

## Enzymatic Saccharification of Oil Palm Mesocarp Fiber (OPMF) Treated with Superheated Steam

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The effectiveness of superheated steam pretreatment on the enzymatic saccharification of oil palm mesocarp fiber (OPMF) was investigated by varying the temperature (140 to 210 °C) and the retention time (20 to 90 minutes). The results showed that superheated steam pretreatment at 180 °C for 60 minutes is the optimum condition for enzymatic saccharification of OPMF. Scanning electron microscopy (SEM) images of the OPMF show that superheated steam pretreatment is able to remove silica bodies. Further characterization by FTIR and TG/DTG analysis of the raw and treated OPMF indicates that the solubilization and removal of hemicelluloses took place after the pretreatment. This suggested that superheated steam pretreatment is an effective method for the alteration of the OPMF structure and enhances the digestibility of the biomass, hence improving enzymatic saccharification.

*Keywords:* Superheated steam; Oil palm mesocarp fiber; Enzymatic saccharification

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### INTRODUCTION

The Malaysian palm oil industry has grown to become a very important agriculture-based industry, in that the country is now the world's second largest producer and exporter of palm oil. Palm oil mills leave behind a huge amount of biomass, such as empty fruit bunches (EFB), palm oil mill effluent (POME), sterilizer condensate, palm fiber, and palm kernel shell. To date, approximately 368 palm oil mills are operating in Malaysia, producing a substantial amount of lignocellulosic biomass. It was estimated that the amount of solid waste produced could reach 39 million tons by the year 2020 (Yusoff 2006).

Even though there are a number of researchers investigating the ways to utilize these wastes, a large fraction of such wastes have yet to be properly used (Yusoff 2006). Oil palm mesocarp fiber (OPMF) is a suitable raw material for ethanol production since it has a high cellulose content. However, the efficiency of current pretreatment methods is low enough that it hinders the the potential use of OPMF. Therefore, it is essential to identify the pretreatment conditions that allow for the maximum recovery of hemicellulose-derived sugar from the subsequent fermentation process, as well as to produce sufficiently accessible cellulose for efficient enzymatic digestion (Bahrin *et al.*

2012). Furthermore, the selection of pretreatment method is highly dependent on the feedstock and its composition. In general, pretreatment can be achieved by physical, mechanical, chemical, or biological methods.

Currently, steaming is one of the most popular methods of pretreatment, especially for hardwoods such as poplar, oak, and aspen (Sanchez and Cardona 2007). In the conventional palm oil milling process, fresh fruit bunches are subjected to sterilization with saturated steam at 275.8 kPa (140 °C) for 75 to 90 min (Corley and Tinker 2003). The residual steam from the process can be used as a pretreatment method instead of being released into the atmosphere. According to Balan *et al.* (2008), the pretreatment step is the most significant contributing factor to the production cost of the bioethanol conversion process. Hence, utilization of the steam will certainly reduce the pretreatment cost of OPMF.

There are a few steaming methods that can be suggested based on the suitability of the substrate and the operating conditions available at the mill. Steam explosion is an example of a physiochemical method, where the biomass is heated for certain period of time. A relief valve is then opened to immediately reduce the reactor pressure to atmospheric pressure, which causes the water in the reactor to vaporize. This causes an explosion to occur, which reduces the biomass particle size and disrupts the cell wall structure (Horn and Eijssink 2010). However, this method is unreliable in terms of the solubilization effects, as steam explosion disrupts the chemical structure of lignin. Thus, this process contributes to soluble fermentable sugars and phenolic substances in hydrolysis, making it less attractive compared to other steam treatment methods.

Besides steam explosion, high-pressure steam treatment is also widely used for the pretreatment of lignocellulosic materials. A previous study has proven that high-pressure steam treatment affects the chemical composition of oil palm empty fruit bunches (OPEFB). The process also involves lower capital investment, lower environmental impact, and eliminates the use of chemicals (Shamsuddin *et al.* 2011). However, the use of such high pressure (up to 3 MPa) is not preferable, because it might be not safe for operation.

Another steaming method that promises better and more efficient results is superheated steam treatment. Superheated steam is unsaturated (dry) steam generated by the addition of sensible heat to saturated (wet) steam. The addition of heat increases the steam temperature above the corresponding saturation or boiling point at a given pressure (Head *et al.* 2010). Superheated steam treatment of OPMF has been at atmospheric pressure similar to other research works (Ferreira-Leitão *et al.* 2010; Berghel 2011). The advantages of superheated steam treatment are the use of an air-free environment (thus enhancing product quality), improved energy efficiency, higher drying rates, and reduced environmental impact when condensate is reused (Markowski *et al.* 2003). Although superheated steam requires a longer treatment time compared to steam explosion and high-pressure steam treatment, the process can be considered a safe operation because it is done at low temperature (Head *et al.* 2010). In addition, superheated steam treatment is a cost-effective method for large-scale purposes.

Based on the issues just described, the main objective of this study is to identify the optimum temperature and retention time for superheated steam pretreatment, to allow for efficient enzymatic saccharification of cellulose, and to investigate the physiochemical properties of OPMF.

## EXPERIMENTAL

### Materials

Oil palm mesocarp fiber (OPMF) was obtained from Seri Ulu Langat Palm Oil Mill (Dengkil Selangor, Malaysia). The OPMF were dried at 60 °C for 24 h prior to the treatment.

### Pretreatment Using Superheated Steam

The pretreatment of OPMF was performed using superheated steam equipment (9F-5200, Naomoto Corporation, Japan), based on the method described by Bahrin *et al.* (2012). Tap water was used to produce the superheated steam. A built-in fan inside the equipment helped to circulate the injected superheated steam uniformly in the heating chamber.

Prior to the treatment process, the OPMF was placed on aluminium foils and oven dried overnight at 105 °C. In each treatment process, exactly 10 g of OPMF was treated at different temperatures (140 °C, 180 °C, 210 °C) and retention times (20, 40, 60, 90 min).

After the process was completed, the chamber was left to reach ambient temperature. The samples were collected and oven dried at 105 °C for 24 h.

### Weight Loss Analysis

The weight of OPMF was recorded before and after each pretreatment process. Prior to the pretreatment, the OPMF was oven dried overnight at 105 °C. Once the pretreatment using superheated steam was completed, the steam injection was stopped and the heating chamber was left to reach the ambient temperature. The treated OPMF samples were then collected, oven dried at 105 °C for 24 h, and the weight loss was recorded.

### Enzymatic Saccharification of OPMF

Saccharification of the OPMF was performed with 5% (w/v) substrate in 50 µM sodium acetate buffer of pH 4.8. Ten Filter Paper Units (FPU) of Celluclast 1.5 L mixed with 50 U/mL β-glucosidase (Novozymes) were used for the saccharification. The process was performed for 24 h in an orbital shaking incubator (ZHWHY-1102C/02, Labwit, China) at 50 °C and agitated at 200 rpm. The fermentable sugars obtained were recovered by centrifugation at 10,000 rpm for 5 min.

The supernatant was taken and tested for total reducing sugar using dinitrosalicylic acid (DNS) based on Miller's method (1959). The cellulose and hemicellulose contents of OPMF were analyzed using acid detergent fiber (ADF) and neutral detergent fiber (NDF), respectively (Goering and van Soest, 1970; AOAC 2002.04 2007). The hydrolysis rate percentage was calculated according to the equation described by Latif *et al.* (1994) as follows:

$$\text{Hydrolysis rate (\%)} = \frac{\text{Total reducing sugar } \left(\frac{\text{g}}{\text{L}}\right) \times 0.9 \times 100}{\text{Substrate } \left(\frac{\text{g}}{\text{L}}\right) \times [\text{hemicellulose} + \text{cellulose } \left(\frac{\text{g}}{\text{g}}\right)]} \quad (1)$$

### Thermal Analysis (TGA)

A thermogravimetric analyzer (TGA/DTA 6200, SII Nano Technology Inc, Japan) was used to investigate the mass loss of the samples. The treated samples were ground

into powdered form in order to avoid heat and mass transfer limitations. During TG/DTA analysis, the samples were heated from 30 to 550 °C at a heating rate of 2721.5 C/min. Purified nitrogen was flushed at a flow rate of 100 mL/min to provide an inert atmosphere for thermal decomposition. Weight loss and heating rates were continuously recorded during the experiment.

### Scanning Electron Microscope (SEM)

A scanning electron microscope (S-3400N, Hitachi, Japan) was used to analyze the morphological structure of the superheated steam-treated OPMF. The samples were mounted on an aluminum stub using double-sided adhesive tape and were sputter-coated (E-1010, Hitachi, Japan) with platinum prior to the morphological examination. The SEM micrographs were obtained at an acceleration voltage of 15-25 kV.

### Fourier Transform Infrared (FTIR)

FTIR experiments were performed using a Spectrum™ GX, 2000R device (Perkin Elmer, USA) at wave numbers 500 to 4000  $\text{cm}^{-1}$ . The instrument was operated at 4  $\text{cm}^{-1}$  resolution, and samples were subjected to 16 scans/s. An attenuated total reflectance (ATR) was applied to obtain information on the surface modification of the OPMF samples.

## RESULTS AND DISCUSSION

The weight loss and composition changes of OPMF are important indicators of the effectiveness of superheated steam treatment. In this study, the overall weight loss of OPMF was varied from 3.41% to 18.17% under different treatment conditions and retention times. As shown in Table 1, it was found that the weight loss increased from 3.41% to 5.23% when treatment time was increased from 20 to 60 min for steam treatment at 140°C.

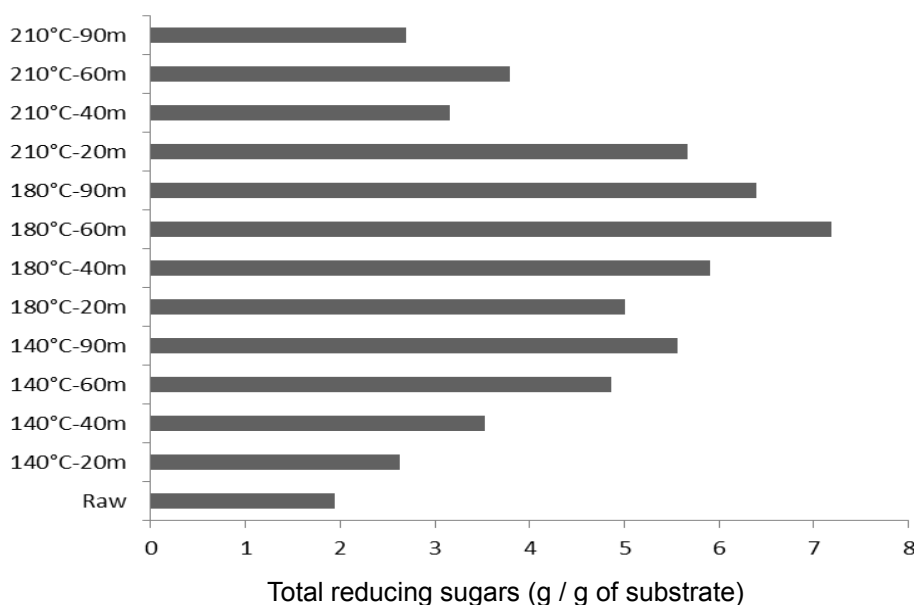
**Table 1.** Weight Loss of OPMF After Superheated Steam Treatment

Temp	Time (min)	Weight Loss (%)
140°C	20	3.41
	40	3.84
	60	5.23
	90	4.74
180°C	20	6.81
	40	7.36
	60	7.61
	90	8.05
210°C	20	9.37
	40	12.31
	60	15.60
	90	18.17

In general, the weight loss of OPMF treated at different temperatures increased steadily with increasing retention time. Increasing temperature also resulted in increased weight loss. The most apparent weight loss occurred at a treatment temperature of 210 °C. At 90 min, the weight loss was the highest (18.17%) compared to 20, 40, and 60 min. During the steam treatment process, a loss in weight indicates the removal of a major part of the hemicellulose from the solid material, which makes the cellulose more susceptible to enzymatic digestion (Bahrin *et al.* 2012). However, a temperature that is too high is not beneficial, as it can deteriorate the structure of cellulose itself. This could be demonstrated from the amount of sugar obtained after enzymatic hydrolysis, as will be discussed in the next section. TG and FTIR analyses made it possible to further determine the selective structural degradation of OPMF components.

### Enhancement of Enzymatic Saccharification

The effect of superheated steam treatment on cellulose digestibility in ligno-cellulosic materials was assessed following the release of reducing sugar (RS). The results of the experiments for superheated steam treatment of OPMF are shown in Fig. 1. Overall, superheated steam treatment enhanced the rate of enzymatic saccharification compared to untreated OPMF, although this effect depended on the temperature and retention time.



**Fig. 1.** Total reducing sugar obtained after 24 hours of incubation

For superheated steam treatment at 140 °C (Fig. 1), 90 min retention time yielded the highest amount of total reducing sugar. The yield of reducing sugar at 180 °C increased by 28.57% when the retention time was increased from 20 min to 60 min. However, the yield decreased slightly when the retention time was further increased to 90 min. The maximum yield of reducing sugar was 7.18 g/g, occurring at a temperature of 180 °C, after 60 min retention time.

Increasing the temperature to 210 °C led to a further decrease in the yield of reducing sugar. The reducing sugar yield decreased again after increasing the retention time. It is possible that under more severe conditions, such as a temperature of 210 °C,

and with increasing retention time, some of the cellulose was solubilised along with hemicelluloses (Bahrin *et al.* 2012). Such severe conditions could also cause a degradation of the physical and chemical properties of the cellulose.

The hydrolysis rate was described as the percentage of soluble sugar produced per amount of substrate (OPMF) used in the saccharification. Table 2 shows the hydrolysis rate for untreated and treated samples for 60 min retention time. The hydrolysis rate between untreated and treated OPMF at 140 °C was not significantly different. However, the highest hydrolysis rate occurred at a temperature of 180 °C, which is 4 times higher than the hydrolysis rate of raw OPMF. Thus, increasing the temperature of superheated steam resulted in a higher hydrolysis rate. However, a temperature of 210 °C should be avoided during superheated steam treatment because it will decrease the hydrolysis rate of OPMF. The samples listed in Table 2 will be further analyzed to determine the morphological changes of the OPMF upon steam treatment.

**Table 2.** Enzymatic Saccharification of OPMF After Superheated Steam Pretreatment for 60 Minutes

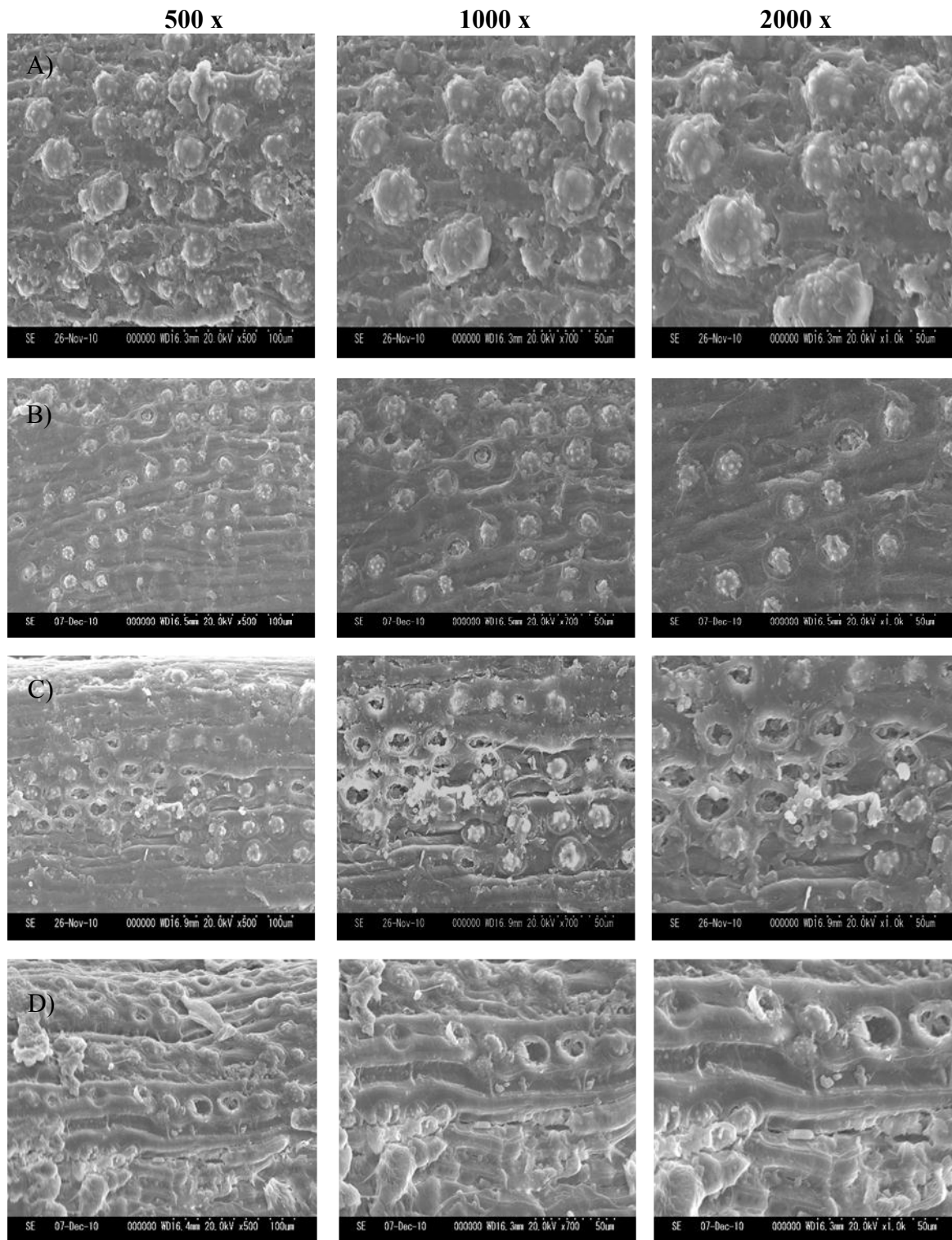
Temperature of OPMF	Hydrolysis Rate (%)
Raw	13.18
140 °C	13.40
180 °C	58.28
210 °C	33.23

### Scanning Electron Microscopy (SEM)

Morphological features of untreated and treated OPMF samples at different temperatures are shown in Fig. 2. Based on the images, untreated samples seem to have had a great number of silica bodies embedded in the OPMF structure. They attached themselves to circular craters, which were spread rather uniformly over the strand's surface. The structure looked very rigid, rough, and solid. The treated OPMF at 140°C showed a marked change, in that some of the materials on the structure seemed to be removed by heat. Some silica bodies inside the pores were partially removed, as is clearly apparent in Fig.2B. However, the energy produced at this temperature was not sufficient to remove a significant amount of silica bodies from the structure. Silica bodies act as a shield to protect the plant structure and concurrently enhance its mechanical strength (Law *et al.* 2007). The presence of silica bodies inside the pores will hinder a fast enzymatic hydrolysis of OPMF.

When the steam temperature was increased to 180 °C, the outer layer of the OPMF's inner structure was distorted and decomposed. The removal of silica bodies seems to be more obvious in Fig. 2C. In addition, some cracks and micropores can be clearly seen on the strand of OPMF structure. The disordered structures of the pre-treated OPMF would be more accessible to and more readily penetrated by hydrolytic enzymes for enzymatic digestion than those of untreated materials. For OPMF treated at 210 °C, the most obvious changes were that its surface looked smooth and clear as almost all the silica bodies were completely removed. As a result, there were holes and cracks along the OPMF inner structure. However, this characteristic was found to be unfavorable for

enzymatic saccharification reactions, because a portion of the cellulose component was degraded.



**Fig. 2.** (A) SEM micrograph of raw OPMF, (B) superheated steam treated OPMF at 140 °C, (C) 180°C, and (D) 210 °C, for 60 min

### Thermogravimetric (TG) Analysis

The obtained TG (weight fraction remaining) and DTG (weight loss rate) curves for untreated and treated OPMF at different temperatures after 60 min of treatment are shown in Fig. 3. The TG curves showed that untreated OPMF samples had a lower thermal stability than the treated samples. After steam treatment, the OPMF was comprised of an increasing percentage of lignin structure due to the removal of cellulose and hemicellulose. Lignin consists of a highly branched structure with many stable C-C carbon chains in its matrix. The lignin residue intensified the thermal stability and thus making it difficult to decompose the treated OPMF.

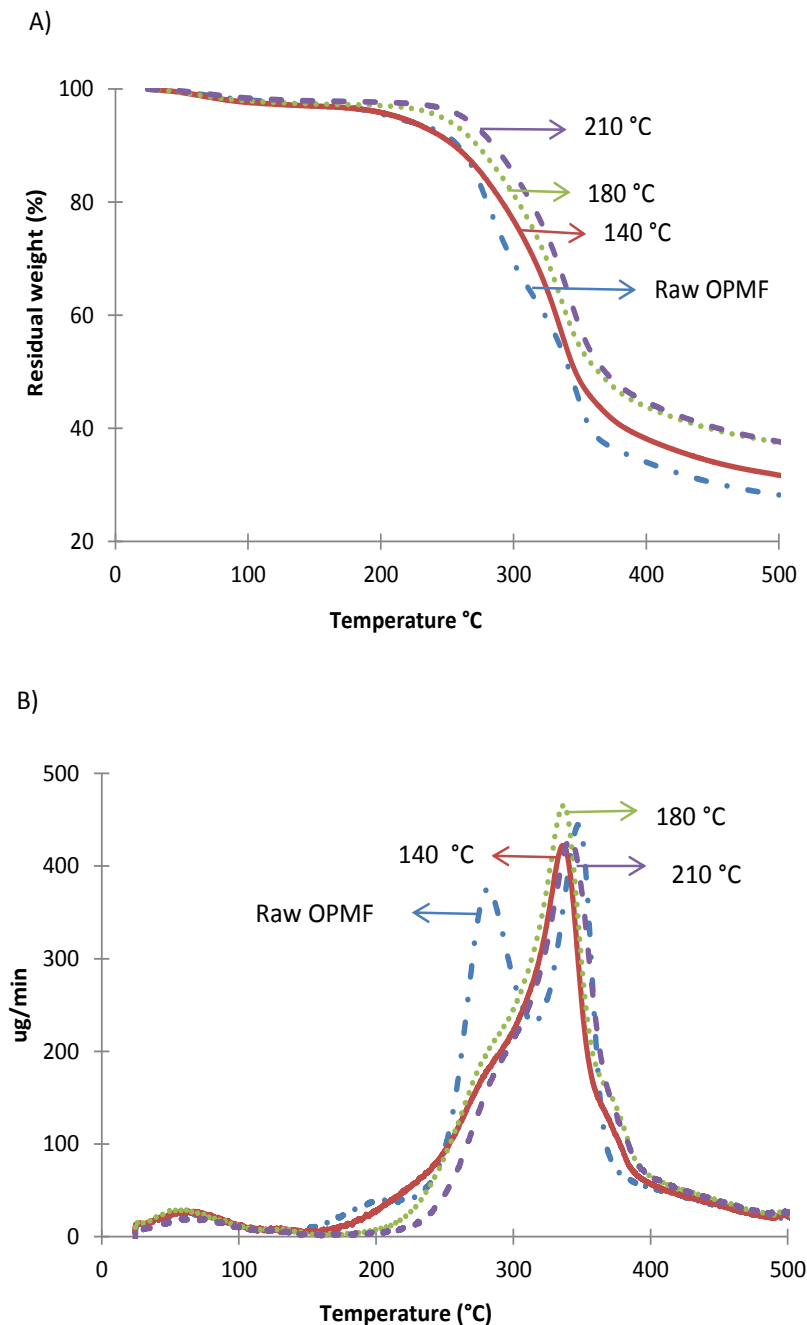


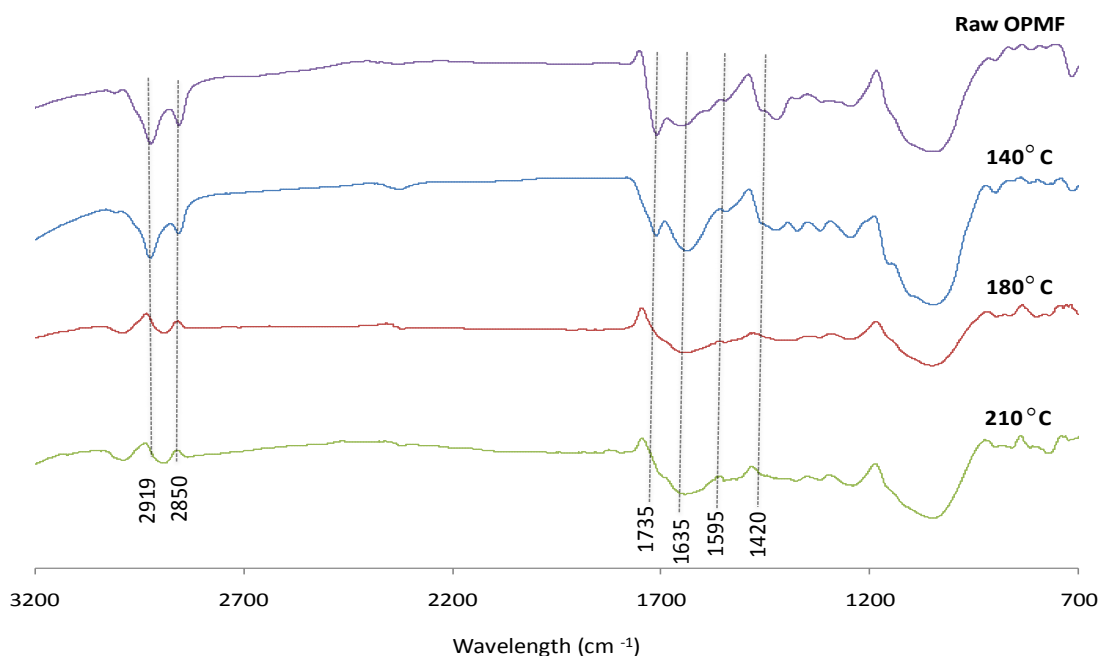
Fig. 3. TG (A) and DTG (B) of raw and treated OPMF after 60 min of treatment



Figure 3B compares the DTG profile of treated and untreated OPMF samples. The figure exhibits a distinguishable peak of a preliminary loss of moisture content at below 100 °C. For untreated OPMF, another peak appears between 250 and 300 °C. The peak most likely corresponds to the hemicellulosic structure of the OPMF. The peak was not present in the case of treated OPMF, indicating that hemicelluloses were degraded in the steam treatment process. According to Fengel and Weniger (1984), hemicellulose has a lower molecular weight compared to cellulose and is easily hydrolyzed. Another prominent peak is at 320 to 390°C, which is attributed to the degradation of the cellulose component and is present for both treated and untreated OPMF. The existence of this peak indicates that the cellulose matrix inside the lignocellulosic material was not significantly affected by this particular treatment.

### FTIR Spectral Analysis

The FTIR spectra of untreated and treated OPMF are given in Fig. 4. An analysis was conducted in order to identify the existing functional group of the samples. The diminished peak at 1710  $\text{cm}^{-1}$  belongs to a carbonyl, which is a component of hemicelluloses. This indicates that the treatment was able to remove a large portion of hemicelluloses. Figure 4 also shows an increase in the absorbance intensity ratio of C-H stretching bands at approximately 2850  $\text{cm}^{-1}$  and 2920  $\text{cm}^{-1}$ . This might occur due to the presence of C-H moieties in the samples.



**Fig. 4.** FTIR spectra of untreated and treated OPMF at 140°C, 180°C, and 210 °C

The most important bands that help to identify the cellulose component are 1420  $\text{cm}^{-1}$ , which can be attributed to the amorphous cellulose and crystallized cellulose II, and 1430  $\text{cm}^{-1}$ , which is attributed to crystallized cellulose I. In the present study the peak intensity at 1420  $\text{cm}^{-1}$  decreased when the treatment temperature was increased from 140°C to 180°C, and the peak seemed to disappear at 210 °C. This indicates that the crystalline cellulose II had been reduced.

**Table 3.** The Main Functional Groups of Three Components

Wave number	Functional group	Functional group/component	References
1420	C-H bending	Amorphous cellulose	Wang <i>et al.</i> 2009
1430	C-H bending	Crystallized cellulose	Wang <i>et al.</i> 2009
1595	Aromatic skeletal	Lignin	Kristensen <i>et al.</i> 2008
1635	O-H bending	Hemicellulose	Smidt <i>et al.</i> 2005
1710	C=O	Hemicellulose	Gastaldi <i>et al.</i> 1998
2850	C-H Stretch	Cellulose and hemicellulose	Wang <i>et al.</i> 2009
2916	C-H Stretch	Cellulose and hemicellulose	Wang <i>et al.</i> 2009

Cellulose is relatively stable compared to other components. However, superheated steam treatment at 210 °C was able to break down its structure. The observation was similar to the results reported by Sun *et al.* (2005). One of the strategies employed in increasing enzymatic convertibility is to decrease cellulose's crystallinity. The lignin band at approximately 1595 cm<sup>-1</sup> was slightly reduced after the superheated steam treatment. The bending mode of absorbed water, which was recorded at 1635 cm<sup>-1</sup>, was gradually diminished when the temperature of superheated steam treatment was increased. Due to these beneficial characteristics, it has been demonstrated that after superheated steam treatment, the intensity of these peaks changes, and the disappearance of some bands reveals that hemicellulose was largely removed. This result is consistent with the thermogravimetric (TG) analysis results described earlier.

## CONCLUSIONS

1. The morphology and structure of raw and superheated steam treated oil palm mesocarp fiber (OPMF) exhibited significant differences. The removal of silica bodies, crater appearances, and microtubular form of OPMF were observed on the surface of treated OPMF.
2. Most of the cellulose and hemicellulose components in OPMF were degraded when the material was treated at 210 °C or higher temperatures. Therefore, superheated steam treatment of OPMF at 210 °C was not favourable for biosugar production.
3. The optimum time and temperature for the pretreatment of oil palm mesocarp fiber (OPMF) were determined to be 180 °C and 60 minutes. Thus, it can be concluded that the superheated steam treatment is an attractive and promising method to enhance enzymatic hydrolysis for bio-sugar production.

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