraction Reported Using Different Seaweed Species and by-Product Material

Parameters	Result				
Raw material	Seaweed *(Gracilaria sp.)*	Seaweed: *Sargassum wightii* *Turbinaria ornata* *Gracilaria edulis* *Gelidiella acerosa* *Kappaphycus alvarezii*	Dates by-product	Kraft pulp -	Rice straw
Extraction method	ultrasound-assisted chemical extraction	acid-base treatment, ultrasound assisted, multistep acid-base treatments	Chemical treatment	Chemical treatment	ultrasound-assisted chemical extraction
Yield (%)	11.65 – 19.97	0.38 – 74.58	11.0	-	33-37
Functional group					
OH stretching	3334	3300	3335	3400	3400
	2916	2921	2897	2900	2900
C−H, CH_2	896		895		897
	558	621	660		
C=O	1734	1609	-	1740	1735
C−O	1059	1162	1055	1060	1245
		1150	1157		1060
Crystallinity Index (%)	68.64 – 89.01	-	54.6	74.3	50.6-65.78
Fibre size (nm)	235.5 – 245.6	-	-	20-300	6-20
Thermal stability (°C)	311-318		-	347	
References		(Muthukumar and Chidambaram, 2023)	(Aisy et al., 2024)	(Filipova et al., 2018)	(Dilamian and Noroozi, 2019)

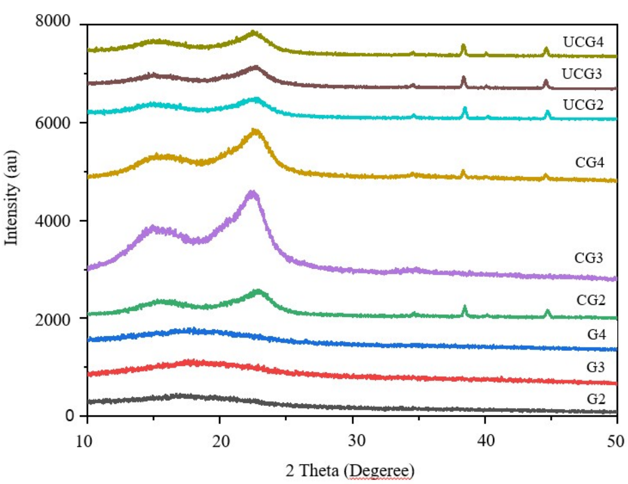

Figure 4. XRD Diffractograms of Three Different Grade Dried *Gracilaria sp.* (G2, G3, G4), Cellulose Extracted (CG2, CG3, CG4), and MFC Sonicated Cellulose (UCG2, UCG3, UCG4)

process, mechanical treatments such as ultrasonication can also affect cellulose crystallinity. Table 5 shows that ultrasonication treatment of cellulose causes an increase in the crystallinity index. Ultrasonication can cause defibrillation or breaking cellulose fibres into smaller particles to open the fibre structure and increase the crystal regularity of cellulose fibres. Defibrillation of fibres with high-speed mechanical friction causes the breaking of bonds between fibrils and the formation of nano- to micro-sized fibres with high surface areas (Nair et al., 2014). Factors such as amplitude, frequency, and duration of ultrasonication also greatly influence the fibre defibrillation process (Dilamian and Noroozi, 2019).

3.5 Particle Size Distribution

The particles use a liquid medium to prevent agglomeration in the particle size analysis using PSA (particle size analyzer). The measured particle size represents the size of individual particles. The obtained particle size data includes four distributions. Figure 5 shows the particle size distribution graph of MFC from the three *Gracilaria sp.* grades. The average particle sizes of MFC are UCG2 <UCG3 <UCG4, with respective values of 234.5±12.7, 237.9± 4.1, and 245.6±2.7 nm. Based on Figure 5, it can be observed that all three samples have the highest frequency in the particle size range of 200–500 nm, accounting for 63.16%, followed by the sizes of 500–1000 nm at 29.53, and 1000–2000 nm at 3.31%. Particle size analysis (PSA) on MFC allows for the measurement of the fibres' diameter and

Table 5. Crystallinity Index of Dried *Gracilaria sp.*, Extracted Cellulose, and MFC

Sample	Crystallinity (%)	Peak (2 Theta)	
		1	2
Dried *Gracilaria sp.* (G2)	39.83	16.59	-
Dried *Gracilaria sp.* (G3)	22.72	17.44	-
Dried *Gracilaria sp.* (G4)	25.97	17.80	-
Gracilaria sp. cellulose (CG2)	45.91	15.53	22.60
Gracilaria sp. cellulose (CG3)	30.44	15.00	22.03
Gracilaria sp. cellulose (CG4)	35.66	15.33	22.47
Sonicated CG2 cellulose (UCG2)	89.01	14.83	22.44
Sonicated CG3 cellulose (UCG3)	71.60	15.10	22.43
Sonicated CG4 cellulose (UCG4)	68.64	14.90	22.46

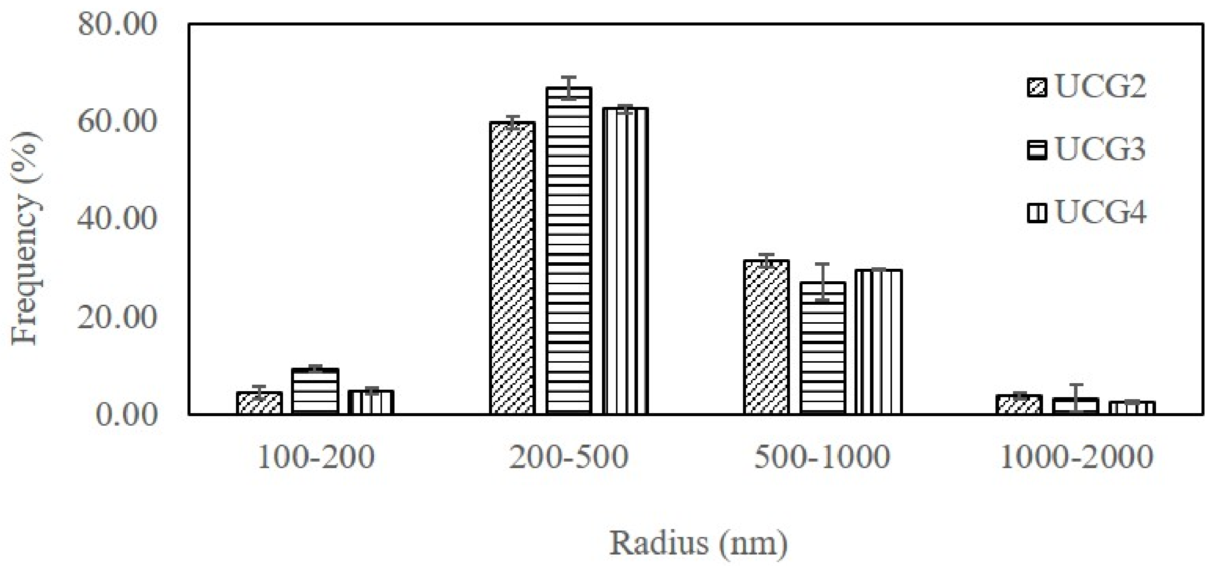

Figure 5. Particle Size Distribution of Three Different Grade Dried *Gracilaria sp.* (G2, G3, G4), Cellulose Extracted (CG2, CG3, CG4), and Sonicated Cellulose (UCG2, UCG3, UCG4)

length. Most particles fall within the 200–500 nm range, with an average size of 235.5–245.6 nm.

3.6 Thermal Properties

Figure 6 shows the heat decomposition results of raw *Gracilaria sp.* with three areas of decomposition. The first area occurred in the temperature range of 50–150°C for all types of *Gracilaria sp.* (G2–G4), which indicates evaporation of moisture or molecularly bound water on the surface of the material (Kumar et al., 2024; Wang et al., 2014). The initial decomposition temperature (T_{onset}) occurs at above 500°C, and the maximum decomposition temperature was above 100°C for all types of *Gracilaria sp.* (G2–G4), as detailed in Table 6. Weight loss was in the range of 11–14%, which indicates the relative water content of raw *Gracilaria sp.* as stated in Table 2, where the moisture content obtained is not much different between 12–15%. A relatively small slope was obtained in the 2^{nd} area with a temperature range of 175–250°C, which indicates the decomposition of impurities and some dissolved lignocellulose and polysaccharides, which have a lower decomposition temperature than lignocellulose (Wang et al., 2014).

The highest T_{onset} and T_{max} were obtained in raw *Gracilaria sp.* G3 at 201.13°C and 225.6°C, followed by G4 and G2. The highest weight loss also occurred in G3 with 5.62%, which showed that the impurity and ash content of the G3 type was greater than that of G4 and G2, as shown in Table 2. The 3^{rd} area is in the temperature range of 250–500°C which was the largest decomposition area where the main biochemical components in seaweed such as polysaccharides, proteins, lipids, pigments, fatty acids, lignocellulose, and minerals, are decomposed (Chanchpara et al., 2023). In this area, the highest maximum decomposition temperature (T_{max}) was obtained in types

G2 and G4 with a value of 286°C, with the most significant weight loss also occurring in type G2 at 42.57%, which indicates that G2 has the largest biochemical components compared to G3 and G4. The composition temperature is related to CAW value (Table 2), where the G2 type has the highest levels compared to G3 and G4. Temperatures above 500°C indicate the decomposition of carbon components and inorganic minerals in seaweed (K, Cl, N, and S) (Roslee and Munajat, 2017). Char residue at 500°C refers to the remaining components of carbon and inorganic minerals where the most considerable char residue is obtained in types G2 and G4. The heat stability of raw *Gracilaria sp.* types G2 and G4 is better than G3.

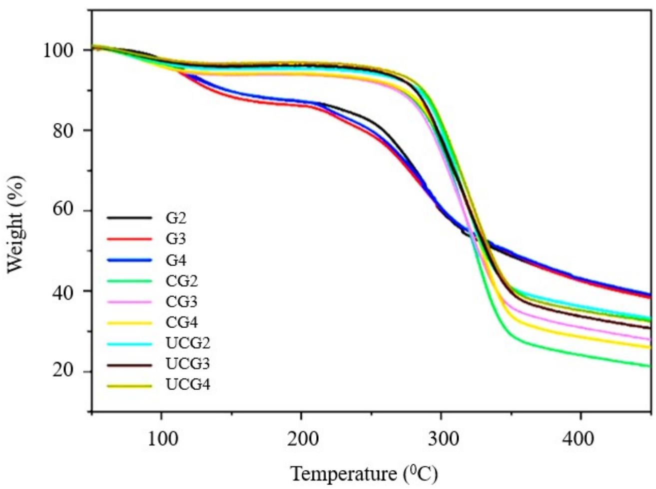

Figure 6. TGA thermogram of Three Different Grade Dried *Gracilaria sp.* (G2, G3, G4), Cellulose Extracted (CG2, CG3, CG4), and MFC Sonicated Cellulose (UCG2, UCG3, UCG4)

Characterization of the heat resistance properties of cellulose extracts (CG2–CG4) from various types of *Gracilaria sp.* can be seen in Figure 6. The decomposition area of the cellulose extract occurs in two areas, i.e. the first area in the temperature range of 50–100°C and the second area in the temperature range of 250–400°C. The first area shows the volatilization event of moisture and water components contained in the cellulose extract. A series of degradation treatments with acids and bases degrade the polysaccharide components and amorphous lignocellulosic components such as hemicellulose and lignin, thereby increasing the cellulose composition and resulting in an increase in decomposition temperature compared to raw *Gracilaria sp.* The second area showed cellulose decomposition with a Tonset of around 231–260°C and a T_{max} of 318–326°C. These results follow several studies stating that cellulose extract from seaweed has a T_{onset} of around 250–280°C and a T_{max} of 300–360°C (Jmel et al., 2019). These heat characteristics are comparable to cellulose extracts from terrestrial biomass where T_{onset} ranges from 220–280°C and T_{max} between 300–370°C. In addition, these results are close to AVICEL commercial cellulose with T_{onset} at 280°C and T_{max} at 365°C (Jmel et al., 2019). Based on these heat characteristics, cellulose extract from *Gracilaria sp.* has the potential to be an alternative source of cellulose from marine biomass. Table 6 shows cellulose from type G4 produces the highest T_{onset} and T_{max}, reaching 237.09°C and 326.57°C, respectively, followed by G2 and G3. The G4 type has better heat resistance than G2 and G3. Char residue at 500°C also decreased compared to raw *Gracilaria sp.*, which shows the decay of inorganic components during the extraction process, as reported by Wahlström et al. (2020) that inorganic components can be reduced during the mechanical process.

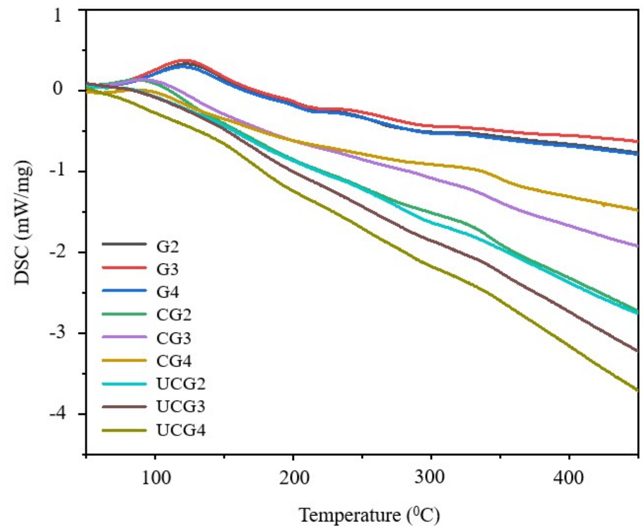

Figure 7. DSC Diffractogram of Three Different Grade Dried *Gracilaria sp.* (G2, G3, G4), Cellulose Extracted (CG2, CG3, CG4), and MFC Sonicated Cellulose (UCG2, UCG3, UCG4)

The thermal degradation behaviour of the samples showed that cellulose microfibrils and cellulose derived from *Gracilaria sp.* showed improved thermal stability compared to raw *Gracilaria sp.* The data showed that there was no significant difference in thermal resistance between cellulose (CG) and cellulose microfibrils (UCG), most likely due to the suboptimal ultra sonification processing of converting cellulose into cellulose microfibrils so that the structural changes that occur during the conversion of cellulose into microfibrils do not run well. Cellulose is a natural polymer consisting of long chains of glucose molecules. In contrast, cellulose microfibrils are formed from many interacting cellulose molecules, consisting of crystalline (regular) and amorphous (less regular) parts.

3.7 Thermal Analysis

DSC analysis can identify the occurrence of phase transformation, crystallization, degradation, and chemical reactions in the test material. The heat changes identified in the material play a significant role in influencing the molecular structure of cellulose; these changes will be reflected in the amount of energy that indicates the occurrence of endothermic and exothermic reactions (Lubis et al., 2019). Table 7 shows a positive DSC

Table 6. Thermal Stability of Dried *Gracilaria sp.*, Extracted Cellulose, and MFC

Code	T_{onset} (°C)			T_{max} (°C)			Weight loss (%)			Char residue at 500 °C (%)
	$T_{onset\ 1}$	$T_{onset\ 2}$	$T_{onset\ 3}$	$T_{max\ 1}$	$T_{max\ 2}$	$T_{max\ 3}$	stage 1	stage 2	stage 3	
G2	58.34	161.33	230.15	118.67	224.34	286.7	11.65	3.77	42.57	36.11
G3	57.99	201.13	241.48	116.24	225.60	284.8	13.97	5.62	40.53	35.82
G4	54.06	196.57	235.72	116.89	219.80	286.8	12.68	4.86	41.09	36.64
CG2	55.07	236.07	-	81.55	323.81	-	6.96	68.87	-	24.23
CG3	55.94	231.93	-	84.67	318.60	-	6.58	62.25	-	26.05
CG4	50.58	237.09	-	84.18	326.57	-	6.29	64.85	-	24.38
UCG2	51.40	238.76	-	75.87	312.90	-	5.39	58.49	-	31.39
UCG3	59.26	239.05	-	77.60	311.02	-	4.32	61.79	-	28.84
UCG4	56.57	239.61	-	77.46	318.70	-	4.12	61.11	-	30.90

Note: Dried *Gracilaria sp.* sp. grade 2 (G2), grade 3 (G3), and grade 4 (G4); cellulose extracted (CG2, CG3, CG4); and sonicated cellulose (UCG2, UCG3, UCG4)

Table 7. Thermal profile of Dried *Gracilaria sp.*, Extracted Cellulose, and MFC

Samples	Endothermic		Exothermic	
	Peak Temp. (°C)	ΔH (mW/mg)	Peak Temp. (°C)	ΔH (mW/mg)
Dried *Gracilaria sp.* (G2)	122.2	0.335		
Dried *Gracilaria sp.* (G3)	121.5	0.376		
Dried *Gracilaria sp.* (G4)	119.5	0.298	218.4	-0.262
			296.3	-0.518
Gracilaria sp. cellulose (CG2)			134.7	-0.520
			34.5	-2.541
Gracilaria sp. cellulose (CG3)			121.9	-0.233
Gracilaria sp. cellulose (CG4)	73.8	0.039	314.5	-1.708
Sonicated CG2 cellulose (UCG2)	85.9	0.013	59.1	-0.024
			331.8	-0.973
Sonicated CG3 cellulose (UCG3)	92.0	0.140		
Sonicated CG4 cellulose (UCG4)	85.2	0.138		

value, which means an exothermic reaction, and a negative DS value, which means an endothermic reaction. The exothermic curve appears to occur in G2, G3, and G4 as well as UCG3 and UCG4, while in samples CG2, CG3, CG4, and UCG2, the exothermic curve is formed after the completion of the endothermic process.

Figure 7 shows that the endothermic process occurs at a lower temperature range, 30–50°C, where UCG2 >UCG3 >UCG4. This figure shows that cellulose from *Gracilaria sp.* that has undergone the sonication process produces a polymer structure with a higher degree of crystallization when compared to cellulose before sonication or even to the raw material. Cellulose fibrils have better thermal stability than other treatments (Mwaikambo and Ansell, 2002). The reaction in the alkalization process is that the NaOH solution dissociates into Na^+, and OH^- reacts with seaweed polysaccharide components other than cellulose. The OH^- ion reacts with the H group on the seaweed polysaccharide substrate to produce water. This event causes the O group to become a free radical and reactive with C to form C−O−C. The C group already has four 'arms', so a series of groups release bonds to the O group. This reaction produces two benzene rings, each with a reactive O group. This reactive O group reacts with Na^+ and dissolves in alkaline solution so lignin is lost when rinsed. In addition, this reaction also produces H_2O. Cellulose is hydrophilic, so H_2O bound by cellulose causes the concentration of O−H bonds to increase.

Figure 7 reveals that the exothermic pattern indicates the presence of a fusion process or crystal formation in *Gracilaria sp.* flour. and occurs in the temperature range of 100–150°C. The same happens at a relatively lower temperature, 50–100°C, where CG4 >CG3 >CG2. This fusion process follows that in cellulose, exothermic thermal properties are read at around 80°C, related to the release of water molecules due to the hydrophilic nature of the polymer (Carrillo et al., 2004). The alkalization process extracts cellulose from *Gracilaria sp.* to remove lignin and hemicellulose. The loss of both compounds

Table 8. Organic Compounds in MFC Processed from Different Grades of Cultivated *Gracilaria sp.*

Names	UCG2			UCG3			UCG4		
	Ret Time	Area %	Height %	Ret Time	Area %	Height %	Ret Time	Area %	Height %
Carbamic acid, monoammonium salt	1.92	9.52	14.68	1.92	11.21	16.16	1.92	10.32	15.01
Glycidol	2.02	3.66	4.49	2.02	4.24	4.84	2.02	3.54	4.14
1-Hydroxy-2-propanone	2.23	7.52	10.64	0	0	0	3.37	7.62	6.68
2,3-Butanedione	2.64	3.77	5.30	2.65	3.30	5.07	2.65	3.55	5.12
1-Hydroxy-2-propanone	3.35	7.52	6.96	3.34	6.91	6.88	3.37	7.62	6.68
1-Nitro-2-propanone	4.79	2.09	2.53	0	0	0	4.81	1.70	1.88
Propanoic acid, 2-oxo-, methyl ester	5.14	3.09	3.21	0	0	0	5.15	2.84	2.71
3,5-Dimethylpyrazole	6.02	1.92	2.43	0	0	0	0	0	0
1,2-Cyclopentanedione	8.16	3.81	2.91	8.15	2.23	1.83	8.17	2.95	2.17
3-Methyl-1,2-cyclopentanedione	10.36	2.16	1.61	10.36	1.46	1.13	0	0	0
1,6-Anhydro-β-D-glucopyranose	19.22	0.39	0.36	0	0	0	20.74	10.82	2.88
D-allose	20.80	16.94	3.98	20.43	10.72	3.34	20.91	7.65	3.91
n-Hexadecanoic acid	26.25	8.74	8.25	26.28	13.30	10.50	26.27	9.29	8.59

Note: UCG2 is sonicated cellulose from *Gracilaria sp.* grade 2, UCG3 is sonicated cellulose from *Gracilaria sp.* grade 3, and UCG4 is sonicated cellulose from *Gracilaria sp.* grade 4.

from the polymer structure will reduce the amorphous nature and increase crystallinity, which is indicated by an increase in the degradation temperature. The DSC curve also identifies the occurrence of an endothermic process which identifies the degradation of cellulose molecules, the process of separating lignin and hemicellulose from the polymer structure, or the process of releasing water molecules related to the hydrophilic nature of the cellulose polymer.

3.8 Py-GC/MS Analysis

Table 8 is a tabulation of the identity of compounds present in MFC from different raw material grades of *Gracilaria sp.* analysed using Pyrolysis-GC/MS. The analysis showed the presence of carbamic acid, glycidol, 1,6-anhydro-β-D-glucopyra nose, D-allose, and n-Hexadecanoic acid in the sample. Carbamic acid appears at the beginning of the retention time in all three chromatograms of samples UCG2, UCG3 and UCG4. Carbamic acid is an organic compound that is not stable under normal conditions. This compound tends to break down into ammonia and carbon dioxide. Theoretically, carbamic acid could form under certain conditions in *Gracilaria sp.* cells, for example, as a by-product of specific metabolic reactions. However, the carbamic acid formed will likely decompose soon due to its instability. So, it is unsurprising that carbamic acid appears early in the retention time. Glycidol is an organic compound that has a unique and interesting chemical structure. An epoxide ring (three members) bonded to a hydroxyl group. This structure gives glycidol its reactive properties and makes it useful in various applications. Glycidol was identified in all three samples at relatively low concentrations.

Chromatograms UCG2 and UCG4 show the presence of 1,6-anhydro-β-D-glucopyranose compounds, meaning cellulose is in the sample. β-D-glucopyranose compounds are the main monomers in the formation of cellulose. Cellulose in land plants and seaweed combines thousands of D-glucopyranose monomers connected through glycosidic bonds. Thousands of β-D-glucopyranose molecules are connected through -β1,4 glycosidic bonds. This bond connects carbon atom number 1 on one glucose molecule with carbon atom number 4 on the following glucose molecule, forming a long, straight chain. This unique structure of cellulose derived from the β-1,4 bond between glucose molecules gives cellulose its characteristic properties, such as strength and stiffness. Sample UCG3 has a different quality from UCG2 and UCG4, so the chromatogram of UCG3 does not show beta D-glucopyranose. Although β-D-glucopyranose is the dominant basic unit of cellulose in plants, red algae such as *Gracilaria sp.* have variations in their cell wall components, including agar and cellulose. The agar produced using *Gracilaria* is a polysaccharide consisting of sugar units similar to glucose but with different glycosidic bonds. Although the cellulose content in *Gracilaria* is not as high as in land plants, the presence of cellulose in this red algae has been proven through various studies. In land plants, cellulose mainly consists of β-D-glucopyranose units connected with β-1,4 glycosidic bonds. This structure produces strong and straight fibres. Agar is a complex polysaccharide consisting of galactose and 3,6-anhydrogalactose units. The glycosidic bonds in agar are more varied than in cellulose, resulting in a more complex and gel-like structure.

Gracilaria sp. has various monosaccharides that make up its polysaccharides, such as galactose, 3,6-anhydrogalactose, and others. Each type of monosaccharide has different potential bioactivities. As a rare monosaccharide, D-allose has potential applications in the pharmaceutical and food fields. Besides the significant monosaccharides galactose and 3,6-anhydrogalactose, *Gracilaria sp.* also contains minor monosaccharides such as xylose, arabinose, and glucose. D-allose, an aldohexose sugar, appeared in the chromatograms of all three *Gracilaria sp.* seaweed samples at 20.4–20.9 minutes retention times. D-allose sugar is a desirable alternative to sucrose as it is 80% sweeter than sucrose, is very low in calories, and is non-toxic. It continues to attract attention due to its many physiological roles, especially its potent antitumour and anticancer properties, which make it a promising option for clinical medicine. However, due to its scarcity of natural sources and difficulty in chemical synthesis, biosynthetic methods must be researched to increase production. n-Hexadecanoic acid and D-allose are significant components that are identified in the three samples of *Gracilaria sp.* n-Hexadecanoic acid, or palmitic acid, is a type of saturated fatty acid that is commonly found in living things, including plants and marine animals such as algae. Several previous studies have mentioned that hexadecanoic fatty acid component found in *Gracilaria sp.* seaweed (Debbarma et al., 2016) has antibacterial and antifungal properties (Idris et al., 2022), antioxidant (Prasedya et al., 2023; Yuan et al., 2019). The analysis showed that D-allose and n-Hexadecanoic acid were the major components in each sample.

4. CONCLUSIONS

Chemically and organoleptically, the characteristics of *Gracilaria sp.* with the three quality levels used in this research showed differences. Cellulose isolated from *Gracilaria sp.* through agar separation, alkalination, bleaching and sonication processes produces MFC. Functional groups cellulose and MFC showed similar FTIR spectrum peaks, differing only in transmittance intensity. The crystallinity index of raw *Gracilaria sp.*, cellulose and MFC is 22–39%, 5–46% and 68–89% respectively. MFC has the most extensive particle size distribution at 63.16% in the 200–500 nm range. Cellulose decomposition can occur with a T_{onset} of around 231–260°C and a T_{max} of 318–326°C. The sonication process produces a polymer structure with a higher level of crystallization than cellulose without sonication or raw *Gracilaria sp.* Py-GC/MS analysis showed that D-allose and n-Hexadecanoic acid were the main components present in MFC from *Gracilaria sp.* with different quality grades.

5. ACKNOWLEDGMENT

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