



## Low-Cost Alkaline Pretreatments and Ultrafine Grinding in Nanocellulose Crystal Extraction from Giant Swamp Taro (*Cyrtosperma merkusii*) Processing Waste



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### Abstract

Agroindustry biomass are unlimited source for lignocellulosic ecofriendly nanocellulose fibers, with potentials as hydrophobic structural enhancers in biopolymer applications. Two major concerns in producing cellulose nano crystal (CNC); are obtaining a source not competing with food and a low-cost effective method to produce CNC. Alkali pretreatment combined with a mechanical treatment was performed on Giant Swamp Taro (GST) starch processing waste. The alkali pretreatment was useful in eliminating hemicellulose. The following disintegration process (ultrafine grinding) resulted GST CNC at the sizes of 449.6 nm to 78.26 nm. CNC results were thin flakes with fibrous network which appeared like nanowhiskers. Traces of coastal ecosystem mineral and rare elements are found in GST CNC as indicated in EDS, after all GST is a coastal plant.

**Keywords:** Alkali treatment; Cellulose nano crystals; *Cyrtosperma merkusii*; Giant Swamp Taro; Mechanical treatment

### 1. Introduction

Agroindustry biomass wastes are an unlimited source of cellulose due to the it's lignocellulosic content. Lignocellulosic are a good source of biodegradable natural fiber with outstanding eco-friendly properties that are now sought after by researchers as a replacement for petroleum-based polymers. Agricultural biomass wastes and forest residues as unlimited as they are in cellulose and lignocellulosic materials are potential replacement materials also for biofuel and feedstock productions as well as resources for value added advance materials without the competing with human – animal food supply chains [1].

Cellulosic biomass would serve as source of Cellulose Nano Crystals (CNC), Cellulose Nano Fiber (CNF) and the nano-sized compounds produced by bacteria namely Bacterial Nano Cellulose [2]. Bacteria, mainly *Acetobacter*, *Bacillus Sarcina*, *Rhizobium*, *Enterobacter*, *Klebsiella* and some gram-negatives such as *Gluconacetobacter xylinus* and *Acetobacter xylinum* were identified as bacteria involved to produce BNC [3].

The many different methods of extraction and the various sources of creates a rich variety in the CNC & CNF results in regard to properties, morphology, particle size, and crystallinity [4,5]. Some sources of biomass that has been studied to date are wastes of potato, wastes of sugar cane,

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EJCHEM use only; Received date 18 October 2022; revised date 14 April 2023; accepted date 18 April 2023

DOI: 10.21608/EJCHEM.2023.169552.7104

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mango seeds, soybean husks, rice husks, coconut fiber, palm oil wastes, water hyacinth, almond shells, pine soft wood and pineapple wastes [6,7,8,9]. Studies have also been reported on CNC and CNF extracted from bamboo, cotton, sugar beet, banana rachis, hardwood and softwood chips [10]. While these are methods of extraction by degrading biomass materials, newer advances also explored the possibilities of synthesizing cellulose nanoparticles from ionic liquid 1-ethyl-3-methylimidazolium acetate for biomedical applications as in controlling drug release [11].

Applications of CNC and CNF to date are mostly as bioproduct fillers with the purpose of enhancing the mechanical properties, physical properties, barrier properties, biocompatibility, biodegradability, the surface area [12,13] and reinforcements [14,15,16], rheological modifier of cellulosic materials [17] and bioproducts such as CMC (Carboxymethyl Cellulose), chitosan, alginates and other biopolymer products. Furthermore, applications of CNC and CNF are also found applied in enzyme immobilization, functional compound wall material encapsulations, automotive industry, electronics and sensor devices [18,19]. At 5-30% concentration, CNC has the capacity to strengthen the crystalline structure of the resulted films or biofilms, thus raising the peak crystallinity which would also raise the tensile strength of the film and the durability towards water [20]. CNC currently are produced from wood fibers, some non-wood fibers, algae, tunicates, microbes, agro-industrial plant or animal biomass wastes and various other plant materials [21]. These materials are low cost and readily available [22,23,24]. Many studies have been conducted to search for naturally abundant, renewable and sustainable cellulosic source materials that are not contending with food materials.

Some of the mechanical extraction techniques of CNC, CNF and CMF are high pressure homogenization, micro fluidization, grinding, cryo-crushing, high intensity ultrasonication, high-speed blender, ultrafine grinding, and ball miller [26,27,28,29,30,31].



**Fig. 1:** Main application of nanocellulose [25]

Chemical hydrolysis methods aside the mechanical techniques are currently the most common method used [9,32,33] and from the biological side of things are the enzymatic hydrolysis method [34]. Studies have combined the methods as such acidic hydrolysis followed by ultrasonication [35,36] or the alkali treatments with the ball miller method [37]. Combinations of treatments can also be acid hydrolysis with cellulase as an enzymatic hydrolysis agent (biological method) to extract CNC from paper and wood pulp waste [38]. Various extractions methods have been developed over time by many researchers, mostly are a combination of chemical hydrolysis (acidic or alkaline) with physical-mechanical techniques, and mostly tends to be high costing methods, thus impacting the production cost of cellulosic nano-micro materials.

A good source of biomass that would not contend with food sources are underutilized crops such as the Giant Swamp Taro – GST (*Cyrtosperma merkusii*) of North Sulawesi. GST corm is mostly carbohydrate (81-83%), starch content is  $65.52 \pm 0.02\%$  with an amylose-amylopectin content of  $29.63 \pm 0.01\%$  and  $32.88 \pm 0.02\%$  respectively. GST has 18.55% fiber content. When producing starch from GST, up to 70% are cellulosic biomass materials that would be considered as wastes residues, therefore are readily materials to be extract for CNC and CNF [39].

This study was to isolate nano cellulose from GST starch production waste (GST debris) and investigate the effect of a low-cost pretreatment process and mechanical extraction method on the properties of the nano cellulose yield. The pretreatment process was carried out to remove

impurities in the GST debris, such as wax and lignin. Making cellulose more accessible to chemical treatments and mechanical treatments. The novelty of this study was the systematical study on pretreatment effects and the combination of alkali hydrolysis combined with ultrafine grinding towards the GST debris for nano cellulose extraction. The influence of pretreatments on the chemicals combine with mechanical extraction and morphology of debris GST was investigated and characterized. The morphology and crystallinity of the obtained nano cellulose were also investigated.

## 2. Experimental

### 2.1. Materials

GST waste/debris are residue biomass material obtained from the GST starch processing. These biomass materials are what is left from the starch extraction process including the GST corm peels and layers of GST corm collected from the cleaning and peeling process of the corm. During the starch extraction process the corm was disintegrated and pressed. The supernatant containing the starch was separated for sedimentation and starch collection while the residues or debris were discarded. These discarded debris were collected for CNC extraction.

### 2.2. Pretreatment of GST debris and biomass conditioning process

The collected GST debris were collected, and sun dried until moisture content was relatively stabil at about 14%. The dried debris was then grounded in a grinder to a particle size of mesh 100. The alkali pretreatment process of debris GST also doubled as delignification and bleaching treatment which was carried out according to methods of [40,41]. Firstly, the cleaned GST debris (2000 gram) was immersed in NaOH solution (15 wt%), 3000 mL, at room temperature and stirred for 24 hours to remove the impurities including pectin, wax and then washed with distilled water until a neutral pH value was achieved. The bleaching procedure was performed by adding the obtained delignated GST debris into the mixture of  $\text{Na}_2\text{OCl}$  (10 wt%) and stirred at room temperature for 12 hours before washing thoroughly with distilled water until neutral pH was once again achieved.

### 2.3. Determination of the chemical composition of debris GST

The chemical compositions of *debris GST* before and after each treatment were determined by NDF and Van Soest to determine the cellulose, hemicellulose, lignin and fiber. Ash content for all debris GST were analysed following the methods outlined in the Indonesian National Standard (SNI 01-2891-1992). While protein and carbohydrate

content were measured with the Dumas combustion method (DuMaster Buchi D-480, Switzerland).

### 2.4. Extraction of GST nanocellulose

GST nanocellulose were produced in Integrated Laboratory of Bioproduct (i-Lab) – LIPI BRIN, Cibinong, West Java. The ultrafine grinding of the pretreated pulp was done with a supermasscolloiderMKCA6-5J grinder (Masuko Sangyo Co., Ltd., Japan) at a speed of 1500 rpm and a temperature of 78–79 °C (measured from the feed cone). First, the pre-ground GST (100 g dry weight) was diluted to a consistency of 1.7% with distilled water and pH was adjusted to 7 [45]. The suspension, which was under continuous stirring with an overhead mixer, was circulated using a peristaltic tube pump (Watson-Marlow Fluid Technology Group, USA) through the silicon carbide stone grinder from the vessel submerged in a hot water bath. The disk gap was reduced gradually from zero gap (contact mode) to  $-90\ \mu\text{m}$  within the first 10 min and then grinding was continued for 50 min, after which the gap was adjusted to  $-100\ \mu\text{m}$  for an additional 60 min of grinding. Due to the evaporation of water, the suspension was diluted by adding hot water from time to time. The average grinding consistency was around 2.2%. Small intermediate samples were collected after 30 min and 90 min of grinding, and at the end of grinding (120 min). Collected sample was then freeze dried for further analysis.

### 2.5. Particle size distribution determination

Particle size analysis was done at Laboratories for Nuclear Materials- PUSPITEK, LIPI-BRIN, Serpong, Tangerang. The measurement of particle size distribution and average particle size of CNC samples was conducted using LB-550 type particle size analyzer (Horiba, Japan) by Dynamic Light Scattering (DLS) using a Non-Invasive Back Scatter (NIBS) technique, with HeNe 4 mW laser source at 633 nm wavelength, and hydrodynamic range of 0.6 nm – 9000 nm.

### 2.6. Fourier-transformed infrared (FTIR) spectra analysis

GST CNC spectra analysis was carried out in the Integrated Laboratory of Bioproduct (i-Lab) – LIPI BRIN, Cibinong, West Java. Fourier-transformed infrared (FT-IR) spectra of nano cellulose samples were collected using a Nicolet 5700 FT-IR spectrometer in the wavenumber range between 4000 and 500  $\text{cm}^{-1}$ , at a spectral resolution of 4  $\text{cm}^{-1}$ . Each sample was palletized with KBr powder to prepare pastille.

### 2.7. X-ray diffraction analysis

The X-ray diffraction analysis of GST CNC was done at the Integrated Laboratory of Bioproduct (i-Lab) – LIPI BRIN, Cibinong, West Java. The X-ray diffraction (XRD) patterns for all CNC samples were characterized with an X-ray diffractometer (X'TRA-055, ARL, Switzerland) using a Cu K $\alpha$  radiation ( $\alpha = 0.154$  nm) at 50 kV and 100 mA. Scattered radiation was detected in the range of  $2\theta = 5-10^\circ$ , at a scan rate of  $2^\circ/\text{min}$ . Based on XRD data, the crystalline index (CI) of the GST CNC samples were then produced by using the method described by [42].

### 2.8. Thermogravimetric analysis (TGA)

Thermal behavior of GST CNC was carried out at the Integrated Laboratory of Bioproduct (i-Lab) – LIPI BRIN, Cibinong, West Java, using the PerkinElmer TGA 4000 (PerkinElmer, USA). GST CNC was heat from  $25.00^\circ\text{C}$  to  $750.00^\circ\text{C}$  at a heating rate of  $10.00^\circ\text{C}/\text{min}$  under inert nitrogen at a flowrate of  $20\text{ml}/\text{min}$ . Weight loss and temperature differences were recorded and plotted simultaneously.

### 2.9. Statistical analysis

Variance analysis of the chemical composition of the GST debris before and after delignification (alkali treatments) data sets were done with the Minitab statistical application. The significant difference among mean values was evaluated using one-way analysis of variance (ANOVA) followed by the Tukey's test at a significance level of ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1. Pretreatment of GST debris and biomass conditioning process

Biomass properties before and after the conditioning pretreatments with sodium hydroxide and delignification process with sodium hypochlorite was reported as in Table 1. Understanding the properties of the biomass at this stage of the process were to benchmark the biomass initial characteristics before further processing to the ultrafine wet grinding. As seen in Table 1. before biomass preconditioning and delignification, fiber (including NDF-ADF), lignin and hemicellulose were significantly higher. After the preconditioning and delignification process, cellulose became more apparent. This was the main target of this process to have the cellulose to be more exposed, while lignin and all other fibers and components would be cleared away.

Lignin concentration after delignification process decreased considerably to  $0.86 \pm 0.03\%$ . Lignin content of GST debris initially wasn't high to begin with ( $4.11 \pm 0.02\%$ ), therefore was an advantage of the GST debris where lignin content is lower than most other biomass sources. Other biomass sources such as pine chips had the initial

lignin content of  $51.19\%$  and beech chips with a lignin concentration of  $17.24\%$ , would result in more effort to lower the lignin concentrations as such by combining microwave treatments with NaCS (sodium cumene sulfonate) solutions [43].

**Table 1**

The initial conditions of the biomass before undergoing ultrafine wet grinding

|                   | Before             | After              |
|-------------------|--------------------|--------------------|
|                   | delignification    | delignification    |
| Dry Matter (%)    | $83.77 \pm 0.01$ a | $83.64 \pm 0.02$ b |
| Ash (%)           | $11.71 \pm 0.03$ b | $48.24 \pm 0.02$ a |
| Crude Protein (%) | $2.19 \pm 0.05$ b  | $4.53 \pm 0.05$ a  |
| Fiber (%)         | $8.73 \pm 0.06$ a  | $6.31 \pm 0.05$ b  |
| Crude Fat (%)     | $0.57 \pm 0.04$ b  | $2.43 \pm 0.02$ a  |
| NDF (%)           | $60.69 \pm 0.06$ a | $12.27 \pm 0.05$ b |
| ADF (%)           | $10.36 \pm 0.06$ a | $8.06 \pm 0.04$ b  |
| Hemicellulose (%) | $50.33 \pm 0.01$ a | $4.22 \pm 0.01$ b  |
| Cellulose (%)     | $6.01 \pm 0.04$ b  | $6.52 \pm 0.01$ a  |
| Lignin (%)        | $4.11 \pm 0.02$ a  | $0.86 \pm 0.03$ b  |
| Silicate (%)      | $0.24 \pm 0.00$ b  | $0.67 \pm 0.01$ a  |

The mean value followed by different letters showed a significant difference at the 5% level ( $p\text{-value} < 0.05$ )

### 3.2. Particle size analysis

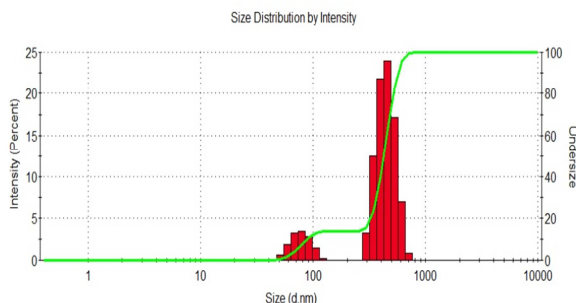
Appearances of the disintegrated pretreated biomass-cellulose (CNC) are as shown in Figure 2. Results of the CNC appears diluted in distilled water (Figure 2a). The needs for distilled water were due to the conditions of the grinder (Figure 2b) where the stones would heat up and necessary to keep adding distilled water to keep the silicon carbide grinding disk stones from heating [45].

Average size distribution of the GST CNC was ranging from  $449.6$  nm to  $78.26$  nm, which was obtained after sodium hydroxide treatment as an alkali activation treatment, then a sodium hypochlorite treatment for the delignification process and followed by ultrafine wet grinding to disintegrate the cellulose (Figure 3). Similarly, to results of CNC hydrolyzed from different cascades of treatments had different size distribution as such acid hydrolysis and ultrasonication process resulted CNC at a size of  $1752\text{nm}$ , acid hydrolysis and enzymatic hydrolysis resulted in  $667\text{nm}$ , and acid hydrolysis followed by enzymatic and sonication processes resulted in diameter of  $401\text{nm}$  [44]. Particle sizes of CNC varies from as small as  $2-20\text{nm}$  to  $100-600\text{nm}$  depending on the stages of treatments performed to activate and condition the biomass to attain the cellulose, in which currently most effective methods are by alkaline process or by acidic, and finally the size disintegration process which can be done by the enzymatic, ball miller, or sonication process [25,44,46]. In this study the cellulose went through a direct mechanical disintegration process by ultrafine wet grinding. Nano cellulose particles synthesized

from ionic liquid 1-ethyl-3-methylimidazolium acetate had particle sizes of 537.4 to 274.9 nm [11]. Therefore, particle size of GST CNC are within the parameters of the many studies of cellulose nano crystals and particles alike.



**Fig. 2:** CNC results (a) from cellulose disintegrations of ultrafine grinding machine (b)

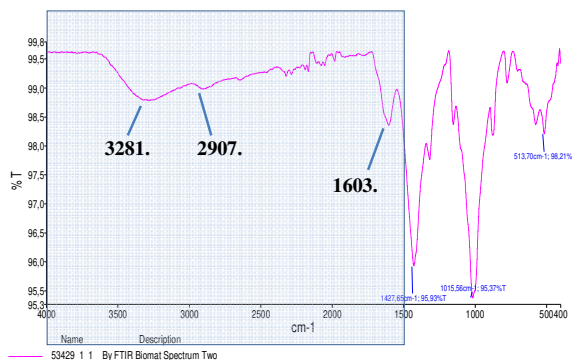


**Fig. 3:** Particle size of GST CNC

### 3.3. Fourier-transformed infrared (FTIR) spectra analysis

**Figure 4** depicts the spectrums from the FTIR spectra of the CNC. Within the functional group regions, it is apparent that the major peaks appeared at  $1500\text{--}1700\text{cm}^{-1}$ , followed by  $2500\text{--}3300\text{cm}^{-1}$ . The peak at  $1602.45\text{cm}^{-1}$  appeared strong which indicates to be C=C stretching, The weak peak at  $2907.19\text{cm}^{-1}$  relates to the presence of stretching of C-H groups. Within the functional group area another weak peak appeared at  $3281.02\text{cm}^{-1}$  which most likely to be related to the presences of hydroxyl groups which would appear at the ranges of  $3300\text{cm}^{-1}$ [45]. C=C groups are known to be related to bands at  $1595\text{--}1605\text{cm}^{-1}$ , at  $1600\text{cm}^{-1}$  are also observed in aromatic rings of lignin [47]. Weak peaks at the  $2908\text{cm}^{-1}$  were

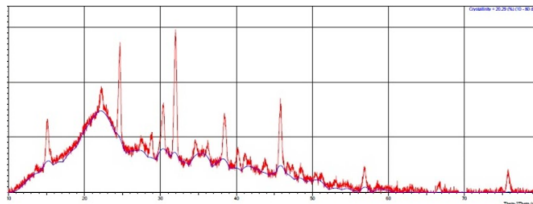
also reported to be the presence of  $\text{CH}_2$  stretching in cellulose nano whiskers, while  $3300\text{--}3600\text{cm}^{-1}$  are commonly attributed to -OH and -NH overlapping in polysaccharides [48].



**Fig. 4:** FTIR spectra of GST CNC

### 3.4. X-Ray diffraction analysis

As described in Figure 5 the XRD results of the obtained CNC appeared to have the strongest peak at  $31.93^\circ 2\theta$ ,  $24.58^\circ 2\theta$  and  $45.74^\circ 2\theta$ . These peaks would be attributed to patterns of cellulose II, as supposed to cellulose I. While the crystallinity was at 20.29. The XRD pattern of native cotton cellulose crystals were  $14.9^\circ 2\theta$ ,  $16.4^\circ 2\theta$  and  $22.5^\circ 2\theta$  and cotton cellulose nano crystals produced by dissolution and regeneration in 1-butyl-3-methylimidazolium chloride ([BMIM]<sup>+</sup>[Cl]<sup>-</sup>) solution resulted in peaks at  $11.7^\circ 2\theta$ ,  $20.1^\circ 2\theta$  and  $21.6^\circ 2\theta$  were identified as cellulose crystalline allomorphs [49]. Alkali treated cellulose beyond 10% NaOH resulted in peaks at  $20.0^\circ 2\theta$  and were identified as cellulose II polymorphs due to the extent of the alkali exposure that induced swellings of the fibres, therefore alkali treatment would act as an activation agent exposing the cellulosic materials to various other treatments such as enzymatic or mechanical treatments[50]. Ball milling and ultrafine wet grinding treatments are common approaches in modifying the surface profiles with less involvement of chemical treatments. But these mechanical methods require a liquification stage which alkali treatments provides this stage while activating the cellulose and exposing the cellulose to better reactivity to the disintegration process [25,37,50].



**Fig. 5:** XRD Diffractogram of GST CNC

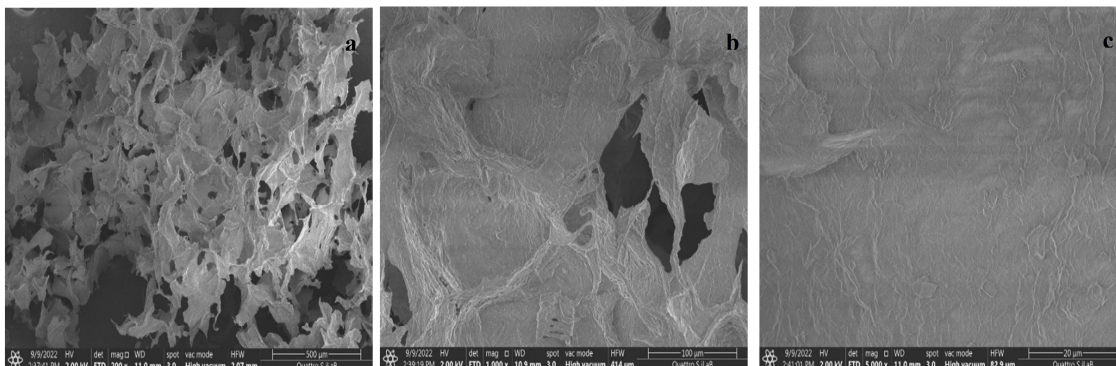
### 3.5. FESEM microstructures of GST CNC

The FESEM microstructures are as shown in Figure 6. The appearance of the CNC in the various magnifications is observed as disorganized, thin flakes held by a fibrous network. At 200x magnifications the CNC could be seen the flakiness of the structure. The GST CNC structure results did not aggregate and appeared consistent as in thin sheets of flakes with a fibrous network. Observing at a deeper magnification (1000x) on a singled-out area, the fibrous network morphology becomes more noticeable as thus with the 5000x magnifications. The fibers seen on the surface of the structure are uneven and unorganized, consistent with the particle size results that are ranges in a widespread range (78.26 nm-449.6 nm). This fibrous rod like structure of CNC is typical for Nano Cellulose structure [51]. While flake-like structure has also been reported and consistent with the presences of cellulose nano whiskers as indicated by the CH<sub>2</sub> stretching on the FTIR results [48].

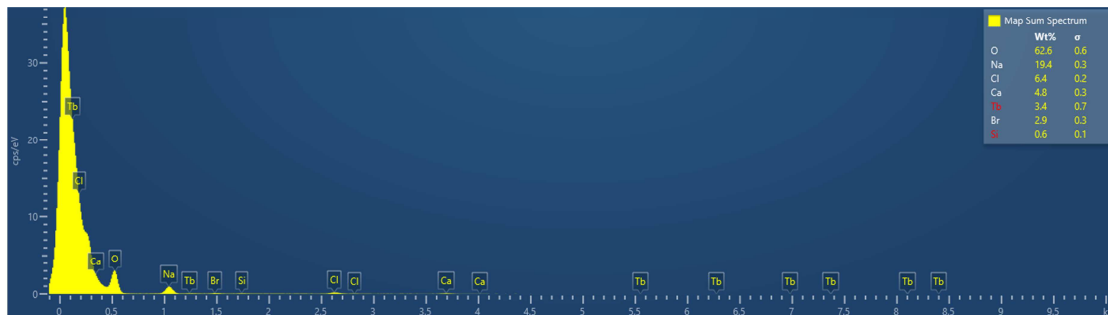
would be fitting with the FTIR results indicating the vibrations of the -OH in polysaccharides. While traces of Cl, Ca and Na are also natural to GST as a coastal species. Tb (terbium) are amongst on the rare earth elements (REE), naturally occurring in marine or coastal ecosystems. This concurs with the fact that GST natural habitat are coastal ecosystem as GST are coastal plants growing amongst the nipah plants behind the mangroves, especially due to that REE bioaccumulation are more apparent in non-mangrove coastal species [52].

### 3.6. Energy dispersive spectrum of GST CNC

The energy dispersive spectrum of the GST CNC presented traces of Tb, Cl, Ca, O, and Na (Figure 7). Presence of O are common as should be the presence of C and H, in which the presences of O



**Fig. 6:** a.FESEM microstructure at 200x magnification; b.FESEM Microstructure at 1000x magnification and c.FESEM Microstructure at 5000x magnification

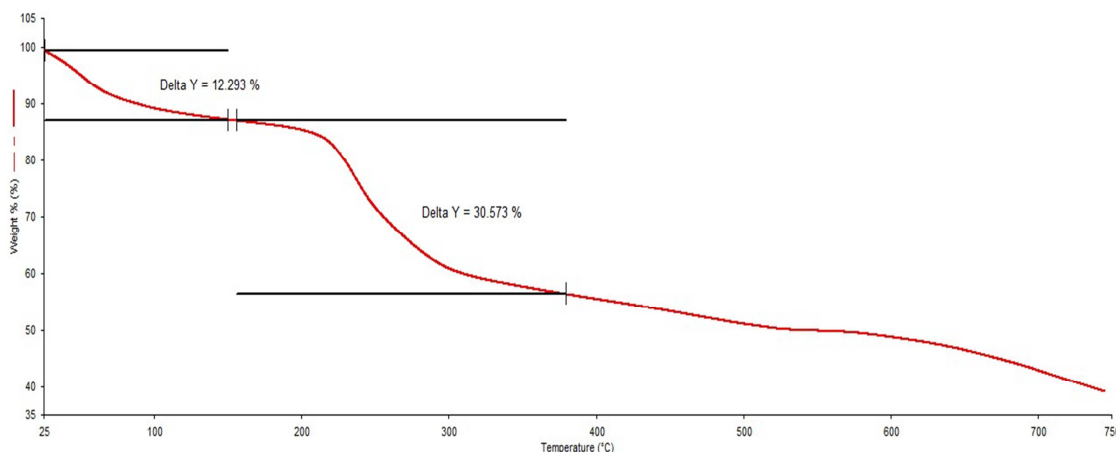


**Fig. 7:** EDS spectra of GST CNC

### 3.7. Thermogravimetric analysis (TGA) Of GST CNC

GST CNC thermostability as expressed in Figure 8 indicates points of moisture loss (evaporation) and degradative weight loss of the CNC. Initial degradation was recorded at 130.49 °C (at 12.293% weight loss). The initial degradation as indicated by various studies pinpoints the process of dehydration (evaporation) and the state of total moisture loss nearing to 100 °C [11,53,54]. The second degradation was noted at 359.87 °C (at 57.134% weight loss or 30.573% weight loss after the initial degradation). Beyond this second degradation point as depicted in

Figure 8 the rate of weight loss appeared as a steady decline indicating a condition of better thermal stability in decomposition rate corresponding to the increase of temperature close to 750 °C, which in this study total decomposition was ended at 745.12 °C and at this point GST CNC still had 39.067% of mass to lose. Studies have also observed a 2-step degradation process although the second degradation point observed were steep and rapid along with the rise in temperature [11,53,54].



**Fig. 8:** Dynamic TGA Curve of GST CNC Temperature vs Weight (%)

### 4. Conclusion

Effectiveness of alkali pre-treatment on GST wastes were mainly in eliminating hemicellulose since lignin in GST wastes weren't significantly apparent in the first place and the following disintegration process using ultrafine grinding resulted GST CNC at the sizes of 449.6 nm to 78.26 nm. CNC results of these low-cost processes were thin flakes with a fibre network which appeared like finely threads nano whiskers. Traces of coastal ecosystem mineral and rare elements are found in GST CNC as indicated in EDS, after all GST is a coastal plant. In response to heat treatments, the GST CNC had relatively better thermal stability compared to other studies but was observed to also have a 2-step decomposition (110°C and 359.87°C).

### 5. Conflicts of interest

There are no conflicts to declare

### 6. Acknowledgment

The authors acknowledge to the Ministry of Education, Culture, Research, and Technology through Hibah Riset Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) 2022, contract number 123/E5/PG.02.00.PT/2022.

The authors acknowledge the facilities, scientific and technical support from Appropriate Technology Research Laboratories (iLab-Integrated Laboratory of Bioproducts), Cibinong, National Research and Innovation Agency through E- Layanan Sains-BRIN.

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