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# Characteristics of cellulose from oil palm mesocarp fibres extracted by multi-step pretreatment methods

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**Abstract.** Characteristics of cellulose isolated from oil palm mesocarp fiber (OPMF) using multi-step pretreatment methods were determined in this study. It was postulated that the succession of pretreatment steps for cellulose extraction from lignocellulosic biomass may affect the cellulose properties. Pretreatment steps involved in cellulose extraction were superheated steam (SHS) treatment, enzymatic hydrolysis and alkaline treatment. OPMF was subjected to SHS treatment to open up the fibres wall structure followed by enzymatic hydrolysis using xylanase for effectiveness enhancement the alkaline treatment afterwards. In order to obtain bleached cellulose, peracetic acid, a totally chlorine free (TCF) chemical was used. Cellulose obtained from these multi-step pretreatment methods had purity of 87.5 %, with crystallinity of 69.2 % and thermally stable up to 360 °C.

## 1. Introduction

Plant based materials provides potential applications such as in biofuel and biosugar production. The unique properties of plant material are mainly due to its carbohydrate composition which could be converted into value-added products. There have been many reports on the conversion of these lignocellulosic materials such as sugarcane bagasse [1-2], corn stover [3], rice straw pulp [4], raw cotton linter [5], sawdust, rice husk and wheat straw [6]; into value-added products. In Malaysia, oil palm biomass have attracted interest from many researchers [7–9]. There are several types of oil palm biomass such as oil palm fronds (OPF), oil palm empty fruit bunch (OPEFB), oil palm trunks (OPT), oil palm shells (OPS) and oil palm mesocarp fibers (OPMF).



Cellulose is a linear polymer of anhydroglucopyranose units (AGU) which is linked through ether bonds, while hemicellulose has mixed monomeric unit of C5 and C6. Commonly, hemicellulose contains more than one sugar type and known as amorphous polysaccharide. For lignin, it has a complex crosslinking polymer with phenylpropane unit [10]. Cellulose was first discovered by a French chemist named Anselme Payen in 1837 who presented a carbohydrate in various plant material yielded with fibrous substance after purification with acid ammonia treatment and the extraction with water, alcohol or ether. Generally, isolation of cellulose from lignocellulosic materials requires harsh conditions considering the complex plant structure. Until today, various methods for isolation of cellulose, such as alkaline treatment, bisulfite/sulfate process, biological treatment, and enzymatic treatment as well as the combination with mechanical treatment have been tested. It was shown that different process resulted in diverse in physical and chemical properties of cellulose. Table 1 shows examples of pretreatment methods used for cellulose extraction from plant biomass with their advantages and shortcoming.

**Table 1.** The advantages and shortcoming from different methods of cellulose extraction.

Method for cellulose extraction	Biomass	Advantages	Shortcoming	References
Hydrogen peroxide and acetic acid combination	Rice straw, pine wood & oak wood	Simple methods	Low purity of cellulose obtained	[11]
Pulping, bleaching (50% hydrogen peroxide) and purifying process (5% potassium hydroxide)	Oil palm frond	Increase the brightness of bleached pulp	High energy consumption and high alkaline charge for pulping process	[12]
Mechanical commutation, steam washing, sulphuric acid treatment, organosolvent extraction and alkaline hydrogen peroxide process.	Sweet sorghum bagasse	Economical and eco-friendly processing	High cost and required many chemical replacement	[13]
Oxygen, hydrogen peroxide and sodium chlorite treatment. Followed by enzymatic saccharification.	Mixed hardwood chips	Attributed to removal of hemicellulose and lignin	Required chlorinated chemicals	[14]
Dewaxed with (2:1, v/v) mixture of toluene and ethanol, alkali treated and acid hydrolysis (2x). Followed by sodium chlorite bleaching.	Areca nut husk fibres	Hemicellulose removal	Many chemical replacement and required chlorinated chemicals	[15]

Recent studies of these pretreatment and delignification process have been investigated by considering the treatment conditions which give less impact on the environment. In this study, multi-step pretreatment methods consisted of enzyme, superheated steam, alkali and chlorine-free bleaching have been used for cellulose isolation from oil palm mesocarp fibre (OPMF). The aim of using combined pretreatment was to obtain highly purified cellulose. Characteristics of the cellulose obtained was studied by using several analytical tools such as by using Wide-angle X-ray Diffraction (WAXD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analyzer (TGA) for determining the cellulose crystallinity, morphology, functional groups and thermal stability.

## 2. Experimental

### 2.1 Materials

OPMF was collected from Seri Ulu Langat Palm Oil Mill, Dengkil Selangor, Malaysia. The samples were thoroughly washed with tap water and sun dried until free from moisture to avoid fungal growth in samples. The dry samples were kept in sealed plastic bag and stored at room temperature prior to further treatment. All chemicals used in pretreatment were of laboratory grade. Commercial xylanase enzyme (pulpzyme HC 2500) was supplied by Novozymes A/S (Denmark).

### 2.2 Multi-step treatment (Superheated steam, Enzymatic hydrolysis, Alkaline treatment & Bleaching)

The superheated steam pretreatment was conducted using a lab scale superheated steam oven (QF-5200C, Naomoto Corporation, Osaka, Japan). In brief, the conditions used were according to the previous study [16]. The temperature and retention time was 260 °C for 30 min. About 100g of SHS treated fibers was soaked with sodium phosphate buffer (pH5.5) of xylanase which has an activity of 2500 AXU/g in standard condition. Hydrolysis using xylanase was conducted at liquid to solid content of 5:1 (w/w) at temperature of 55 °C with agitation speed of 160 rpm for 24 hours. Alkaline treatment was conducted using 10 % (w/v) sodium hydroxide (NaOH) and heated at 90 °C for 60 min. The ratio of solid to liquor fixed at 1:10 (w:w). Finally, bleaching was conducted using peracetic acid (PA) solution. PA solution was developed by mixing acetic acid and hydrogen peroxide at equal volume ratio (5:5) [11] with addition of catalyst using 98 % sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) at 1.1 % (w/v). The samples were vigorously stirred for 24 hours at 70 °C.

Solids obtained after multi-step pretreatments were washed with distilled water until the pH became neutral. Characteristics of samples obtained from each stage of treatment were determined.

### 2.3 Characterization of untreated and treated OPMF

**2.3.1 Chemical composition analysis.** Chemical composition analysis of fibres was conducted based on Technical of Pulp & Paper Industry (TAPPI) Standard Test Method. Composition included the testing of moisture content (T264 om-88), extractives (T204 cm-97), lignin content (T222 om-88), cellulose and hemicellulose content (TAPPI useful method 249-75) [17].

**2.3.2 Thermo gravimetric Analysis (TGA).** Thermal stability of fibres was determined by conducting thermogravimetric (TG) analysis on a Thermo gravimetric/ Differential Thermal Analyzer (TG/DTA) (model: EXSTAR6000, Hitachi High-Tech Science Corporation, Tokyo, Japan). The samples were heated from 40 °C to 550 °C at heating rate of 10 °C/min under nitrogen flow of 100 ml/min.

**2.3.3 Scanning Electron Microscopy (SEM) analysis.** The morphology of sample surface was examined using a scanning electron microscopy (SEM) model JCM-6000PLUS NeoScope Benchtop SEM (JEOL Ltd., Japan). Prior to observation, the samples were coated with Pt for 30 s by using Hitachi Ion Sputter E-1030. The view was conducted at several magnifications: 500X, 2000X and 8000 X with beam voltage of 15 kV.

**2.3.4 Wide Angle X-ray Diffraction (WAXD) analysis.** Crystallinity of samples was determined using a Rigaku MiniFlex 600 bench top X-ray Diffractometer operated at 40 kV and 15 mA and the measurement condition was in 2 $\theta$  range of 5 to 50° with scanning speed at 3.6 L/min (about 40 minutes). The crystallinity index (CrI) was calculated by the subtraction of total intensity ( $I_{002}$ ) at 2 $\theta$ =23 and the amorphous scatter ( $I_{am}$ ) at 2 $\theta$ =18 by divided with  $I_{002}$  and represent as percent of crystallinity [18].

**2.3.5 Fourier Transform Infrared Spectroscopy (FTIR) analysis.** The functional groups of fibers were determined using Nicolet iZ10 FT-IR spectrometer in standard analyses using transmission or ATR technique. The spectral resolution was scanned in the range of 700 to 4000 cm<sup>-1</sup>.

### 3. Results and Discussion

In this study, cellulose extraction from OPMF was conducted in multiple steps with the combination of superheated steam pretreatment, enzymatic hydrolysis, alkaline treatment and peracetic acid (PA) bleaching.

#### 3.1 Effect of chemical composition in each stage of multistep treatment

Chemical composition of fibre samples is shown in Table 2. The data is presented as the mass fraction (%) of each component of cellulose, hemicellulose, lignin and extractives presence in untreated and in each stage of treated samples. Untreated OPMF contained 28.2 %, 32.7 % and 32.4 % cellulose, hemicellulose and lignin, respectively. Extractives in raw OPMF were about 6.5 %. Extractives may indicate the presence of oil and wax in fibers which are available at small amount in biomass. The fibers were then subjected to multistep pretreatment with the aim to remove the non-cellulosic component by increasing the cellulose fraction in fibres. The purpose of using combined treatments was to break down the bondings between non-cellulosic components like hemicellulose and lignin, without degrading the cellulose.

It is seen that hemicellulose content was decreased from 32.7 % to 20.2 % after SHS treatment. The composition of lignin increased after SHS treatment due to condition in which lignin depolymerized while in condensation at high temperature and limited the removal of lignin [19]. It has been reported that lignin isolates from pretreated biomass such as using superheated steam exhibited better enzyme binding on fiber surface compared to directly use enzyme on untreated biomass [20]. Therefore, subsequent treatment using enzymatic hydrolysis would enhance the removal of recalcitrant hemicellulose and at the same time extract the cellulose. The xylanase helped to loosen the link of xylan or known as recalcitrant hemicellulose in between cellulose and lignin [21-22]. As a result, the hemicellulose greatly reduces within treatment to 12.7 %. The use of xylanase also potentially accelerates the delignification process towards fibers. By this reason, the fibres subject to delignification using alkaline chemical which results in swelling the fibres and partly removal of lignin. The removal of some lignin cause the loose of network binding for fibre bundles [23]. The extensive lignin removal proposed at final stage of bleaching using PA. PA bleaching was taken place to remove remaining lignin component by reaction of oxidation which the lignin depolymerized at temperature of 70 °C and consequently more dissolving of lignin. Thus, the fraction of cellulose revealed and take place the highest percentage of cellulose content which gives the purity of cellulose from OPMF about 87.5 %.

**Table 2.** Chemical composition (%) for lignocellulose component of OPMF in each multistep treatment.

Multi-step stage	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)
Raw OPMF	28.2 ± 0.8	32.7 ± 4.8	32.4 ± 4.0	6.5 ± 0.1
SHS pretreatment	27.3 ± 0.0	20.2 ± 0.0	42.0 ± 0.3	6.0 ± 0.9
Enzymatic hydrolysis	33.6 ± 0.1	12.7 ± 0.0	48.3 ± 0.1	5.41 ± 0.7
Alkaline treatment	39.9 ± 1.2	11.4 ± 1.0	37.6 ± 0.2	2.6 ± 0.0
PA bleaching	87.5 ± 1.2	12.5 ± 1.1	0.0 ± 0.0	0.0 ± 0.0

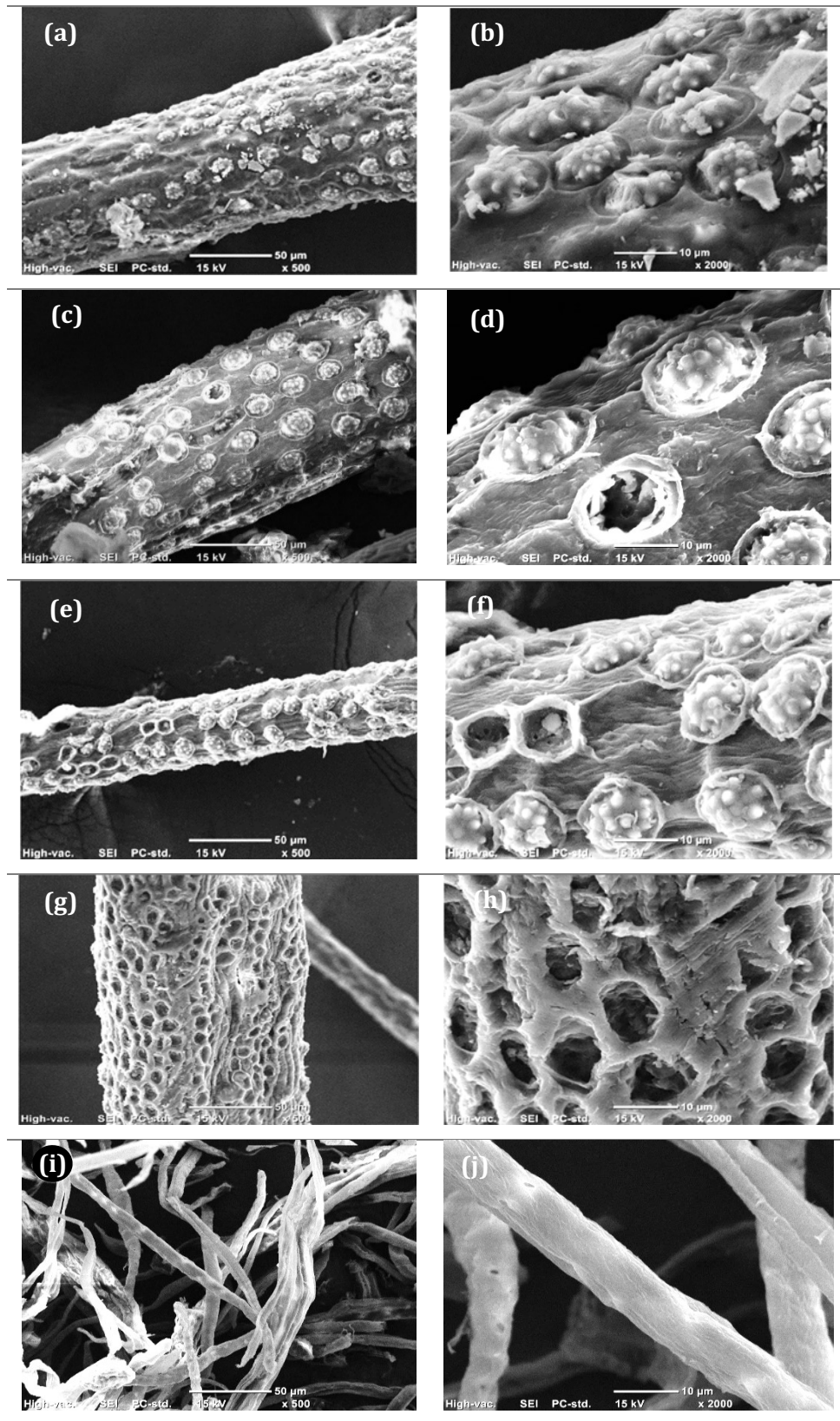
\*Note: Results are representative of three replicate analysis

#### 3.2 Morphological changes at each stage of pretreatment

In case of morphology, the effect of pretreatment on fiber surface can be clearly evaluated using a standard resolution of SEM micrograph in Figure 1. The physical structure of the raw OPMF can be seen clearly, i.e. the silica bodies were highly attached in pores. SHS treatment was found to remove the silica deposits, as being reported by [9]. Further treatment by xylanase which removed some amount of hemicellulose resulted in smaller size fibrils. The subsequent alkaline treatment contributed to ultimate removal of silica bodies, which opened the pores. In this stage, the size of fibres reduced to 10 micrometer due to separation of fiber matrix. PA bleaching which removed lignin eventually



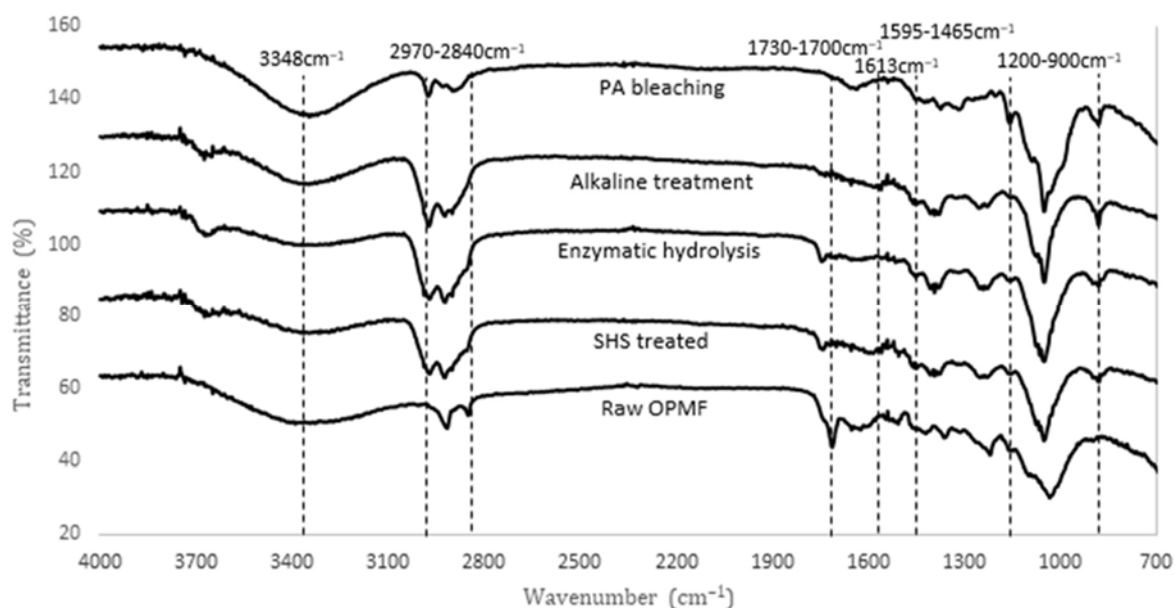
disrupted the structure of the lignocellulose, leaving small size microfibrils consisted of mainly cellulose (Table 2).



**Figure 1.** Morphology of OPMF treated from each pretreatment step: (a) raw OPMF at 500x; (b) raw OPMF at 2000x; (c) superheated steam treated at 500x; (d) superheated steam treated at 2000x; (e) enzymatic treated at 500x; (f) enzymatic treated at 2000x; (g) alkaline treated at 500x; (h) alkaline treated at 2000x; (i) PA bleaching at 500x; (j) PA bleaching at 2000x.

### 3.3 Functional groups of untreated and treated OPMF

The changes of functional groups in sample from each treatment stage were characterized by using ATR-FTIR. As shown in Figure 2, the OPMF fibres had a strong and broad hydrogen bond of O-H stretching at  $3348\text{ cm}^{-1}$  region which indicated as cellulose group with water absorption [12,24]. The C-H stretch vibration was notably low at  $2840\text{--}2970\text{ cm}^{-1}$ . The peak intensity at around  $1700\text{--}1730\text{ cm}^{-1}$  indicates as the presence of C=O stretch which apparent to hemicellulose or lignin position [8,24–26]. There was no peak corresponded to the hemicellulose and lignin components after alkaline treatment and PA bleaching, which indicated almost complete removal of the components. The disappearance of the peak in PA-bleached sample corresponds to mainly cellulose. As discussed earlier, the rises of cellulose indicates the removal of non-cellulosic component. The aromatic skeletal of C=C at  $1613\text{ cm}^{-1}$  corresponded to existing of lignin which can be observed clearly for raw OPMF fibers [8,26]. The following region around  $1429\text{ cm}^{-1}$ ,  $1463\text{ cm}^{-1}$ ,  $1509\text{ cm}^{-1}$  and the rest were assigned to  $\text{CH}_3$ , C=O stretch and C=C stretch or aromatic skeletal of lignin. Whereas, the peak intensity at around  $900\text{--}1200\text{ cm}^{-1}$  apparent to be strong as fibres are treated which corresponds to C-O stretch, C-O-C stretch and C-H bending which can be defined as cellulose and hemicellulose [12].



**Figure 2.** Band characteristic of FTIR spectra after each stage multistep treatment.

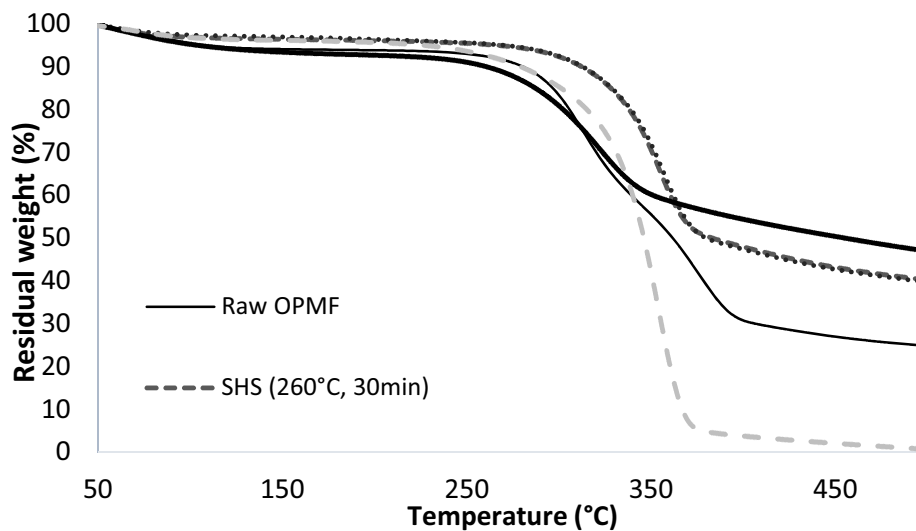
### 3.4 Thermal stability of untreated and treated OPMF

In correlation with other analysis, the prediction of cellulose, lignin and hemicellulose composition can be analyzed using a TGA. Thermal stability and decomposition peak of the cellulose fiber are presented Figure 3. As shown in the figure, weight loss within temperature range of  $50\text{ to }100\text{ }^{\circ}\text{C}$  can be attributed to water content [10, 27]. It is interesting to note that the thermal stability of the OPMF treated samples varied according to the treatment step, which could be related with the chemical composition of the samples. Based on TG thermogram, raw OPMF showed multi-step degradation, which could be attributed by cellulose, hemicellulose and lignin degradation. [28] reported that



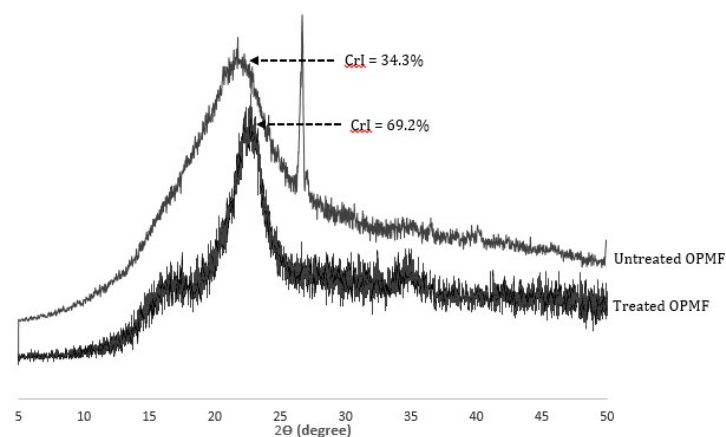
hemicellulose is degraded at lower temperature due to its amorphous structure with low molecular mass.

Subsequent pretreatments of SHS followed by enzyme hydrolysis resulted in fibres with higher thermal stability with  $T_{max}$  (from DTG thermogram) at around 350 °C. On the other hand, despite of further hemicellulose removal by alkaline treatment, thermal stability of alkaline-treated sample was reduced. This could be contributed by reduced lignin composition and cellulose degradation at high concentration of alkali. TG thermogram showed complete fibre degradation for fibre obtained after PA treatment, indicating that the fibre obtained after the last step of treatment consisted of mainly cellulose.



**Figure 3.** TGA thermogram of OPMF samples obtained from multi-step pretreatment.

Crystallinity of untreated and multi-step pretreated OPMF are conducted by WAXD as shown in Figure 4. Untreated OPMF showed broader peak which attributed to amorphous cellulose. After multi-step pretreatment, sharp peaks at 16° and 23° was observed indicating the crystalline fraction of OPMF cellulose. Crystallinity of the OPMF fibre increased from 34.3 % (before pretreatment) to 69.2% (after multi-step pretreatment). The increase of crystallinity is mainly due to the removal of amorphous structure of hemicellulose and lignin [29]. As shown in diffraction pattern, the strongest intensity was found in treated fibers which eventually affected the crystallinity of cellulose.



**Figure 4.** WAXD analysis of untreated and multi-step pretreated OPMF.

#### 4. Conclusions

Results from this study exhibited that cellulose extraction could be achieved by combination of multiple-step pretreatment methods. Each step of pretreatment affecting differently on the fiber, resulted in fibers with diverse characteristics. It was interesting to note that thermal stability was greatly affected after each pretreatment stage, which is mainly due to the chemical composition of the fiber after pretreatment which resulting increment in cellulose purity from 28.2 % to 87.5 %. Produced cellulose obtained after pretreatment had a crystallinity of 69 %, suggesting the suitability of the sample for further applications such as in biocomposite or nanocellulose production.

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