International Journal of Biological Macromolecules 131 (2019) 858–870

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

# Novel processing parameters for the extraction of cellulose nanofibres (CNF) from environmentally benign pineapple leaf fibres (PALF): Structure-property relationships

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ARTICLE INFO

*Article history:*
Received 27 November 2018
Received in revised form 9 February 2019
Accepted 20 March 2019
Available online 21 March 2019

*Keywords:*
Pineapple leaves waste
Lime juice hydrolysis
Ball milling
Cellulose nanofibres

ABSTRACT

Development of bio-nanoreinforcement is gaining momentum worldwide nowadays since it contributes much to the green materials era. This paper presents a proposal for reusing the agro waste to produce cellulose nanofibres in an ecofriendly way. The present work aims to isolate cellulose nanofibres (CNF) from pineapple leaf fibres (PALF) via green-cost effective route using lime juice for acid hydrolysis and ball milling for defibrillation. PALF is considered as a waste material after the cultivation of fruits. But PALF is rich in cellulose, fully biodegradable and renewable which makes it a perfect candidate for the extraction of CNF. The main objective of the present work is to avoid the use of hazardous acids and reduce the high energy consumption for the isolation of cellulose nanofibres. Nowadays the use of these green methods is relevant in order to prevent pollution in the environment. The extracted fibres have been characterised by FT-IR, XRD, FESEM, HRTEM, AFM, DLS and Thermal analysis. FT-IR results show the isolation of cellulose nanofibres by losing hemicellulose and lignin. In XRD, the increase in crystallinity (77%) is a clear indication of removal of lignin and hemicellulose present in amorphous region. The Thermal analysis reveals that the thermal stability increases for the isolated nanofibres. The maximum degradation temperature observed for isolated CNF is 344.49 °C. The surface morphological analysis (AFM, FESEM and HRTEM) showed that lime juice hydrolysis and ball milling have been a successful method for the isolation of cellulose nanofibres which can be used an effective reinforcement for preparation of polymer nanocomposites. The diameter of CNF from FESEM, HRTEM and DLS obtained was around 30–85 nm, 10–50 nm and 420 nm respectively. Present work points out the chances of recycling the agro waste (pineapple leaves) to extract cellulose nanofibres and thereby preserving nature in an environmentally benign way. Analysis of production cost reveals that the proposed green methodology is economic, able to produce CNF on a large scale and can be utilized in food industry, paper making, biomedicine and machinery tools.

## 1. Introduction

Indian economy mainly relies on agriculture. Natural fibres extracted from agricultural residue can contribute much to the economy of a nation. In order to reduce the waste generated from the agricultural crops, large number of innovative ideas has been developed by researchers to utilise it. Global warming is a serious issue generated as a result of fossil fuel burning. Nowadays researchers have been focussing on natural polymers to reduce these diverse problems. Several methods are used for producing nanomaterials but the environmental issues created by the methodologies are regarded as a serious issue. The utilization of agricultural residue and green methodologies are the perfect solutions for reducing the degradation of environment. The unique properties of natural fibres are biodegradability, environmental friendly, non-carcenogenic, low cost, no health hazards and easy to handle. These properties made them to compete with synthetic fibres since they have adverse effect on the nature. Natural fibres found many applications in the field of automotives, structural components, and packaging. In order to maintain the ecological balance of environment large number of research works are going on to use these natural fibres derived from the agro waste.

Even though the awareness of cleaner production and sustainability is increasing the proper waste management of agricultural residues all around the world is decreasing. By adopting green methodologies will improve eco-efficiency, lower energy consumption, reduces emissions to the environment, retards the use of toxic chemicals and reduces waste generated. Nanocellulose exhibits broader market value but the use of expensive precursors, toxic chemicals, and high energy consumption make environmental impact.

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https://doi.org/10.1016/j.ijbiomac.2019.03.134
0141-8130/

Cellulose is one among the natural fibres present in plants, animals, bacteria etc., which are widely used in the field of automotives, furnishing etc. [1]. It is having excellent mechanical properties, high aspect ratio, biodegradable, renewable and nontoxic. In 1870 Hyatt Manufacturing Company synthesized Celluloid, the first thermoplastic polymer made by treating cellulose with nitric acid. Cellulose is comprised of cellobiose (a dimer of glucose) units consisting of anhydroglucose linked together by β-1,4 linkages [2]. Cellulose appears like fibrous arrays found in wood, plants etc. It consists of linear polymer with hydroxyl groups which are responsible for strong bonding. Cellulose in nanoscale dimension is referred as "nanocellulose". Nanocellulose can be classied into cellulose nanocrystals (CNC), cellulose nanowhiskers (CNW), cellulose nanofibrils (CNF) and bacterial cellulose (BC).

CNF contains bundles of fibres with a diameter in nanometre range and length in micrometers. Unlike CNC fibrils of CNF can exist as both in amorphous and crystalline form. CNF can be obtained by means of mechanical, chemical and a combination of both [3]. Mechanical treatments involve homogenization, grinding, milling etc. An example for chemical treatment is TEMPO oxidation. In CNC, the fibrils are in crystalline form and can be prepared by means of acid hydrolysis [4]. Gram-negative bacteria (Acetobacterxylinum or Gluconacetobacterxylinum) is the major source for bacterial cellulose [5]. Cellulose contains carbon, hydrogen, and oxygen and it can be included in the class of carbohydrate. The unique feature of cellulose is that it can be prepared and can be divided into monosaccharides by means of hydrolysis [6].

Cellulose is a rigid, stable polymer due to inter and intramolecular hydrogen bonding and its solubility is very low in solvents and does not exhibit melting point [7]. Nano sized cellulose extracted from plant fibres show enhanced properties when it is combined with polymer matrix. In 1983 Turbak et al. [8] found that cellulose nanofibre can bring about change in rheology when it is associated with food, cosmetics, paints, pharmaceutical products etc. Due to its hydrophilicity it can excellently combine with hydrophilic polymers. The diameter of cellulose nanofibres may vary based on their source and processing parameters. Fibrous cellulose with a thickness of 2–10 μm and length of thousands of microns generated during biosynthesis of plants are termed as microfibrils [9]. From wood it shows a thickness of 3–5 nm [10]. There are other sources for cellulose such as tunicates, plant fibres, microorganisms like algae, fungi, bacteria etc., leaf like sisal, cotton fruit etc. Use of cellulose nanofibres (CNF) has increased tremendously due to its potential applications in the field of packaging, composites, electronic films, drug delivery, water treatments etc. Due to the high surface area, CNF can be used as perfect reinforcements for preparing polymer nanocomposites.

Enormous studies are still going on for the extraction of CNF from different sources (wood pulp, sugarcane bagasse, cotton fibre, banana peel etc.) using different methods [11–14]. For the extraction of cellulose nanofibres, different methods can be employed [15–20]. The properties of the nanocellulose extracted from the natural sources strictly rely on the isolation methods and the source used. Microfibrillated cellulose (MFC) are those fibres with large diameter. The range of diameter is actually determined by degree of fibrillation. MFC with a diameter bellow 100 nm is named as cellulose nanofibres or nanofibrils, or nanofibrillated cellulose and it can be obtained by the application of high pressure homogenization or by surface modification [21]. By disintegrating natural cellulose mechanically the least diameter obtained is about 10 nm [22]. There are mainly two mechanical methods associated with CNF production namely conventional and non- conventional methods. Conventional methods include homogenization, grinding etc. while non- conventional include blending, cryocrushing, ultrasonication etc. CNF synthesized using different techniques will show various grades in their morphology, surface chemistry, crystallinity etc.

Due to the unique characteristics of cellulose nanofibres like high aspect ratio, large surface area, biodegradability, high mechanical strength, it can be used as a perfect nanofiller for reinforcing polymer matrix [23–28]. CNF found many applications in the field of electronic devices [29], porous membranes [30], barrier films [31,32], Ultrafiltration membranes [33], and adsorption materials [34].

Pineapples are considered as a favourite fruit around the world. But the other parts of the plant (leaves, stem, root) are considered as an agricultural residue. After the harvesting of fruits the disposal of pine apple leaves found to be a serious issue for the farmers worldwide. The proper management of this agro waste will promote the economy and preserve the nature. Nowadays softwood and hardwood are the major sources of nanocellulose. But it is less available and highly expensive. The pineapple leaf fibres derived from the leaves are long and strong which can be used as an efficient replacement for softwood due to its ease of availability and low cost. Pineapple leaves are rich in cellulose but it has not exploited much for the extraction of nanocellulose. Pineapple leaf fibres are mainly composed of larger quantity of cellulose (81.27%) and lower quantities of hemicellulose (12.31%) and lignin (3.46%) [35]. Due to higher cellulosic content PALF shows superior mechanical properties, compared to all natural fibres it has high tensile strength and Young modulus. Some researchers isolated CNF from PALF using steam explosion method [24,36].

The general procedure for extracting cellulose nanofibres include alkali treatment, bleaching, acid hydrolysis followed by defibrillation using conventional or non-conventional methods. All the methods are very expensive and require high energy. Herein we report a cost effective ecofriendly method for the isolation of cellulose nanofibres from pineapple leaf fibres. For acid hydrolysis, mineral acids are usually used which is very hazardous process and expensive. Commonly used acids for hydrolysis include Sulphuric acid, Nitric acid, Phosphoric acid or their mixtures. The problems associated using these mineral acids are corrosion of the equipment, deterioration of cellulose and requirement of large amount of water. By using mineral acids there is a chance of breaking amorphous regions of the cellulose resulting in the formation of small needle like Cellulose nanocrystals. The use of mineral acids will create more effluents and need large amount of water for neutralization. This limits its industrialization [37].

Recent studies showed that CNF are used as dietary fibres since it is nontoxic, able to absorb harmful substances in the body, helps digestion and decreases the amount of cholesterol in the body [38]. Scientists are developing new green strategies for the production of CNF. Enzymatic hydrolysis is the one eco-friendly method but the low efficiency, high cost, time consuming process and low yield limited its applications. Moreover Arviddson et al. [39] compared the environmental impact of pre-treatments done to obtain CNF. They found that carboxymethylation of CNF creates environmental impact since this route uses large amount of organic solvents derived from the crude oil. While CNF produced via TEMPO mediated oxidation reduced environmental hazard but the high cost of TEMPO catalyst retards its commercialization.

Here comes the importance of natural acids like citric acid. Citric acid present in lime juice act as the hydrolysing agent. Citric acid is an organic acid which is extensively used for the extraction of pectin, a non-cellulosic part that wrap around the cellulose [40–42]. The use of lime juice is ecofriendly and economical since it protects environment from the issues caused by the corrosive acids. It also reduces the amount of water needed for neutralization, safe for the operators and no health issues. Present work deals with the acid hydrolysis of bleached pulp using lime juice followed by ball milling the hydrolysed pulp for obtaining the cellulose nanofibres.

The major problem faced to commercialize CNF is the high energy consumption during the production process and expensive machines (homogenizer, microfluidizer, grinding). Due to the environmental concern, Haishun and co-workers prepared funtionalised CNF by hydrolysing with formic acid and size reduction by homogenization [43]. But softwood (raw material) and the homogenizer are highly expensive. Compared to homogenization, microfluidization, and grinding, ball milling is not a common technique used for the production of CNF. This machine requires less energy and is economical. Ball milling is a technique which is used for the size reduction of materials from macro to nanoscale. This is a top down process which brings deformations to the materials. The principle behind size reduction relies on the energy applied between the material and the milling media. CNC have been isolated from cellulose using organic acid assisted ball milling technique was reported [44]. They observed that the isolated CNC exhibits high thermal stability and high yield. Ball milling is found to be an effective method for the production of nanocellulose since it can break the longitudinal axis of cellulosic structure [45,46]. Thus the crystallinity of cellulose is reduced there by resulting in the isolation of cellulose nanofibres [47–50]. Furthermore compared to other mechanical equipments ball milling can produce high quantity of CNF at room temperature and pressure at low cost and energy consumption [51].

Based on the drawbacks of previously mentioned reports, the present work focus on the isolation of CNF from pineapple leaf fibres (PALF) via green cost effective route using lime juice for acid hydrolysis and ball milling for defibrillation. The pretreatments we have employed were; alkali treatment, bleaching, followed by acid hydrolysis (using lime juice) and finally ball milling for size reduction. To best of our knowledge acid hydrolysis using lime juice and ball milling for defibrillation has not been employed for the isolation of CNF elsewhere. The detailed characterisation of isolated CNF will be done using various spectroscopic and microscopic studies (FT-IR, XRD, FESEM, HRTEM, AFM and Thermal analysis). It is expected that acid hydrolysis using lime juice and ball milling is an effective method for the isolation of CNF. The efficiency of the new route for the isolation will be analyzed. The green technology proposed will reduce the pollution (air, water, soil) of the nature by turning the useless agricultural residue to a highly potential resource.

## 2. Materials and methods

### 2.1. Materials

Pineapple leaves and lemons were collected from local sources. The reagents used for chemical treatments of pineapple leaves were NaOH (analytical grade, Merck, India), glacial Acetic acid (analytical grade, Merck, India), $NaClO_2$ (analytical grade, Lobachemie) purchased and used.

### 2.2. Methods

#### 2.2.1. Chemical treatments

Pineapple leaves were collected from local sources and washed, dried in sunlight, powdered using mixer grinder. The powdered samples were first treated with 2% sodium hydroxide solution and magnetically stirred for 4 h at 100 °C. Washed with distilled water several times and dried in air oven. The process repeated for two times. After alkali treatment the samples undergone bleaching with acetate buffer (27 g of NaOH and 75 mL glacial acetic acid diluted to 1 L of distilled water) and aqueous $NaClO_2$ (1.7 wt%). It is repeated for five times in order to remove the lignin and hemicellulose. The bleached sample appeared to be white in colour.

#### 2.2.2. Lime juice hydrolysis

For the sake of eco-friendliness, the use of strong acids have been avoided for hydrolysis and adopted a green protocol for acid hydrolysis i.e., lime juice is used for hydrolysis. Lime juice is diluted with distilled water (1:7) and added to 2 g of bleached pineapple leaf fibres. The pH of the diluted lime juice is 2.5. This is stirred using a mechanical stirrer with a speed of 4000 rpm for 2 h. Then the suspension was washed several times with distilled water to remove the excess acid.

#### 2.2.3. Ball milling

The colloidal suspension was ball milled in Planetary ball mill consisting of plastic container of 500 ml capacity with ceramic balls of different dimensions (10 mm and 20 mm). The ratio of ball to suspension weight is 18:1. The ball milling was carried out for 1.5 h and 3 h. We have got white cream like colloid and used for analysis. Figs. 1 and 2 show schematic illustration of synthesis of cellulose nanofibres.

### 2.3. Characterisations

#### 2.3.1. Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy was recorded using PERKIN ELMER ATR infrared spectrometer in the range of 400–4000 $cm^{-1}$. The FT-IR spectra were taken by making pellets of powdered samples (raw, alkali treated, bleached, hydrolysed, ball milled) mixed with KBr.

#### 2.3.2. X-ray diffraction analysis (XRD)

The crystallinity of the samples (raw, alkali treated, bleached, hydrolysed, ball milled) were recorded on Bruker AXS D8 Advance with Cu $K\alpha$ radiation with an angle range 5°–80° ($2\theta$ angle range) at a wavelength of 1.541 Å, an operating voltage of 45 kV and a current of 35 mA.

The crystallinity index of the different samples was calculated using Segal method which is mentioned in the equation [52].

$$I_{cr} = \left[I_{(200)} - I_{(am)}/I_{(200)}\right] \times 100$$

$I_{cr} \rightarrow$ crystallinity index

$I_{(200)} \rightarrow$ maximum intensity of (002) lattice diffraction at $2\theta = 22.5^{\circ}$

$I_{(am)} \rightarrow$ intensity diffraction of amorphous region at $2\theta = 15.8^{\circ}$

#### 2.3.3. Thermogravimetric analysis

The thermal properties of the samples were analyzed using Perkin Elmer, Diamond TG/DTA. About 10 mg of the sample put on the alumina cup and heated at a rate of 20 °C/min in Nitrogen atmosphere.

#### 2.3.4. Atomic force microscopy (AFM)

The surface morphology of isolated nanofibres were characterised with Atomic Force Microscopy (WITec GmbH, Ulm, Germany) in contact mode at room temperature. The suspension of cellulose nanofibres was sonicated well and then analyzed.

#### 2.3.5. Field emission scanning Electron microscopy (FESEM)

The morphology of samples was analyzed using Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM) at acceleration voltage of 30 kV and Probe current of 1 pA–200 nA. All the samples (raw, alkali treated, bleached, hydrolysed, ball milled) were sputter-coated with gold to avoid charging.

#### 2.3.6. Dynamic light scattering analysis (DLS)

The average particle size distribution of the isolated cellulose nanofibres were carried out using Malvern zetasizer instrument (Malvern Instruments Ltd). The suspension of cellulose nanofibres were ultrasonicated before analysis. The following conditions maintained for analysis was Dispersant Name: Water, Dispersant RI: 1.330, Temperature (°C): 25 °C, Viscosity (cP): 0.8872. The measurements were conducted triplicated and the average of the measurements was reported.

#### 2.3.7. Transmission electron microscopy (TEM)

The transmission electron micrographs of dilute dispersion of cellulose nanofibres were obtained on a High-Resolution Transmission Electron Microscope, JEM-2100 HRTEM. A drop of suspension of each sample was placed on a carbon film-coated copper grid, dried up and examined.

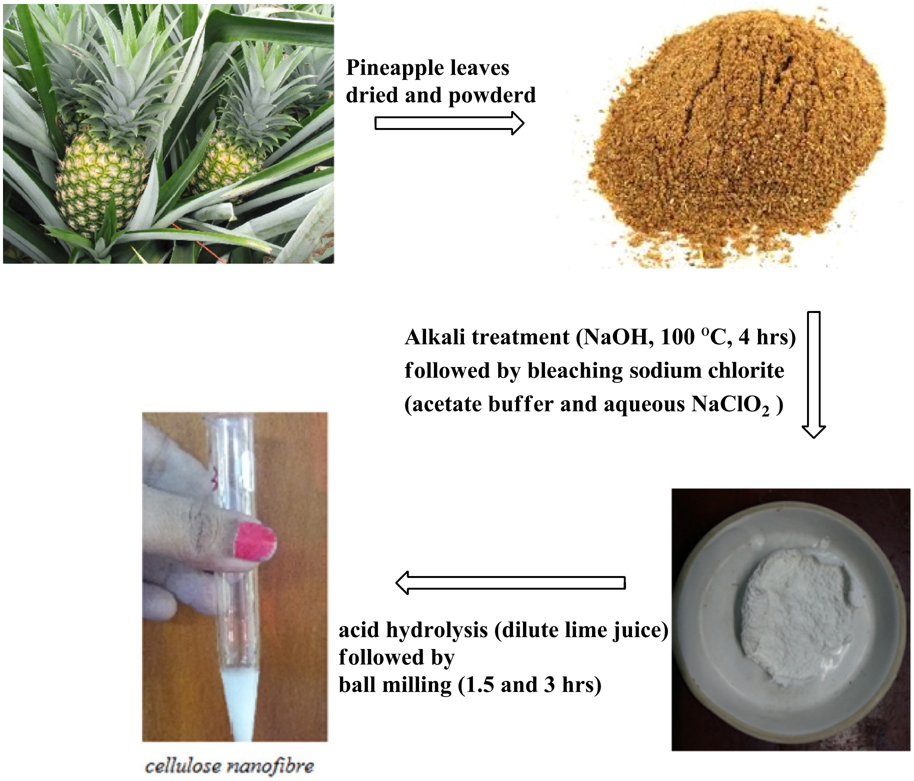


**Fig. 1.** Schematic illustration of synthesis of cellulose nanofibres.

## 3. Results and discussions

### 3.1. Characterisation of cellulose nanofibre (CNF)

#### 3.1.1. FT-IR characterisation of CNF

Plant fibres are rich in cellulose, hemicellulose and lignin. It means it contains several oxygen containing groups in different forms like alcohols, acids esters, ketones etc. The raw fibres are composed of both cellulosic and noncellulosic regions. The pre-treatments are able to remove the noncellulosic components attached with the cellulosic components. The purpose of alkali treatment is to remove pectin, lignin and hemicelluloses associated with pure cellulose [53,54]. This is due to the breakage of ether linkages found in noncellulosic regions. The alkali treatment and bleaching will result in the swelling of the microfibres which indeed helps the breakage of noncellulosic part during lime juice hydrolysis. The peak at 3300 cm$^{-1}$ shows the presence of hydroxyl group. This makes cellulose hygroscopic in nature [55]. The peak at 2900 cm$^{-1}$ shows polysaccharide group as it is slightly visible in raw pineapple leaves. The intensity of this peak is higher for CNF since the noncellulosic part was removed and due also due to the purity of isolated CNF [56]. The band at 1035 cm$^{-1}$ indicates the stretching frequency of C—H and C=O group of cellulose [57]. Many of the authors found that the increase in the intensity of this peak attributes to the removal non cellulosic parts bound around the cellulose [58,59]. The two peaks at 1430 and 895 cm$^{-1}$ show the crystalline band of cellulose which is a clear indication of the presence of cellulose after chemical treatments [60,61]. The intensity of the peak increases after ball milling which shows that hemicelluloses and lignin are lost without losing cellulose. The increased intensity of peak at 2900 cm$^{-1}$ after ball milling points out the asymmetric stretching vibration of $CH_2$ group in cellulose. All these results clearly indicate that cellulose content is maintained throughout the process losing hemicelluloses and lignin. Fig. 3 shows the FT-IR spectrum of different treatments of pine apple leaves (raw, alkali treated, hydrolysed, ball milled).

#### 3.1.2. X-ray diffraction analysis (XRD)

Pineapple leaf is composed of 3.46% lignin, 12.31% Hemicellulose, 81.27% cellulose [35]. The two major peaks appeared in diffractogram is $2\theta = 15.8$ and 22.5°. Higher crystallinity is the key factor which determines better reinforcement with the polymer matrix. Here the intensity of peak increases after lime juice hydrolysis and ball milling which is a clear indication of removal of lignin and hemicelluloses present in amorphous region. Similar observation was reported where the oxalic acid assisted ball milling of cellulose fibres up to 3 h increased the crystallinity of nanocellulose [44]. The increase in the intensity of peaks after each treatment is almost similar to earlier reports [62]. The crystallinity of cellulose can be attributed to the hydrogen bonding among the –OH groups. In the case of raw fibres cellulose is wrapped by the noncellulosic domains which are the reason for the lower crystallinity. The increase in crystallinity after lime juice hydrolysis and ball milling is a clear indication of removal of lignin, pectin and hemicelluloses.

The crystallinity of CNF produced from bamboo using microwave liquefaction followed by ultrasonication and chemical treatments is less compared to the green protocol we adopted [63]. Ultrasonication has disrupted the crystalline domain which is the reason for the decrease in crystallinity. The application of high pressure homogenization also decreased the crystallinity [64]. The crystallinity obtained was higher than CNF from pineapple leaves [24,36], pine [65], pinecone [66], and eucalyptus kraft pulp [67]. From the XRD results it is clear that the green and economic method we adopted was successful for the isolation of CNF. Fig. 4 shows the XRD spectrum of different treatments of pineapple leaves (raw, alkali treated, hydrolysed, ball milled). The crystallinity index calculated is shown in Table 1. After each treatment the crystallinity index increases gradually. These results were in good accordance with the findings of many researchers who employed different methods for the isolation of cellulose nanofibres [68–70]. Higher crystallinity improves better reinforcement with the polymer matrix.

*Raw pineapple leaves* *alkali treated pineapple leaves*

*Bleached pineapple leaves* *Cellulose nanofibres*

**Fig. 2.** Photographic images of different stages of pineapple leaves under chemical treatments.

### *3.1.3. Thermal analysis*

In order to study the thermal stabilities and degradation properties of treated and untreated pineapple leaves TGA was carried out. Fig. 5 [(a) and (b)] shows the TGA and DTG of various samples after treatments undergone by pineapple leaves. A small weight loss found in the region of 35–150 °C is due to the removal of water molecule absorbed into the fibre. Hemicellulose, Pectin and Lignin have low degradation temperature so the raw pineapple leaves exhibited a weight loss at 200 °C compared to the treated fibres and on the DTG curve it showed a maximum peak at 310.47 °C which can be due to degradation of cellulose (Table 2). The second degradation temperature is at about 270 °C which is due to the cellulose-hemicellulose depolymerisation. The third degradation temperature is above 380 °C because of the deterioration of lignin and carbon residues. The thermal degradation studies of CNC hydrolysed by sulphuric acid and oxalic acid-ball milled samples were compared and they found that the latter showed higher thermal stability [44]. Almost similar results were obtained for several authors [71–73]. The DTG curve of alkali treated, bleached, acid hydrolysed, ball milled fibres (1.5, 3) were 359.51 °C, 353.83 °C, 358.31 °C, 346.29 °C respectively which all attribute to the decomposition of cellulose (Table 2).

The higher decomposition temperature can be related to the partial removal of noncellulosic parts. Moreover the lower residue after treatments can be related to the removal of lignin and hemicelluloses. The higher degradation temperature is due to the higher thermal stability of isolated CNF. This result is in accordance with increase in crystallinity obtained from XRD results. The higher degradation temperature for CNF obtained from empty fruit bunch using chemo-mechanical treatment is 339 °C [74]. Nanocellulose extracted from PALF using high pressure homogenization and ultrasonication showed lesser degradation temperature (320 °C) [55]. Therefore highest thermal stability attained for CNF was due to the hydrolysis using lime juice followed by ballmilling for defibrillation. Fig. 6 shows DTA spectrum of different treatments of pineapple leaves (raw, alkali treated, hydrolysed, ball milled). The differential thermal analysis shows two major endothermic peaks. The first peak below 100 °C is due to the removal of water content. This peak is present for all type of fibres. A major degradation is observed after 350 °C which is due the depolymerisation, loss of hydroxyl groups and decomposition of hemicellulose and lignin. The endothermic peak at 359.5 °C for bleached fibres, 362 °C for hydrolysed fibres, can be attributed to the degradation of glycosidic linkage of cellulosic chains [75]. The shift in peaks relates to the removal of waxy materials bound

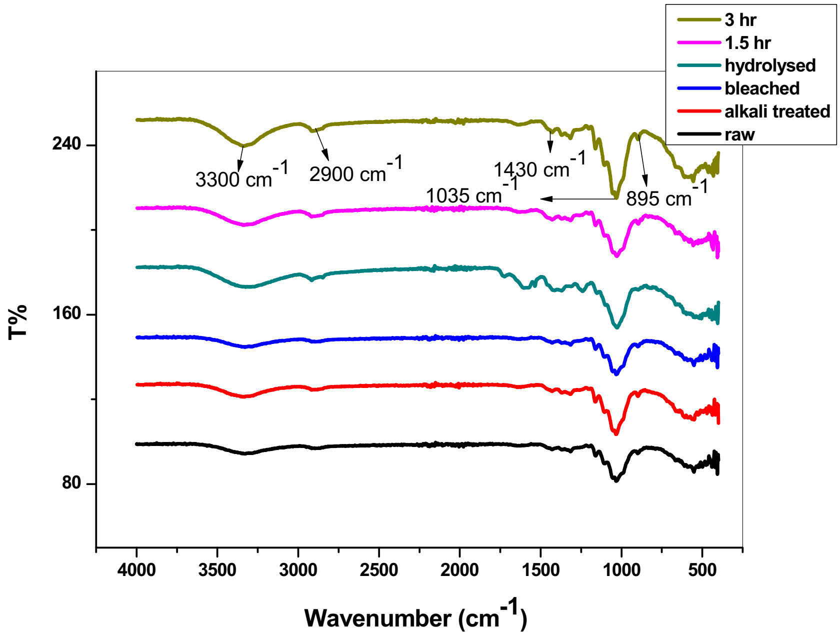


**Fig. 3.** FT-IR spectrum of different treatments of pine apple leaves (raw, alkali treated, hydrolysed, ball milled).

around the pure cellulose after each treatment. The increase in thermal stability is due to loss of noncellulosic materials and higher crystallinity of isolated CNF. All the thermal analysis reports point out that the Lime juice hydrolysis and Ballmilling found to be a novel processing method for the isolation of cellulose nanofibres.

### *3.1.4. Atomic force microscopy (AFM)*

From the atomic force microscopy the surface morphology of the nanofibril can be identified. AFM images show the formation of long fibres after ball milling the samples for 3 h. As a result of agglomeration of individual nanofibres, it is difficult to measure the exact diameter of the isolated nanofibre. The AFM image of nanocellulose consists of two regions namely; shiny region and dull region [76]. The shiny region corresponds to the crystalline part of the cellulose and dull region reveals the amorphous part. The AFM image clearly shows these two regions since CNF is composed of both crystalline and amorphous domains. Unlike the TEM image here we observed the stacking of individual fibres resulting in the formation of small aggregates. This stacking of individual fibres will result in the difficulty to measure the aspect ratio and size of the fibres. AFM results confirmed that lime juice hydrolysis is effective for the partial removal of noncellulosic domains like lignin, pectin and hemicelluloses. This result is in agreement with the AFM results obtained for extraction of dietary fibres from cactus powder using green methods [77]. Similar results were obtained for other authors [78,79]. Lime juice hydrolysis cleaved glycosidic linkage among the glucose units. Lime juice hydrolysis followed by ball milling degraded of noncellulosic impurities leading to extraction of cellulose nanofibres. Thus the combination of lime juice hydrolysis and ball milling effectively removes the impurities embedded over the cellulose. The average surface roughness is found to be 3.6741 nm. Fig. 7 shows the AFM image of isolated Cellulose nanofibres after 3 h ball milling.

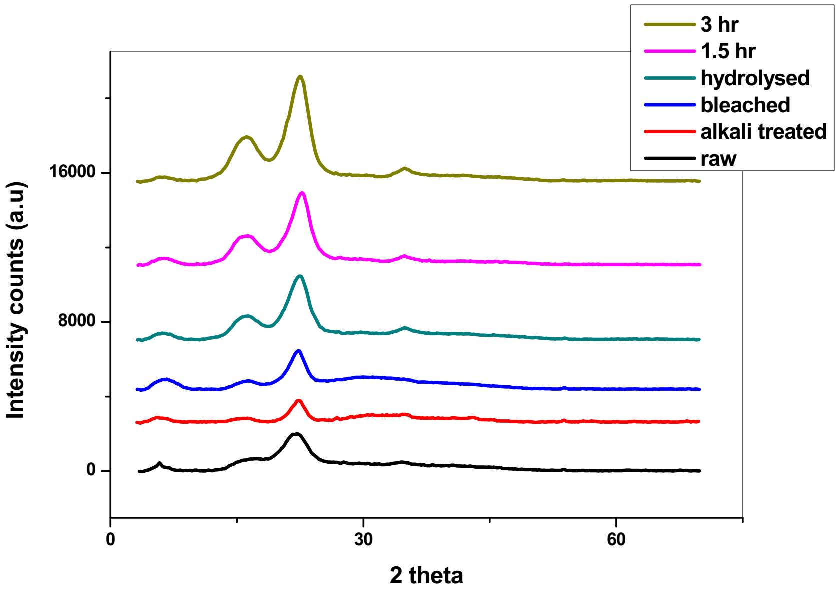


**Fig. 4.** XRD spectrum of different treatments of pine apple leaves (raw, alkali treated, hydrolysed, ball milled).

**Table 1**
Crystallinity index calculated for pine apple leaves (raw, alkali treated, hydrolysed, ballmilled).

| SI no | Name of the samples | Crystallinity index (%) |
|---|---|---|
| 1 | Raw | 57 |
| 2 | Alkali | 62 |
| 3 | Bleached | 63 |
| 4 | Hydrolysis | 65 |
| 5 | 1.5 | 75 |
| 6 | 3 | 77 |

**Table 2**
TGA/DTG profile of different treatments of pine apple leaves (raw, alkali treated, hydrolysed, ball milled).

| Sample | At 350 °C (wt%) | Residue at 700 °C (wt%) | DTG peak point |
|---|---|---|---|
| Raw | 40.936 | 18.243 | 310.47 |
| alkali treated | 55.906 | 9.311 | 359.51 |
| Bleached | 47.546 | 5.306 | 353.83 |
| Hydrolysed | 52.486 | 0 | 358.31 |
| 1.5 h | 47.364 | 12.936 | 346.29 |
| 3 h | 41.586 | 8.657 | 344.49 |

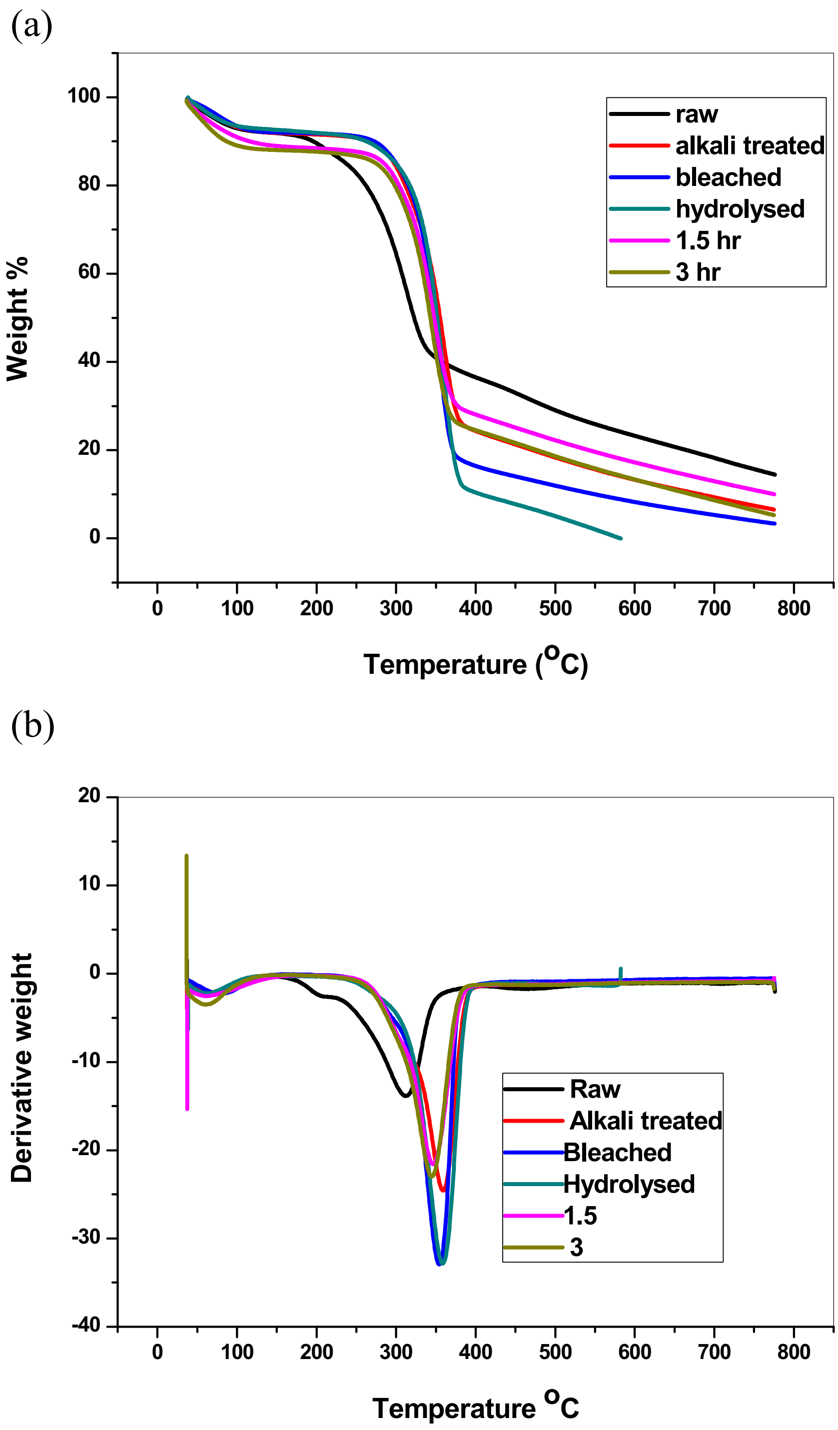


**Fig. 5.** (a) Thermogravimetry analysis (TGA) and (b) Derivative thermo-gravimetric analysis (DTG) of different treatments of pine apple leaves (raw, alkali treated, hydrolysed, ball milled: 1.5 h, 3 h).

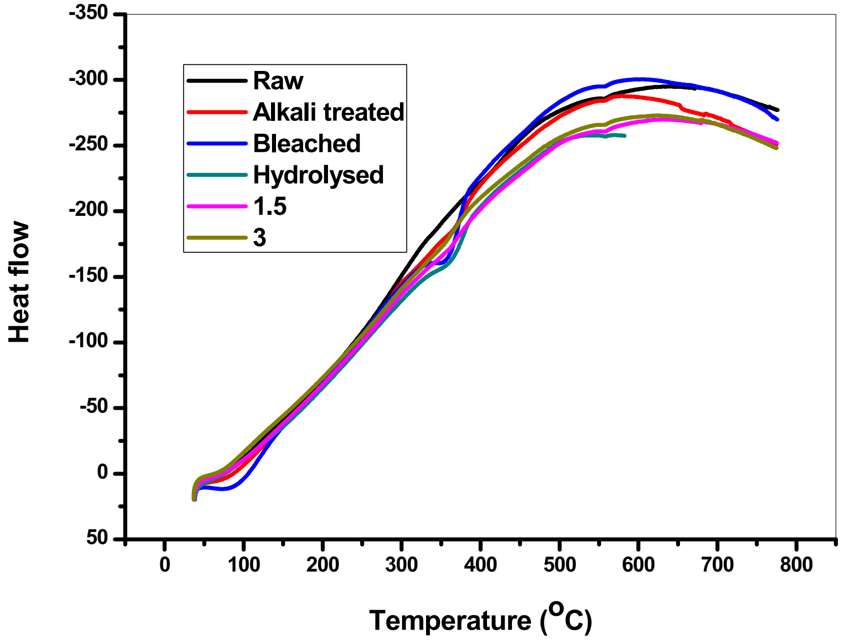


**Fig. 6.** DTA of different treatments of pine apple leaves (raw, alkali treated, hydrolysed, ball milled: 1.5 h, 3 h).

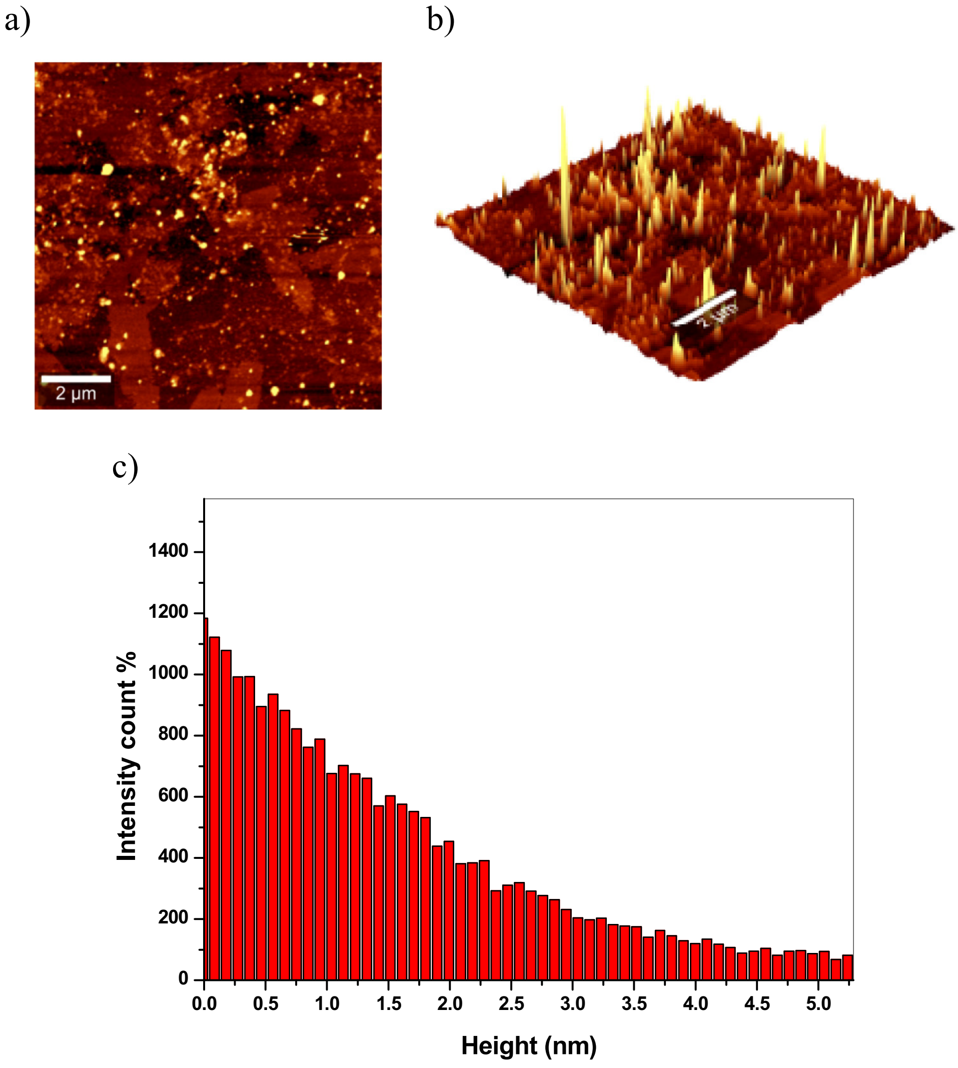
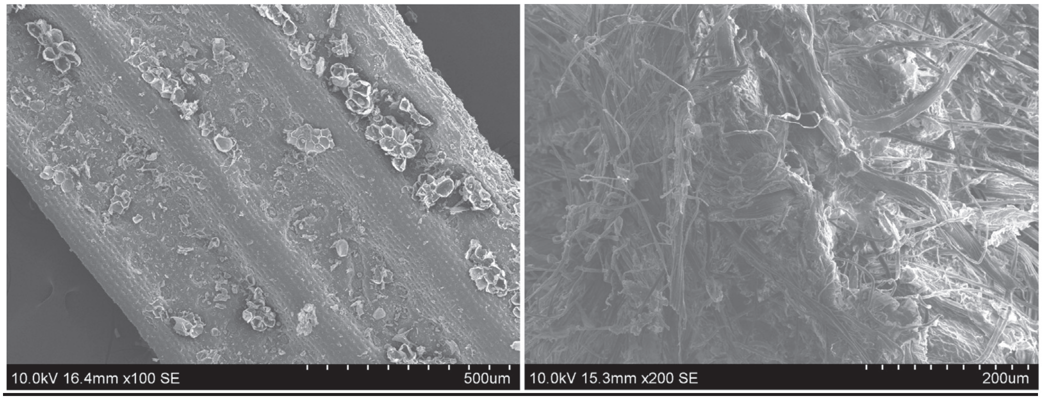


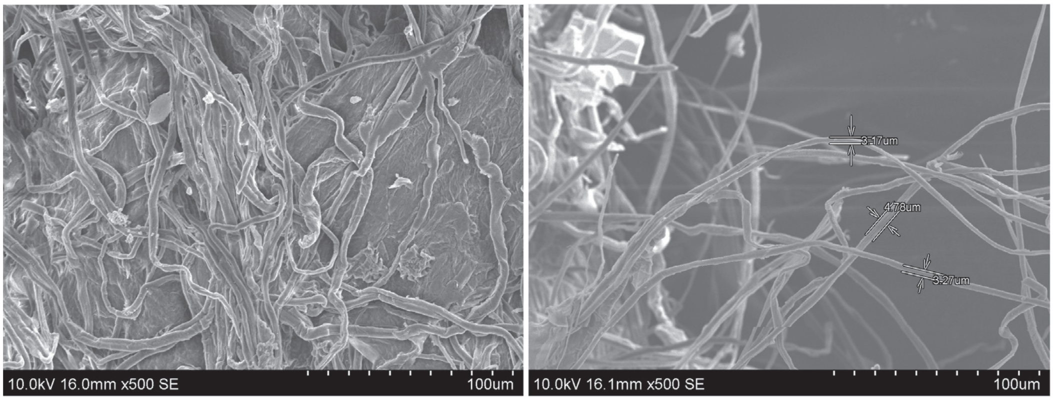
**Fig. 7.** AFM of cellulose nanofibre: a) 2D image b) 3D image c) histogram.

### 3.2. Field emission scanning electron microscopy (FESEM)

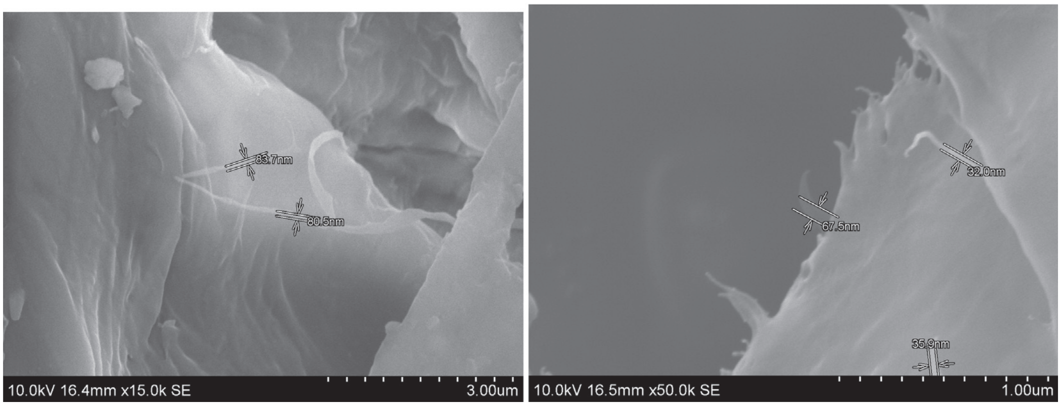
Field Emission Scanning Electron Microscopy is an effective tool to analyze the changes in the surface of pineapple leaves after each chemical treatments. Raw pineapple leaves which seemed to be in a rod like shape due to wrapping of lignin, hemicelluloses, pectin over the surface of cellulose nanofibres. After the alkali treatment the surface became rougher due to the partial removal of these cementing materials. Bleaching of the fibre resulted in the partial defibrillation which can be clearly viewed, as the surface became smoother. Almost similar reduction in size of individual fibres after alkali treatment and bleaching were obtained for other researchers [58]. Acid hydrolysis using lime juice shows cellulose microfibril with a diameter of 3.27–4.78 μm. The resultant cellulose microfibril have been ball milled for 1.5 h and 3 h. Ball milling for 1.5 h shows the defibrillation of the fibre with an average diameter of 80.5–83.7 nm. After 3 h of ball milling the diameter again reduced to around 32 nm. The FESEM image of CNF isolated from PALF by steam explosion and homogenization got long fibre like network [80]. The FESEM image of CNF isolated from oil palm mesocrap fibre using an extruder shows 80–100 nm diameter for individual fibres, which is



*a)Raw pineapple leaves* *b)alkali treated pineapple leaves*



*c)Bleached pineapple leaves* d)*Hydrolysed pineapple leaves*



*e)Cellulose nanofibreball milled(1.5 hr)* *f) Cellulose nanofibreball milled(3 hr)*

**Fig. 8.** FESEM of different treatments of pine apple leaves (raw, alkali treated, hydrolysed, ball milled).

higher than the result we obtained [81]. Due to the aggregation of individual cellulose fibres, similar morphological images were obtained for other authors [82,83]. The FESEM image of CNF isolated from municipal waste showed larger aggregates [84]. From the FESEM result it is clear that chemical treatments, lime juice hydrolysis and ballmilling have been a successful method for the isolation of cellulose nanofibre. Fig. 8 shows FESEM of different treatments of pineapple leaves (raw, alkali treated, hydrolysed, ball milled).

### 3.3. Dynamic light scattering analysis (DLS)

Dynamic Light Scattering Analysis is commonly used to measure the average size distribution of solid particles, suspensions, and solutions. The major drawback of this analysis is that all the samples were considered as spherical particles. But in many studies this technique is widely used for rough analysis or for comparison of results. In most of the cases higher size distribution was observed for DLS measurements compared to other surface analysis techniques [80,85,86]. The strong hydrophilic nature of nanocellulose suspension will lead to aggregation since DLS measures the hydrodynamic diameter of the suspension [87]. The tendency of agglomeration will lead to the formation of larger spheres which in turn increases the particle size by several nanometers. The presence of large number of hydroxyl groups are the reason of the formation of aggregates. For larger particles the Brownian motion will be slower hence the intensity of scattering light will be higher [88,89].

The average particle size distribution of isolated cellulose nanofibres was found to be 420 nm. The larger size can be attributed to the aggregation of isolated nanofibres as a result of hydrogen bonding among the hydroxyl groups present in the cellulose nanofibres. The average particle size of CNF obtained from banana peel and banana bract using microwave digestion, ballmilling followed by ultrasonication is 1285 nm and 972 nm respectively which is higher than what we obtained [90]. The nanocellulose obtained from peanut shell showed average size distribution from 1 to 1000 nm [91]. Therefore the economic and eco-friendly method we used was effective for CNF extraction. Fig. 9 shows DLS of cellulose nanofibres ball milled for 3 h.

### 3.4. High resolution trasmission electron microscopy (HRTEM)

Cellulose nanofibres isolated from the pineapple leaf fibres were examined by High Resolution Trasmission Electron Microscopy (HRTEM). The HRTEM image revealed the size, shape and the morphology of the isolated nanofibrils. The isolated CNF appeared to be like a mesh. The average diameter of individual fibrils is found to be 10-50 nm. The diameter was calculated using *image J software*. Due to the presence of large number of hydroxyl groups CNF had a tendency for agglomeration. The acid hydrolysis using lime juice and ball milling maintained the amorphous domain and the crystalline domain intact. Moreover it reduced the size of the individual fibrils from micron to nano scale. Lime juice helps to remove the noncellulosic parts that wrap around the pure cellulose. The effective size reduction is caused by the forces (impact, shear, compression, friction) acting between the ceramic balls and the microfibres. The size distribution of CNF isolated from PALF using steam coupled acid treatment is 5–55 nm which is similar to our result [36]. The TEM image of CNF obtained from raw jute fibres have an average diameter of 50 nm and length of few micrometers [92]. Chemical pretreatments followed by microfluidization of hemp fibres successfully isolated nanofibres [93]. From the TEM image they observed that the isolated nanofibres are having uniform dimension around 20–50 nm. Many authors reported similar TEM image [94–98]. The average diameter of CNF obtained from citrus colocynthis seeds was only in micrometers which showed that the green strategy we employed for CNF isolation is superior [99]. From the TEM image it is clear that lime juice hydrolysis and ball milling found to be an effective tool for the defibrillation of nanofibrils. Fig. 10 shows HRTEM of Cellulose nanofibres ball milled for 3 h.

### 3.5. Analysis of the green production of CNF and its cost effectiveness

The present work aims on the green production of CNF from agro waste in an economic and eco-friendly way. There are several methods reported for the production of Cellulose nanofibres from agricultural residues such as refining & cryocrushing [100], High pressure homogenization [101], ultrasonication [102], microfluidization [103]. The use of mineral acids for hydrolysis, highly energy consuming and expensive equipments for defibrillation makes the production of CNF very cost intensive and nature harming. Furthermore the CNF produced from commercial cellulose is also expensive.

CNF produced via proposed green method yields high purity CNF which is good as the commercial one. The proposed method is free of mineral acid and expensive instruments which makes the isolation process eco-friendly and low cost. Several extraction methods are reported for isolating CNF from different sources. The cost of commercial CNF is too high and the other extraction methods are also expensive. Therefore the method we proposed to isolate CNF from agro waste is highly cost effective and environmentally safe. In the present study lime juice is used instead of mineral acids for hydrolysis. For the sake of energy conservation we avoided the use of high energy consuming equipments for size reduction of micro fibres to nanoscale. Instead we employed simple ball milling technique for producing nanofibres. As in the case of steam explosion high pressure is applied for defibrillation but the proposed method does not the use of high pressure. By analyzing the results the CNF we produced were as good as commercial one.

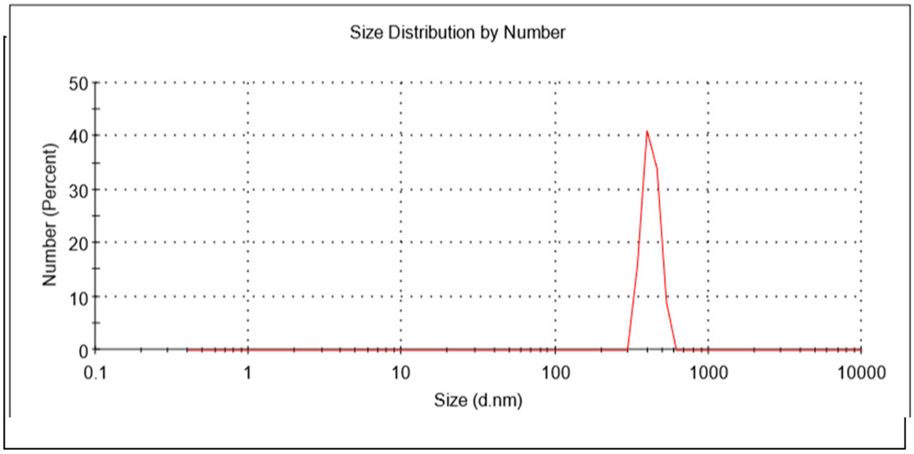


**Fig. 9.** DLS of Cellulose nanofibres (3 h ball milled).

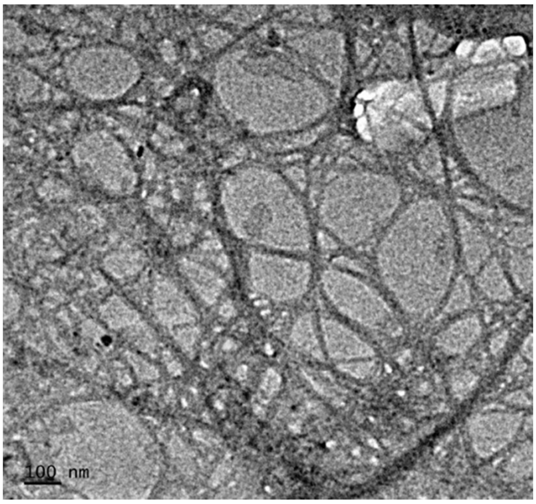


**Fig. 10.** HRTEM of cellulose nanofibres (3 h ball milled).

The comparison of cost estimation of CNF produced from agro waste using proposed green method and commercial CNF are listed in Table 3. It is observed that the proposed method is cheaper and less energy consuming than other methods. Due to the potential value of CNF in industry, the present work gives a way for utilizing the agro waste to produce CNF in much cheaper and greener strategy.

## 4. Conclusions

The proposed work mainly focus on exploring green protocol for the utilization of biomass to highly potential material. This method is total mineral acid free and uses less energy for CNF production. The proposed green method is devoid of any health hazards and environmental issues. Cellulose nanofibres were successfully isolated from pineapple leaves utilizing cost effective green route: lime juice and ball milling. The method adopted for the isolation of CNF was found successful. FT-IR results give evidence for the isolation of cellulose nanofibres by losing hemicelluloses and lignin. XRD results showed an increase in crystallinity (77%) which is a clear indication of removal of lignin and hemicelluloses present in amorphous region. Higher crystallinity is the key factor which determines better reinforcement with the polymer matrix. Here the intensity of peak ($2\theta = 15.8$ and $22.5°$) increased. The slight tendency of agglomeration is evidenced by the higher particle size observed in DLS studies. The thermal analysis revealed thermal stability increases for the isolated nanofibres. The isolated CNF showed the maximum degradation temperature at 344.49 °C. The surface morphological analysis (FESEM, AFM and HRTEM) results showed the topological changes occurred during chemical treatments, lime juice hydrolysis and ball milling. From FESEM, HRTEM and DLS the diameter of CNF obtained was around 30–85 nm, 10–50 nm and 420 nm respectively. The studies revealed that the extracted CNF has high crystallinity and thermal stability which can be used an effective reinforcement for the preparation of polymer nanocomposites. Instead of using several mineral acids, Lime juice can cleave cellulose-non cellulose strong bond which results in the defibrillation of the fibres. During ball milling the microfibres collide with the ceramic balls inside the container, the impact force, shear force, compression, and frictional force developed resulted in the size reduction. The cost estimation of isolated CNF have done and compared with commercial CNF. We found that the proposed green method is economical and environmentally sustainable for the production of high quality CNF. All the results showed the novel processing techniques we adopted were found to be useful for the isolation of CNF without causing any harm to the nature.

**Table 3**
Laboratory level analysis of production cost of CNF (per kg) produced via green strategy using PALF as a source.

| Materials used | Cost per kg |
|---|---|
| Pine apple leaves | Zero cost |
| Sodium hydroxide pellet | 11.69$ |
| Glacial acetic acid | 10.38$ |
| Sodium chlorite | 19.17$ |
| Lemon | Zero cost |
| Ball milling energy cost | 0.91$ |
| Total cost | 42.15$ |
| Market price of CNF | 200$ |
| Net profit | 157.85$ |

Our findings reveal a new path for the isolation of cellulose nanofibres exploring the utilization of agricultural residue and green chemistry. Present study mainly focused on the relevance of ecofriendly methods for the isolation of nanocellulose thereby reducing the environmental issues and to increase its application in biomedical fields, packaging, electronic devices etc. The use of agro waste, mineral acid free, eco-friendly isolation methods and low cost raw materials makes this novel processing technique more environmentally benign and sustainable.

## Acknowledgement

The authors are grateful to the financial support from Kerala State Council for Science, Technology & Environment (KSCSTE) Thiruvananthapuram, Kerala, under SRS scheme, Department of Science & Technology (DST), New Delhi for the facilities provided to SreeSankara College, Kalady under DST-FIST programme (No. 487/DST/FIST/15-16).

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