PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYL CELLULOSE (CMC) FROM PINEAPPLE LEAVES

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Abstract

Pineapple leaves are one of the abundantly available waste materials and used for production natural fibres. Cellulose was isolated from pineapple leaf fibres (PALF) by mechanical and chemical treatments using alkaline, inorganic salts and acids. Cellulose was then converted to carboxymethyl cellulose by an alkalization and etherification process, using various concentrations of sodium hydroxide (10 %, 20 %, 30 % and 40 % w/v) and sodium monochloroacetate (SMCA), in isopropyl alcohol medium. All the carboxymethyl cellulose (CMC) obtained from various concentrations of NaOH were investigated on yield percent, solubility of water and degree of substitution to get the optimum CMC. These results indicate that the optimum reaction of alkalization was reached at 30 % NaOH. The physicochemical properties of optimum CMC such as moisture content, pH and solubility were also determined. Characterization of raw sample, bleached sample, cellulose and CMC were carried out by analyzing the XRD pattern, spectra of FT IR and SEM photomicrographs. The XRD analysis showed that the native cellulose was transformed into an amorphous phase, as evidenced from the characteristic peaks that had almost disappeared. FT IR analysis indicated that, in addition to the main characteristic bands of cellulose, CMC showed new characteristic absorption bands at 1591 and 1413 cm^{-1}, which are associated with the anti-symmetric and symmetric stretching vibrations of COO^-**,** respectively. In SEM analysis, it can be seen the significantly changes from cellulose to CMC. These results confirmed the carboxymethylation process from cellulose.

Keywords: Pineapple leaf fibres, cellulose, carboxymethyl cellulose, alkalization, etherification

Introduction

Plant fibres are mainly composed of cellulose, hemicellulose and lignin (Moran *et al.*, 2008). There are many plant fibres available which has potential to be applied in industries as raw materials such as pineapple leaf, coir, abaca, sisal, cotton, jute, bamboo, banana, hemp and talipot. Among them pineapple leaf fibres (PALF) is one of the waste materials in agriculture sector, which is widely grown in India as well as Asia.

Commercially pineapple fruits are very important and leaves are considered as waste materials of fruit which is being used for producing natural fibres. Fresh leaves yield about 2 to 3 % of fibres. Fibers has white in colour, smooth, glossy as silk, medium length fibres with high tensile strength. It has a softer surface than other natural fibres and it absorbs and maintains a food colour. The chemical composition of PALF constitute holocellulose (70-80 %), lignin (5-12 %) and ash (1.1 %) (Yogesh and Hari, 2017).

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to many thousands of β (1→4) and is the most abundant renewable material resources on earth. Lingin and hemicellulose are amorphous in structure while cellulose is semicrystalline (Yang *et al.*, 2007).

In general, cellulose extraction can be divided into immersion method, chemical method and biological method. Natural cellulose was extracted from pineapple leaf (PAL) by using chemical method with steaming process (Fu *et al.*, 2013). To obtain pure cellulose, the raw material is treated with alkali and bleached. The chemical treatment breaks intermolecular and intramolecular hydrogen bonding between the hydrogen group of cellulose and hemicellulose and

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can increase the hydrophilicity (Abraham *et al.,* 2011). However, alkali treatment and bleaching do not significantly increase the crystallinity of cellulose fibres. Subsequently, acid hydrolysis can increase crystallinity and reduce the diameter of fibres (Mahardika *et al.,* 2018).

The steam process results in a hydrolysis of glycosidic bonds in the hemicelluloses and to a lesser extend in the cellulose. It also leads to a cleavage of hemicellulose-lignin bonds. The reactions result in an increased water solubilization of hemicelluloses and in an increased solubility of lignin in alkaline or organic solvents, leaving the cellulose as a solid residue (Cherian *et al.,* 2011).

Cellulose must be converted into its derivatives. One of the most common derivatives is carboxymethyl cellulose (CMC). CMC is manmade modified cellulose, a linear, long chain, water soluble and anionic polysaccharide (Mondal *et al.,* 2015). The preparation of CMC involves two reaction steps, which are alkalization and etherification process. In the alkalization process, the cellulose is treated with NaOH, often in the presence of inert solvent (ethanol or isopropanol), which acts both as a swelling agent and as a dilutant which facilitates good penetration to the crystalline structure of cellulose. In etherification step the alkali cellulose is reacted with monochloroacetic acid (MCA) or sodium monochloroacetate (SMCA) to form carboxymethyl cellulose ethers (Tasaso, 2015).

The aim of the research work is to prepare and characterize carboxymethyl cellulose via extracted cellulose from pineapple leaf fibres. The obtained CMC will be used as composite films for packaging application in next phase of the research work.

Materials and Methods

Sample Collection

Pineapple leaf was collected from farm of Shaw Pyar Village, Pathein Township, Ayeyarwady Region. Other requiring chemicals were purchased from chemical store. Distilled water was used as the solvent in all analyses.

Extraction of Pineapple Leaf Fibres

The pineapple leaf fibres (PALF) from the pineapple leaves can be extracted by manual or mechanical methods. The most common and effective way of the extraction of PALF was the manual method, and it was used in this research work. Firstly, pineapple leaves were washed with water. Then, a plate was used to scratch and remove skin of the leaf from the surface. The fibres were detached after skin removal. After that, the fibres were washed with distilled water and dried in sunlight. Dry fibres were cut into small pieces.

Isolation of Cellulose from Pineapple Leaf Fibres

Cellulose was isolated from PALF by steam explosion process along with mild chemical treatment including alkaline extraction, bleaching and acid hydrolysis. PALF were treated with 2 % NaOH (with fibres to liquor ratio of 1:10) in an autoclave and kept at 120 ˚C temperature and 138 kPa pressure for a period of one hour. Pressure was then released immediately. The fibers were washed in distilled water until the alkaline solution was completely free from the fibres.

The steam exploded fibers were bleached using a mixture solution of 0.65 M NaOH and 1.30 M glacial CH_3COOH, mixed with 12 % NaClO solution in 1:3 ratio. The bleaching was repeated six times. After that, the bleached fibres were treated with 11% $H_2C_2O_4$ acid in an autoclave at 120 ˚C temperature and 138 kPa pressure. The pressure was then released

immediately. The autoclave was again set to reach 138 kPa and the fibres were kept under that pressure for 15 min. The pressure was released and the process repeated eight times.

The fibres were then taken out and washed until the fibres were free from acid. The processed cellulose fibre was suspended in distilled water and kept stirring with a mechanical stirrer for about 4 h until the fibrils were dispersed uniformly. Finally, the cellulose fibre was sonicated for 30 min at room temperature. The fibres were then filtered using a filter paper and dried in an oven at 60 ˚C.

Preparation of Carboxymethyl Cellulose via Cellulose Isolated from Pineapple Leaf Fibres

Carboxymethyl cellulose was prepared from cellulose according to the procedure. First of all, 5 g of cellulose powder was weighed and added to 150 mL of isopropanol with continuous stirring for an hour. Then, 15 mL of (10 %, 20 %, 30 % and 40 % w/v) NaOH was added dropwise into the mixture and further stirred for an hour at room temperature. The carboxymethylation was started when 6 g of MCA (monochloroacetate) was added with continuous stirring for another 1.5 h. The mixture was covered with aluminum foil and placed into the hot air oven at 60 ºC for 3.5 h.

The slurry was subsequently soaked in 100 mL of methanol for overnight. On the next day, the slurry was neutralized with 90 % of acetic acid to pH 7 and then filtered. The final product was washed for three times by soaking in 50 mL of ethanol for 10 min to remove undesirable by-products, and then it was washed again with 100 mL of absolute methanol for the last time. The obtained CMC from cellulose pineapple leaf fibre (CPALF) was filtered and dried at 60 ºC to constant weight and kept in a dry place.

Determination of degree of substitution (DS) of CMC

Sodium carboxymethyl cellulose was converted to the acid form (H-CMC) by adding an aqueous solution of 6 mL of 6 N HCl per 2 g of the sample, with continued stirring for 30 min. The dispersion was filtered in order to remove the excess acid. The precipitate was washed with methanol. Then the precipitate was again dispersed in acetone, filtered, dried and ground.

The obtained H-CMC was used for the DS determination. About 0.5 g of the H-CMC sample was dissolved in 20 mL of 0.2 N NaOH and 50 mL of distilled water was also added. The solution was transferred to a 100 mL volumetric flask, which was then filled up to the mark with distilled water. 25 mL of the solution was transferred to an Erlenmeyer flask and diluted by addition of 50-100 mL of bi-distilled water. The excess of NaOH was back-titrated with standard 0.05 N HCl using phenolphthalein indicator. The titration was repeated three times and the average value of the HCl volume was used for the calculations. The milli-equivalents of consumed acid per gram of the sample were calculated as the following equation.

$$A = \frac{(B \times C) - (D \times E)}{F}$$

Where,

A = milli-equivalents of consumed acid per gram of specimen
B = milliliters of added sodium hydroxide
C = normality of sodium hydroxide
D = milliliters of consumed hydrochloric acid
E = normality of hydrochloric acid
F = specimen grams used

The degree of substitution (DS) was then calculated as follows:

$$DS = \frac{(0.162) \times \mathrm{A}}{1 - (0.058 \times \mathrm{A})}$$

Where:

162g/mol is the molar mass of an anhydroglucose unit (AGU),
58 is the net increase in the mass of an AGU for each carboxymethyl group substituted.

Characterization of the Prepared Samples

The physicochemical properties (moisture, pH and solubility) of cellulose and carboxymethyl cellulose were determined by analytical method. The crystallinity index was calculated by using XRD analysis. The structural characterization of CPALF and CMC were characterized by using FT IR. The morphological structure of prepared samples was characterized by SEM.

X-ray diffraction (XRD) analysis was carried out using Rigaku X-ray Diffractometer, RINI 2000/PC software, Cat. No 9240 J 101, Japan. Copper tube with nickel filter was used. The diffraction pattern was recorded in terms of 2θ in the range of 10-70 °.

FT IR spectrum was recorded in the range of 4000-400 cm^{-1} by using 8400 SHIMADZU, Japan FT IR spectrophotometer.

The scanning electron microscopy (SEM) images were recorded by using JSM-5610 Model SEM, JEOL-Ltd., Japan.

Results and Discussion

Physicochemical Properties of CMC

The degree of substitution (DS) of CMC obtained in this research work was in the range of 2.0705-2.1303, as present in Table 1. The DS and yield percent of CMC increased with increasing in concentration of NaOH from 10 % to 40 % and attained a maximum DS of 2.1303 with yield (182 %) in 30 % NaOH concentration. Above 30 % NaOH, the DS and yield percent were found to be decreased. It can be seen that the CMC obtained by using 30 % NaOH was more soluble in water than other CMC. According to the results, CMC obtained by using 30% NaOH concentration was selected as the optimum CMC. The physicochemical properties of optimum CMC are shown in Table 2. Moisture content analysis was conducted to calculate the total solid in the sample. The moisture content of CMC obtained from PAL is 7.4329 %. According to the standard procedure, the moisture content of CMC should not be higher than 12 %. So, CMC obtained by using 30 % NaOH was considered as pure CMC because it was within the moisture content (%) of standard CMC. The pH of the 1 % (w/v) CMC solution was in the range of 6 to 8. This CMC was soluble in water and forms viscous solution with water but insoluble in ethanol and methanol. These tests can be confirmed that the optimum CMC is obtained.

Table 1 Yield Percent and Degree of Substitution of CMC Samples with Various Concentrations of NaOH

Concentration of NaOH (%)	Solubility in water	Yield (%)	Degree of Substitution (DS)	pH
10	slightly soluble	133	2.1019	6.64
20	slightly soluble	158	2.1103	6.70
30	soluble	182	2.1303	7.14
40	slightly soluble	165	2.0705	6.77

Table 2 Physicochemical Properties of Optimum CMC (30% NaOH)

Test	CMC
moisture content (%)	7.4329
pH	7.14
viscosity (cP)	40.39
water	+
ethanol	-
methanol	-
DS	2.1303

(+) soluble, (-) insoluble

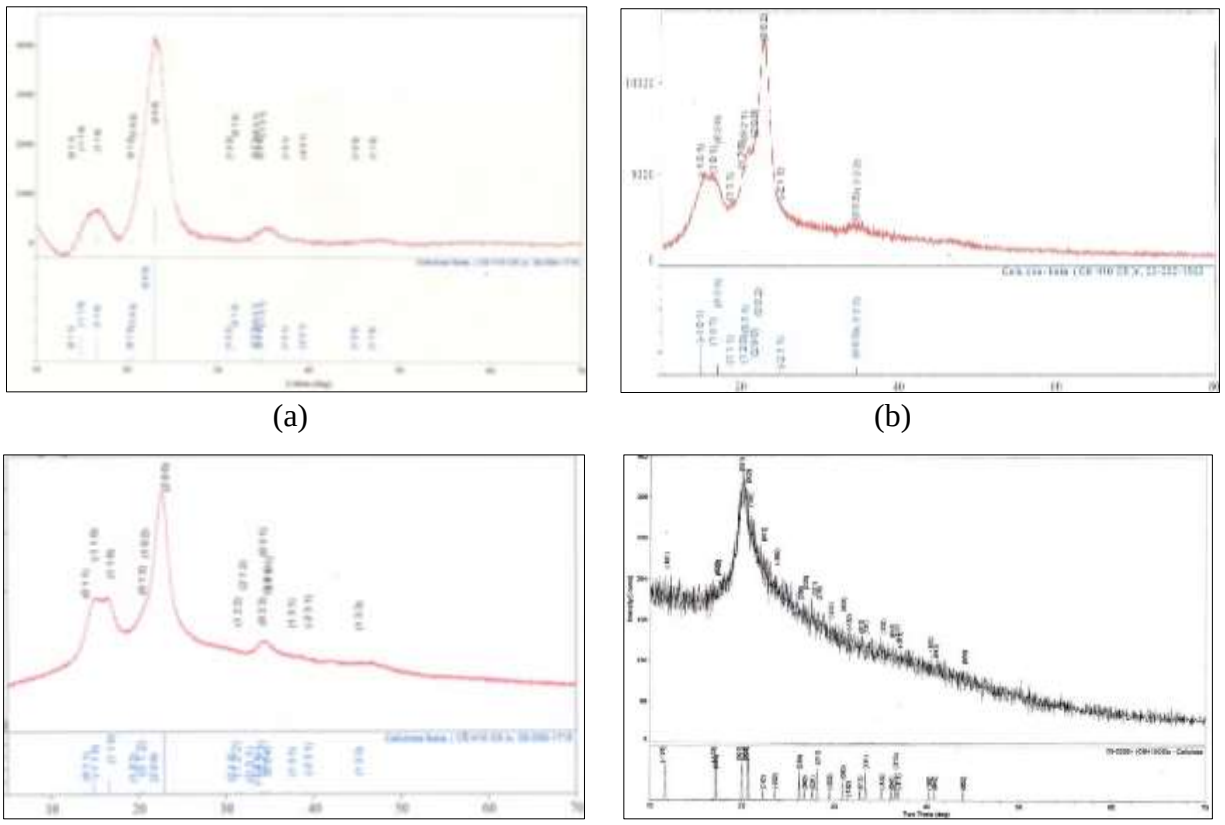
XRD Analysis

XRD diffractometer was used to determine the index of crystallinity (C_I) of PALF at each stage in the process (raw, bleaching, acid hydrolysis and carboxymethylation). The crystallinity index percent was calculated by using the following equation, by measuring the peak height of the crystalline region (I_{200}) and the amorphous region (I_{am}).

$$C_I\ (\%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100\ \%$$

Where, I_{200} is the maximum intensity of the peak for cellulose I ($2\theta = 22^\circ$-23°) and cellulose II ($2\theta = 18^\circ$-22°). I_{am} represent the minimum intensity of diffraction attributed to amorphous cellulose I ($2\theta = 16^\circ$-19°) and cellulose II ($2\theta = 13^\circ$-15°). Figures 1(a), (b), (c) and (d) show the XRD patterns of the raw pineapple leaf fibres (RPALF), bleached pineapple leaf fibres (BPALF), cellulose pineapple leaf fibres (CPALF) and carboxymethyl cellulose (CMC), respectively. Among them, the peak of Figure 1 (d) appears at $2\theta = 20.51^\circ$ and $2\theta = 14^\circ$, suggesting the characteristic of cellulose II. The characteristic diffraction peaks of the rest were observed at the values of $2\theta = 22.67^\circ$ and 18.5°. Therefore, the structure of RPALF, BPALF and CPALF was considered to be typical cellulose I because they have the characteristics of amorphous and crystalline regions.

The crystallinity index percent for all samples was calculated by peak height method and represented as shown in Table 3. The C_I of the RPALF was calculated as 60.5 % and increase in case of BPALF to 75.2 % (due to the removal of hemicellulose and lignin as amorphous part), and 89.05 % in case of CPALF in which remaining amorphous part was removed during acid hydrolysis. It was found that the C_I of CMC decreased as 51.4 %. The decreased of crystallinity on the alkalization and carboxymethylation process of cellulose were due to the cleavage of hydrogen bonds and this also results in the extending the distance between cellulose molecules. Therefore, all characteristic peaks of cellulose have been disappeared and transformed into an amorphous phase.



(a) (b)

Figure 1 XRD diffractograms of (a) raw pineapple leaf fibres RPALF
(b) bleached pineapple leaf fibres BPALF
(c) cellulose pineapple leaf fibres CPALF and
(d) carboxymethyl cellulose CMC

Table 3 Crystallinity Index Percent of all Samples

Samples	Crystallinity Index (%)
RPALF	60.50
BPALF	75.12
CPALF	89.05
CMC	51.40

FT IR Analysis

FT IR spectra of raw PALF, bleached PALF, cellulose PALF and prepared CMC are shown in Figure 2 (a), (b), (c) and (d). The band assignments of all samples are described in Table 4. FT IR spectroscopy was used to confirm that the lignin and hemicellulose have been removed during cellulose isolation process through analysis of its functional group. Figure 2 (a), (b) and (c) present the results of the FT IR analysis of raw PALF, BPALF and CPALF. Based on the FT IR spectrum, there are several peaks in the raw samples which is not found in the spectrum of cellulose. The characteristic peaks of raw sample were observed at 1244 cm^{-1}, 1514 cm^{-1} and 1729 cm^{-1}. The absorption peak range of 1310-1210 cm^{-1} is derived from C-O stretching vibration of aryl group in lignin. Lignin presented characteristic peaks in the range 1600-1500 cm^{-1} corresponding to the aromatic skeletal vibration. The C=O stretching vibration of carboxylic groups of hemicellulose and lignin is around 1765-1715 cm^{-1}. The peaks of BPALF and CPALF are almost nearly the same. The main spectral bands of cellulose were found around at 1425 cm^{-1} and 897 cm^{-1}.

FT IR spectrum of prepared CMC showed new characteristic absorption bands at 1591 and 1413 cm^{-1}, which correspond to the anti-symmetric and symmetric stretching vibration of COO^{-}. Peaks observed at 3263 and 3351 cm^{-1} indicate the OH stretching bands for FT IR spectra of prepared CMC and isolated cellulose. Peaks at 2876 and 2898 cm^{-1} arise from CH stretching of CH_2 and CH_3 groups in the prepared CMC and isolated cellulose respectively. Carboxymethyl cellulose and cellulose have similar functional groups according to the FT IR spectra.

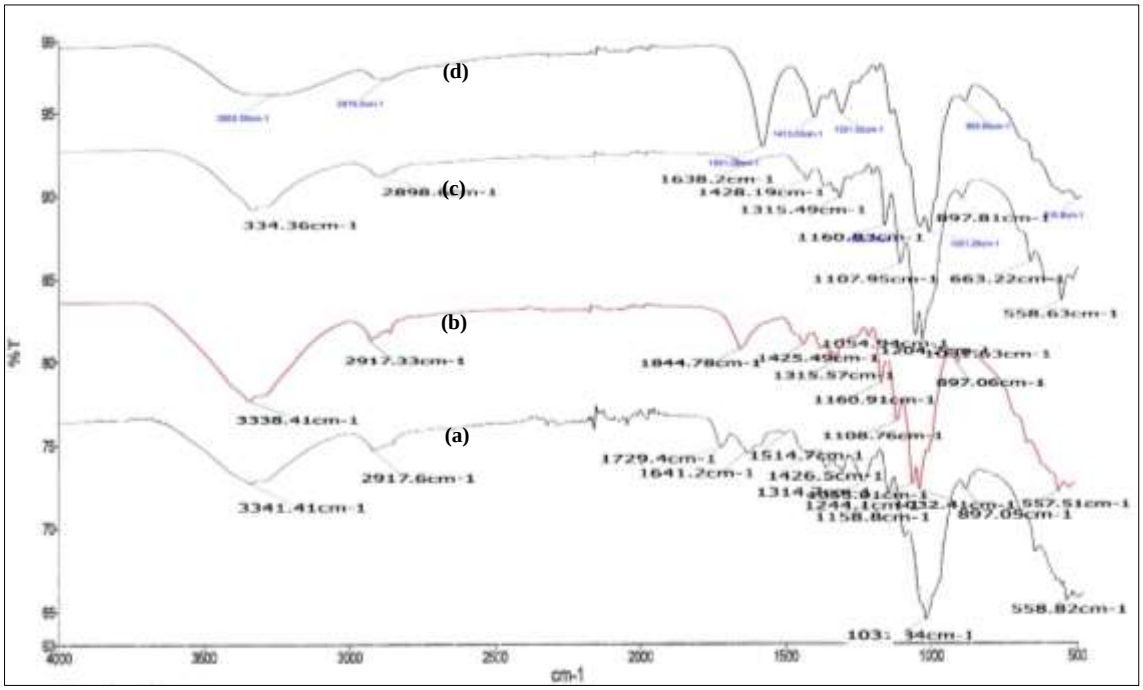


Figure 2 FT IR spectra of (a) raw pineapple leaf fibres RPALF
(b) bleached pineapple leaf fibres BPALF
(c) cellulose pineapple leaf fibres CPALF and
(d) carboxymethyl cellulose CMC

Table 4 FT IR Band Assignments of RPALF, BPALF, CPALF and CMC

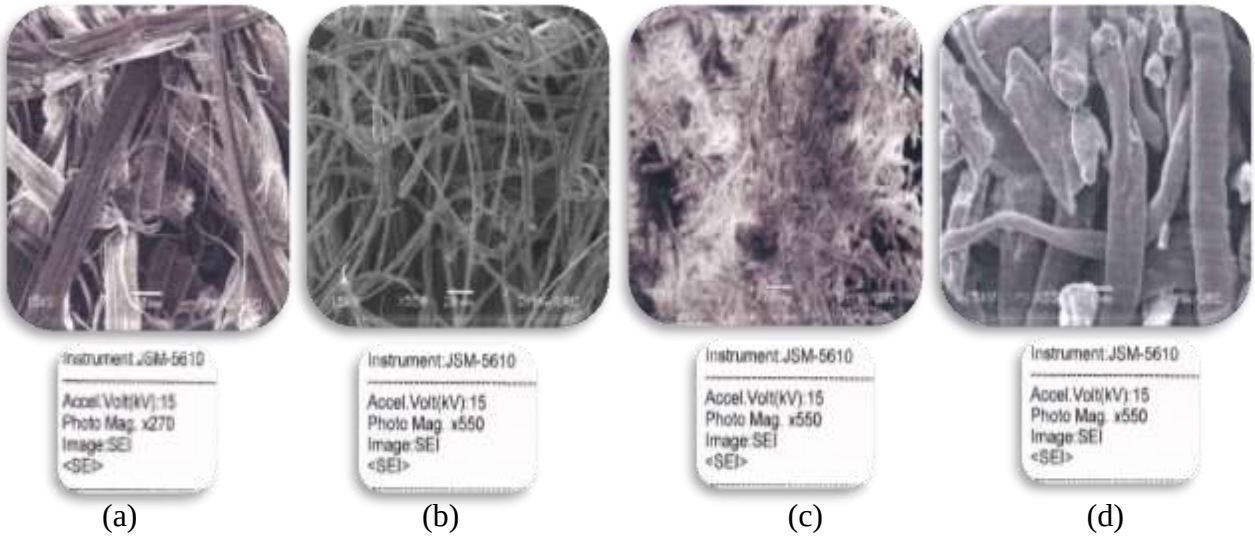
Observed wavenumber (cm^{-1})				Literature * wavenumber (cm^{-1})	Band Assignment
RPALF	**BPALF**	**CPALF**	**CMC**		
3431	3338	3334	3263	3600-3200	O-H stretching
2918	2917	2890	2876	2980-2850	C-H stretching (ketone and carbonyl)
1729	-	-	-	1765-1715	C=O stretching of ester
1641	1644	1638	-	1665-1620	O-H bending
-	-	-	1591	1650-1550	COO^- stretching (anti-symmetric)
1514	-	-	-	1600-1500	C=C stretching (aromatic ring in lignin)
-	-	-	1413	1440-1435	COO^- stretching (symmetric)
1427	1425	1428	-	1430-1420	CH_2 scissoring motion in cellulose
1314	1315	1315	1321	1390-1319	C-O-H bending
1244	-	-	-	1310-1210	C-O stretching (aryl group in lignin)
1158 1031	1160 1032	1160 1054	1052	1200-1000	C-O-C stretching (symmetric)
897	897	897	898	937-897	β (1-4) glysosidic linkage between the glucose unit in cellulose

***** Silverstein *et al*., 2003

SEM Analysis

In order to further investigate the structural changes in the fibres, SEM micrographs of the RPALF, BPALF, CPALF and CMC are shown in Figure 3 (a), (b), (c) and (d). According to the SEM images, the surface morphology of all samples except CMC is composed of several microfibrils. These images visually suggest the partial removal of hemicellulose, lignin and pectin after high pressure chemical treatment, which are the cementing materials around the fibres bundles. The raw PALF was found to be aggregate and microfibrils are still bound to one another due to the presence of lignin and hemicellulose components. After bleaching treatment, the bonding between lignin and hemicellulose had been broken due to the removal of the amorphous content in microfibril bundle. The CPALF image shows a reduction in fiber size after acid hydrolysis. The CPALF sizes are much smaller than the other fibres samples before treatments. This explanation is also supported by XRD crystallinity index data. It can be seen from SEM micrographs that high pressure steam treatment helps in fibres separation and fibrillation.

The surface morphology of prepared CMC can be clearly seen that the obtained products are rod like structure and surfaces are more extended than the cellulose. It had long and narrow strand characteristics. This image clearly showed that the conversion of cellulose to CMC leads to changes in its ribbon shape.



(a) (b) (c) (d)

Figure 3 SEM micrographs of (a) raw pineapple leaf fibres RPALF
(b) bleached pineapple leaf fibres BPALF
(c) cellulose pineapple leaf fibres CPALF and
(d) carboxymethyl cellulose CMC

Conclusion

Cellulose fibres were isolated from pineapple leaf fibres through alkali treatment, bleached and acid hydrolysis. Furthermore, isolated cellulose successfully converted to carboxymethyl cellulose (CMC) using various concentration of NaOH in the range from 10 % to 40 % and etherified with sodium monchloroacetate (SMCA) in isopropanol medium. To get the optimum condition of CMC from isolated cellulose, this cellulose was treated by using 15 mL of 30 % NaOH, 6 g of SMCA in 150 mL of isopropanol solvent at room temperature. In this research work, the highest DS and yield percent of CMC obtained by using 30 % NaOH concentration were 2.1303 and (182 %). So, this concentration regarded the optimum concentration of CMC. Each step from the raw sample to the CMC were characterized by XRD, FT IR and SEM. XRD analysis confirmed that the crystallinity index percent of CMC decreased in comparison with that of cellulose and other samples due to the cleavage of hydrogen bonds and this also results in the extending the distance between cellulose molecules. FT IR analysis showed that, in addition to the main characteristic bands of cellulose, new characteristic absorption bands for CMC at 1519 cm^{-1} and 1413 cm^{-1}, which related to the anti-symmetric and symmetric stretching vibration of COO^{-}. According to the SEM analysis, the significantly changes from raw sample to CMC were clearly observed in each chemical reaction.

This investigation showed that the chemical process was more efficient and effective process for the preparation of CMC from cellulose. Prepared CMC is suitable for developing biopolymer composite film at the second part of the study.

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