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Isolation of nanocellulose from pineapple leaf fibres by steam explosion

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ABSTRACT

Steam explosion process is employed for the successful extraction of cellulose nanofibrils from pineapple leaf fibres for the first time. Steam coupled acid treatment on the pineapple leaf fibres is found to be effective in the depolymerization and defibrillation of the fibre to produce nanofibrils of these fibres. The chemical constituents of the different stages of pineapple fibres undergoing treatment were analyzed according to the ASTM standards. The crystallinity of the fibres is examined from the XRD analysis. Characterization of the fibres by SEM, AFM and TEM supports the evidence for the successful isolation of nanofibrils from pineapple leaf. The developed nanocellulose promises to be a very versatile material having the wide range of biomedical applications and biotechnological applications, such as tissue engineering, drug delivery, wound dressings and medical implants.

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1. Introduction

The promising performance of cellulose nanofibres and their abundance encourages the utilization of agricultural waste residue, which acts as the main source of cellulose. The use of natural fibres such as banana pseudo-stem, oil palm empty fruit bunch and rayon fibre in some polymers has demonstrated promising results in the past, especially in the presence of the interfacial bonding agents (Facca, Kortschot, & Yan, 2007; John & Anandjiwala, 2009; John, Francis, Varughese, & Thomas, 2008; Liu et al., 2008; Luz, Gonçalves, & Del'Arco, 2007). The utilization of this biomass for processing of novel composites has attracted growing interest because of their ecological and renewable nature characteristic. Indeed enormous interest in development of new composite materials filled with natural fibres has been shown importance in industries such as automotive, construction or packaging industry.

Pineapple leaf fibre (PALF) is an important natural fibre that exhibits high specific strength and stiffness. The fibres have a ribbon-like structure and consist of a vascular bundle system present in the form of bunches of fibrous cells, which are obtained after mechanical removal of all the epidermal tissues. PALF is of fine quality and its structure is without mesh. The fibre is very hygroscopic, relatively inexpensive and abundantly available. The superior mechanical properties of PALF are associated with its high cellulose content and comparatively low microfibrillar angle (14°). Due to the unique properties exhibited by pineapple leaf fibre (PALF) they can be used as excellent potential reinforcement in composite matrices (Lopattananon, Panawarangkul, Sahakaro, & Ellis, 2006). PALF is being examined with a view to replace glass fibre in low priced products, especially building materials. Profound research has to be done to discover the potential applications of pineapple leaf fibres in high performance polymer composites.

In tropical countries, pineapple is one of the fibrous plants available in abundance. Pineapple leaf at present is a waste product of pineapple cultivation. Hence, without any additional cost input, pineapple fibres can be obtained for industrial purposes. These fibres are found to be multicellular and lignocellulosic. They are extracted from the leaves of the plant Ananus cosomus belonging to the Bromeliaceae family by retting. The superior mechanical properties of pineapple leaf fibres are associated with their high cellulose content (Devi, Bhagawan, & Thomas, 1997).

Steam explosion is found to be an effective method for the separation of nanofibres from biomass (Cherian et al., 2008). High pressure steaming followed by rapid decompression is called the steam explosion. The steam explosion process includes saturating the dry material with steam at elevated pressure and temperature followed by sudden release of pressure, during which the flash evaporation of water exerts a thermo mechanical force causing the

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material to rupture. To reduce the content of non-cellulosic compounds that cement the fibre aggregates, steam explosion can be used as a pretreatment. By this method, the nanofibrils are liberated from the bundles as individual entities to increase their reinforcement ability in composites.

In the present study, the nanodimensional cellulose embedded in pineapple fibres is successfully extracted for the first time applying acid coupled steam treatment. The main goal of the present work is to focus on the chemical, crystalline and morphological changes during steam explosion treatment. The structural and physicochemical properties of the pineapple leaf fibres were studied by environmental scanning electron microscopy (ESEM), atomic force microscopy (AFM) and transmission electron microscopy (TEM) and X-ray diffraction (XRD) techniques.

2. Materials and methods

2.1. Materials

The materials used for the study includes pineapple leaf fibres were supplied by the company Superpolpa from Iaras–SP, Brazil, NaOH (analytical purity), acetic acid (analytical purity), sodium hypochlorite (analytical purity), oxalic acid (analytical purity), obtained from Merck and Co., Inc.

2.2. Isolation of cellulose nanofibrils

Pineapple fibres were chopped into uniform size of approximately 10 cm. The fibres were treated with 2% NaOH (fibre to liquor ratio 1:10) in an autoclave and kept under 20 lb pressures for a further period of 1 h. Pressure was released immediately. The fibres were removed from the autoclave and the fibres were washed in water until it was rid of alkali. The steam exploded fibres were bleached using a mixture of NaOH and acetic acid (27 and 78.8 g respectively) and a mixture of 1:3 sodium hypochlorite solutions. The bleaching was repeated six times. After the bleaching the fibres were thoroughly washed in distilled water and dried. The steam exploded bleached fibres were treated with oxalic acid of 11% concentration in an autoclave until it attained a pressure of 20 lb. The prsessure was released immediately. The autoclave was again set to reach 20 lb and the fibres were kept under that pressure for 15 min. The pressure was released and the process repeated eight times. The fibres were taken out washed until the washings no longer decolorized $KMnO_4$ solution to make sure that the washings are free from acid. The proceeded nanofibrils were suspended in water and kept stirring with a mechanical stirrer of type RQ-1.27 A and 8000 rpm for about 4 h until the fibres are dispersed uniformly.

2.3. Characterization

2.3.1. Chemical estimation

Chemical compositions of fibres were estimated according to the following ASTM procedures: α-cellulose (ASTM D1103-55T), lignin (ASTM D1106-56), holocellulose (ASTM D1104-56) and moisture content (ASTM D4442-92).

2.3.2. X-ray diffraction (XRD)

Wide-angle X-ray diffraction data were collected using a Rigaku 200B X-ray diffractometer (45 kV, 100 mA) equipped with Cu Kα radiation (λ = 0.1541 nm) to investigate the XRD spectra of the steam exploded nanofibres (obtained by air drying of the nanofibre suspension). The crystallinity determination was made using a powder X-ray diffraction method (PXRD).

2.3.3. Scanning electron microscopy (SEM) analysis

Scanning electron micrographs of virgin and treated fibres were captured using Philips SEM 515. In this case, the samples were coated with platinum using the sputtering technique.

2.3.4. Environmental scanning electron microscopy (ESEM) analysis

Philips XL30 FEG ESEM was also used to observe the morphology of dried nano film with an accelerating voltage of 30 kV. The nanofibres were analyzed using water suspension of the nanofibres. A drop of suspension of nanofibres was kept onto a carbon film and left to dry in a silica gel ambient for 12 h followed by coating with platinum using the sputtering techniques. The film was sputter-coated with platinum using a Baltec SCD050 sputter coater for enhanced conductivity.

2.3.5. Transmission electron microscopy (TEM) analysis

Transmission electron micrographs of cellulose fibres were taken with a Philips CM 30 transmission electron microscope with an acceleration voltage of 75 kV. The nanofibrils were deposited from an aqueous dilute dispersion on a micro grid covered with a thin carbon film (~200 nm). The deposited fibres were subsequently stained with a 2% uranyl acetate solution to enhance the microscopic resolution.

2.3.6. Scanning probe microscopy (SPM) analysis

Cellulose nanofibrils were observed using atomic force microscopy, NanoScope IVa, Multimode SPM (Veeco Inc. Santa Barbara, USA), in tapping mode. Calibration was performed by scanning a calibration grid with precisely known dimensions. All scans were performed in the air with commercial Si nanoprobes SPM tips with a resonance frequency of about 300–330 kHz. Free amplitude (A_0) of about 20 nm and a set-point ratio (rsp) between 0.4 and 0.6 were used. rsp is the ratio between the set-point amplitude (Asp) and A_0. Image processing including flattening was made. The images were obtained simultaneously in tapping mode at the fundamental resonance frequency of the cantilever with a scan rate of 0.5 line/s using a j-type scanner. The free oscillating amplitude was 3.0 V, while the set-point amplitude was chosen individually for each sample. Sample images were scanned on at least five different areas of nanofibrils. Only one representative image of sample is shown. For SFM analysis of cellulose nanofibril sample for characterization was prepared by pipetting a 0.12 g/L aqueous whisker suspension was allowed to dry on a freshly cleaved mica surface. The sample was allowed to dry in room temperature overnight.

3. Results and discussion

3.1. Chemical estimation

Table 1 showed the chemical composition of raw, steam exploded and bleached fibres of PALF. It is clear from Table 1 that the raw fibre has the highest percentage of hemicellulose and lignin and lowest percentage of α-cellulose. When the PALF fibres undergo steam treatment a decrease in the hemicellulose and lignin components present in the PALF fibre. This proves that during the steam explosion substantial breakdown of the lignocellulosic structure takes place resulting in improved defibration. The partial hydrolysis of the hemicellulosic fraction and depolymerization of the lignin components occur during the steam explosion giving rise to sugars and phenolic compounds that are soluble in water as reported by other authors (Cara, Ruiz, Ballesteros, Negro, & Castro, 2006; Fernández-Bolaños, Felizón, Heredia, Guillén, & Jiménez, 1999). When the raw fibre is subjected to steam explosion in the alkaline medium the hemicellulose and lignin components present in the raw fibre will dissolve out. Yamashiki et al. (1990) proposed an

Table 1
Chemical composition of different stages of pineapple leaf fibers.

Material	α-Cellulose (%)	Hemicellulose (%)	Lignin (%)	Moisture content (%)
Raw PALF	81.27 ± 2.45[a]	12.31 ± 1.35	3.46 ± 0.58	10.52 ± 0.48
Steam exploded PALF	93.45 ± 2.79	3.72 ± 0.73	2.08 ± 0.47	10.64 ± 0.36
Bleached PALF	98.63 ± 0.54	0.53 ± 0.03	0.77 ± 0.44	10.80 ± 0.50

[a] Mean ± SD (n = 3).

explanation for the solubility of steam exploded cellulose in NaOH solutions, suggesting that during the steam explosion, there is a partial breakdown of the intramolecular hydrogen bond at the C-3 and C-6 positions of the glucopyranose unit, and these results in significant variations in the network and strength of the hydrogen bonds of the cellulose hydroxyls. However, the complete removal of these components does not take place. The hydrolysis of glycosidic linkages in hemicellulose and the ether linkages present in lignin are catalyzed by acetic acid formed from the acetyl groups present in hemicellulose (autohydrolysis) at the high temperature.

During the explosion, some changes occur in the arrangement of macromolecular chains. Slight rearrangement of macromolecular chains and the reduction of some polar groups made it more difficult to absorb water and swell in fibre thus the dissolving rate of fibre slowed down. In addition, the explosion treatment might have removed some loose substance in the fibre surface leaving the hard structure that was difficult to dissolve in the caustic solution (Cara et al., 2006). At the end of the process, the samples are explosively discharged, providing additional mechanical defibrillation. The cellulose is depolymerized and defibrillated.

The percentage cellulose content of raw pineapple leaf fibre increases when it undergoes steam explosion. When the steam exploded fibres undergo bleaching process we can see that there is a further decrease in the percentage of hemicellulose and lignin and increase in the percentage of α-cellulose component present in the fibre. The final fibres obtained after bleaching process is found to have a high percentage (98.63%) of cellulose content. Therefore, the reinforcement ability of these fibres is expected to be much higher than other fibres, which have less percentage of cellulose content. It is clear from the table that the treated fibres obtained after bleaching process also has trace amounts of hemicellulose and lignin components. From raw fibre to the bleached fibre, we can see that there is an increase in the percentage of moisture content present in the fibres. Steam explosion resulting in a higher availability of the hydroxyl groups distributed either on the surface of the cellulose crystals or in the non-ordered regions. This is due to the increase in percentage of cellulose content during the process. Since, in pure cellulose, each unit has three free –OH groups, the moisture absorption rate increases with cellulose content. When the fibres are exposed to alkaline medium induces swelling and to develop strongly hydrophilic ionic groups on the fibre surface which also promote the absorption of moisture.

Steam explosion process, which combines mechanical and chemical actions, causes lignocellulosics to undergo physical and chemical changes, which include defibrillation of lignocellulosics into individual fibres and partial depolymerization of hemicellulose and lignin. Hemicellulose and cellulose degrade through the hydrolysis of glucoside bonds while lignin degrades through radical reactions involving its units. Hemicellulose and lignin can be removed from the pretreated solid residue by successive extractions, first with water and then with bleaching solution.

During the steam explosion with 2% NaOH, the steam penetrates into the microfibril bundles of PALF. It removes the natural and artificial impurities, waxes and pectins, causes the dissolution and leaching of fatty acids and removes most of the lignin component of the fibre. Steam explosion processes results in the hydrolysis of hemicellulose within the fibre, the interfibrillar region is likely to be less dense and less rigid. The hydrolyzed sugars can be filtered off by washing. After washing, a residue of cellulose and lignin will be left. The solubility of non-cellulosic components depends on the molecular weight of the substances. So the aim of the pretreatment of the fibres is to decrease the intermolecular binding possibilities, which in turn leads to the increase of solubility of non-cellulosic components. In the presence of alkali solution, the carboxylic groups present in pectin ionize to form sodium carboxylate, which is soluble in the solvent medium and can be filtered off. This result in the decreasing tendency of intermolecular hydrogen bond formation. The concentration of alkali solution should be carefully controlled so that it cannot degrade the cellulose content. The 2% alkali solution is found optimum to avoid the degradation of cellulose residue.

The second step of the steam explosion process is bleaching treatment. The main aim of bleaching treatment is to remove the lignin present in the steam treated pulp. Lignin is believed to be linked with the carbohydrate moiety through two types of linkages. One is alkali sensitive and another alkali resistant. The alkali sensitive linkage forms an ester type combination between lignin hydroxyls and carboxyls of hemicellulose uranic acid. The ether type linkage occurs through the lignin hydroxyls combining with the hydroxyls of the cellulose. The degradation of lignin leads to the formation of hydroxyl, carbonyl and carboxylic groups. These groups help in solubilizing the lignin content in the alkali medium and there by facilitates the purification of cellulose. After bleaching, the cell walls of the fibres are individualized but the nanofibrils are still associated within the cell wall. So an acid coupled steam treatment is employed in order to individualize the nanofibrils from the cell wall. This novel treatment leads to the individualization of the nanofibrils having the high cross-linked structure having a web-like form comprising the individual helicoidal arrangement with precise resemblance to cellulose ordered chains, destroying the links and twists present in the microfibrils. These links are accounted for the amorphous region of microfibrils. The removal of this amorphous region by the acid coupled steam explosion increases the crystallinity index of nanocellulose residue. The enhancement in the properties of the nanofibrils is associated with increased crystallinity index, increased surface area and decreased degree of polymerization.

3.2. X-ray diffraction studies

XRD studies of the treated and untreated pineapple fibres were done to investigate the crystalline behavior of the fibres. From the XRD graphs (Fig. 1), it is clear that the treated PALF fibres show a crystalline nature. The fibres show increasing orientation along a particular axis as the fibres are treated under different processing conditions. The sharp peak in the X-ray diffraction pattern of the acid treated fibre exhibits a higher crystallinity due to the more efficient removal of non-cellulosic polysaccharides and dissolution of amorphous zones. Accordingly, the above results demonstrate that hydrolysis took place preferentially in the amorphous region. This increase of crystallinity after acid treatment has been reported by several authors (Azizi Samir, Alloin, Paillet, & Dufresne, 2004; Tang, Hon, Pan, Zhu, Wang & Wang, 1998).

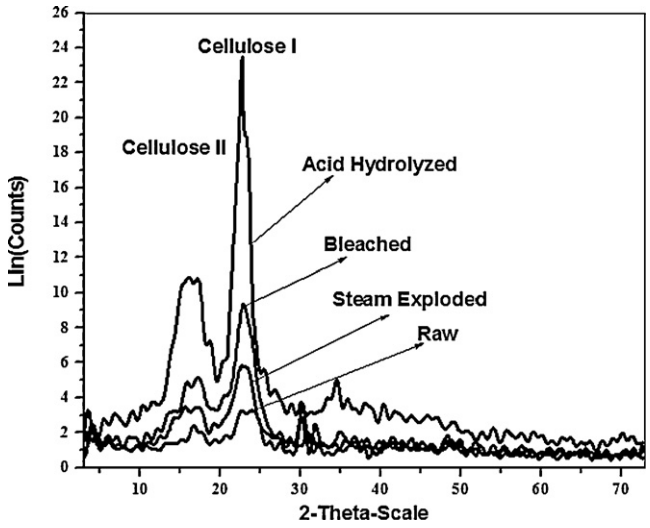

Fig. 1. X-ray diffractometry of different stages of PALF fibre.

By contrast, the crystalline part of acid treated fibre corresponds to cellulose I (Nelson & O'Connor, 1964) and shows highest scattering intensity at 22.7°. A comparison of $\theta/2\theta$ scans obtained for raw to acid treated fibres reveals differences in crystallinity and cellulose I/cellulose II content. Fig. 1 reflects that raw fibre exhibits a slight shoulder at a scattering angle of 22.7° indicating the presence of cellulose I. This shoulder becomes more prominent in steam treated, further more intensified in bleached and finally develops into an obvious intensity peak in acid treated fibre. The ratio of scattering intensity at 22.7° vs. intensity at 20.4° ($I_{22.7^\circ}/I_{20.4^\circ}$) is indicative of cellulose I vs. cellulose II content. In raw to acid treated fibres, this ratio increases from 0.43 to 0.57 and finally to 0.93 (Table 2). The progressive increase of the ratio $I_{22.7^\circ}/I_{20.4^\circ}$ may be interpreted as an increase in the proportion of cellulose I crystallites with respect to cellulose II crystallites. Assuming a linear relationship between the ratio $I_{22.7^\circ}/I_{20.4^\circ}$ and cellulose I/cellulose II content, a rough estimate of the former was calculated (Table 2). Based on this estimate, which considers only the peak height of the respective most intense reflection, the cellulose I part of the crystalline cellulose in the composite's increases from 35.9% in steam treated fibre to 73.6% in acid treated fibre. This signifies that with an increase in the amount of cellulose I (Table 2), progressively an increase in percentage crystallinity is observed. Increasing amounts of cellulose I facilitate the elevated increase in crystallinity of the overall material. This corresponds well to the fact that enhancement in the amount of cellulose I/cellulose II causes intensification in the overall crystallinity.

Table 2
Proportional ratio of cellulose I crystallites with respect to cellulose II crystallites.

Fiber stage	$I_{22.7^\circ}$	Crystallinity (%)	$I_{22.7^\circ}/I_{20.4^\circ}$
Raw fiber	$I_{22.7^\circ} = I_{am} = 10.5$	–	–
Steam treated fiber	16.4	35.97	0.43
Bleached fiber	22.9	54.18	0.57
Acid treated fiber	39.8	73.62	0.93

3.3. Morphological analysis

The effect of pretreatment and the structural changes of the steam exploded and bleached PALF fibres were obtained from SEM studies. Fig. 2(a) reveals the SEM of raw PALF. From the figure, it can be seen that the fibrils are associated in bundles and the surface of the fibre is found to be smooth due to the presence of waxes and oil. Fig. 2(b) shows the SEM photograph of the PALF after steam explosion treatment. During the steam explosion with alkali at a high temperature, the hemicellulose is hydrolyzed and become water soluble. The lignin gets depolymerized. As a result defibrillation of the fibre occurs because of the removal of the cementing materials which can be seen from the SEM micro photograph [Fig. 2(b)].

Fig. 2(c) shows the SEM micrograph of the PALF after bleaching. Bleaching helps to remove most of the lignin present in the pineapple leaf fibre, which helps in further defibrillation. Sodium hypochlorite and sodium acetate buffer allows the removal of lignin and tannin. Lignin is rapidly oxidized by chlorine. Lignin oxidation leads to lignin degradation and leads to the formation of hydroxyl, carbonyl and carboxylic groups, which facilitate the lignin solubilization in an alkaline medium.

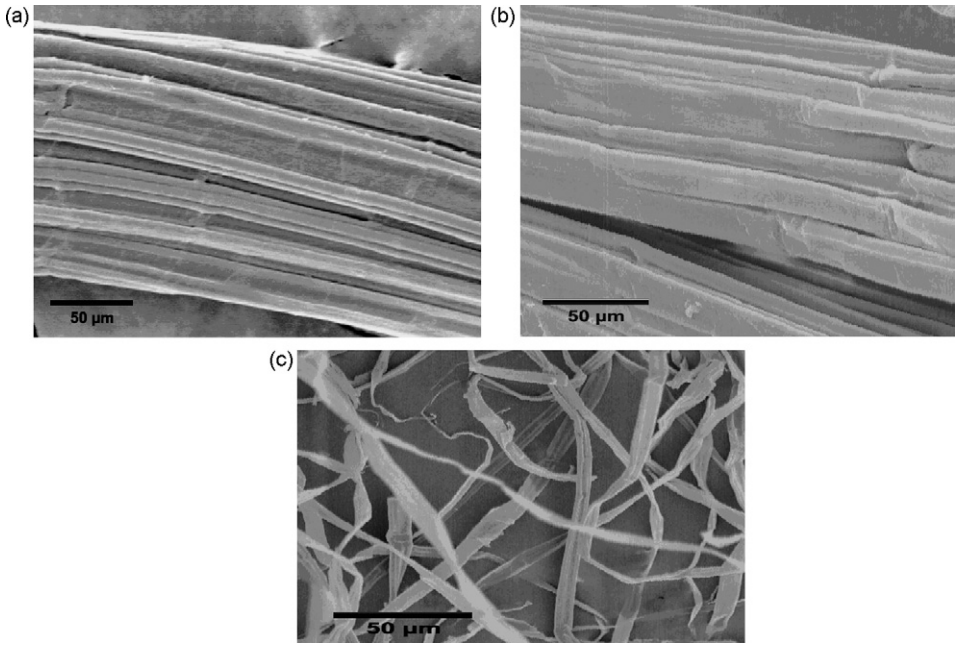

Fig. 2. Scanning electron micrographs of (a) raw PALF fibre, (b) steam exploded PALF fibre and (c) bleached PALF fibre.

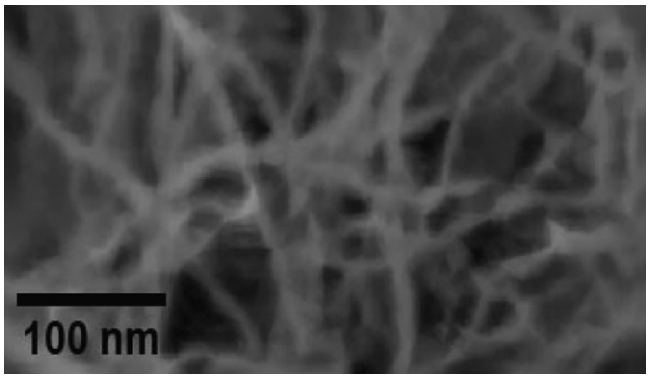

Fig. 3. ESEM photograph of the individualized nanofibrils.

Acid coupled steam treatment after bleaching process helps to disintegrate the fibrils further. Fig. 3 shows the ESEM photographs of the individualized microfibrils after acid treatment. It can be seen from the ESEM that the size of the PALF is reduced from 5 μm to less than 50 nm. Thus acid coupled steam explosion helps in defibrillating the fibre diameter to nano range.

The TEM micrographs (Fig. 4) also support the evidence for the isolation of individual nanofibres from PALF. The size distribution of isolated PALF nanofibres is shown in Fig. 5. The aspect ratio (fibril length to the diameter ratio) is one of the most important parameters in determining reinforcing capability of the nanofibres. Aspect ratio (50) of the extracted cellulose nanofibres was estimated from transmission electron micrographs. In some cases total fibril length was not visible. Therefore, only the visible portion was considered for the calculation in TEM graphs (provide statistical significance of this assumption).

AFM image (Fig. 6) proves that the acid coupled steam defibrillation leads to individualization of the cellulose nanofibres from the cell wall without degrading them. Atomic force micrographs and transmission electron micrographs suggested that only few lateral associations occur between adjacent nanofibres. Nanofibres are much more clearly defined probably because of the removal of pectic polysaccharides. The nanofibres obtained after the efficient

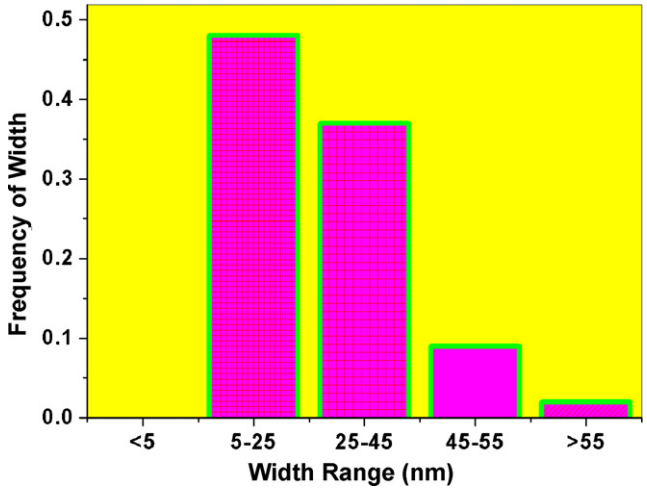

Fig. 5. Size distribution of PALF nanofibres.

mechanism of reduction in size (to submicron level) of acid treated PALF fibres seem to be more interwoven and their lengths were estimated between 200 and 300 nm. It was observed that most of the nanofibres had a diameter range of 5–60 nm. By the fibre image statistical analysis, the average width of obtained nanofibre was 32.5 nm based on averaging 100 individual fibres.

Nanocellulose isolated from pineapple leaf fiber (PALF) is very hygroscopic with various characteristics desired for biomedical applications, such as high cross-linking, uncomplicated chemical structure and non-toxic. For biomedical applications, physical cross-linking has the advantages of not leaving residual amounts of the toxic cross-linking agent. Due to its unique nanostructure and properties, PALF nanocellulose is a natural candidate for numerous medical and tissue-engineered applications since it is both durable and biocompatible. The non-woven ribbons of nanocellulose closely resemble the structure of native extracellular matrices, suggesting that it could function as a scaffold for the production of many tissue-engineered constructs. The successful mass production of PALF nanocellulose will eventually become a vital

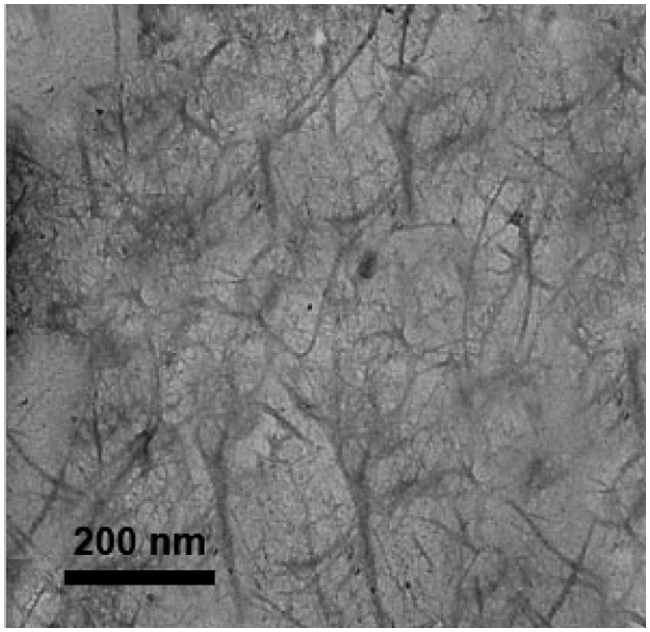

Fig. 4. Transmission electron micrograph of cellulose nanofibres.

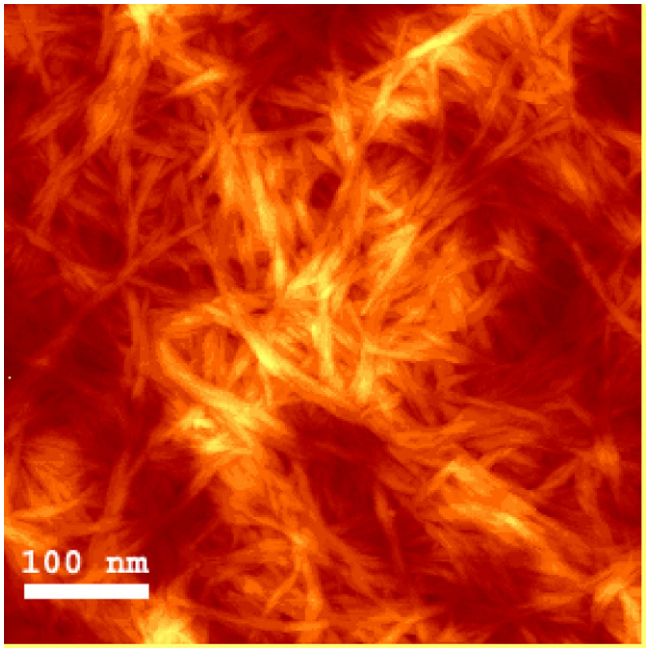

Fig. 6. Atomic force microscopic image of cellulose nanofibrils of PALF.

biomaterial and will be used in the creation of a wide variety of medical devices and consumer products.

4. Conclusions

The study has been done to investigate the effectiveness of the high pressure hydrothermal process on pineapple leaf fibre to individualize the embedded cellulose from macro to nano scale. The steam explosion process resulted in the isolation of PALF nanofibres having a width in the range of 5–60 nm. The high pressure defibrillation contributed a unique morphology of the interconnected web-like structure of nanofibres. Chemical analysis of PALF after each stage of purification showed an increase in cellulose content and a decrease in lignin and hemicellulose content. Successive bleaching helped with the cellulose purification. The characterizations of the fibre morphology in each stage by electron microscope support the individualization of the fibres from macro to nanodimension. AFM and TEM support the evidence for the development of nanofibrils having the width in the range of 5–60 nm. The separated nanocellulose promises to have many new biomedical applications including, surgical wounds, tissue and organ engineering and medical implants such as artificial heart diaphragms, heart valves, joint prostheses, vascular grafts, etc.

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