*bioresources*.com

# PHYSICOCHEMICAL PROPERTY CHANGES AND ENZYMATIC HYDROLYSIS ENHANCEMENT OF OIL PALM EMPTY FRUIT BUNCHES TREATED WITH SUPERHEATED STEAM

Ezyana Kamal Bahrin,[a] Azhari Samsu Baharuddin,[b,*] Muhammad Faizal Ibrahim,[a] Muhammad Nafis Abdul Razak,[a] Alawi Sulaiman,[c] Suraini Abd-Aziz,[a] Mohd Ali Hassan,[a] Yoshihito Shirai,[d] and Haruo Nishida [d]

The effect of superheated steam treatment on oil palm empty fruit bunches (OPEFB) was investigated in terms of physicochemical property changes and enzymatic hydrolysis enhancement. The experimental treatment was carried out at different temperatures (140-210°C) and durations (20-90 min). Results showed that as the superheated steam temperature and time increased, the size distribution also changed, resulting in more small particles. Analysis on the surface texture, color, and mechanical properties of the treated OPEFB also showed that significant changes resulted due to the superheated steam treatment. In support to this, Fourier Transform Infrared (FTIR) spectroscopy and thermogravimetric (TG) analyses showed that solubilization and removal of the hemicelluloses component also took place. As a result of this phenomenon, higher total sugar and glucose yield was achieved once the treated OPEFB was subjected to enzymatic hydrolysis. This suggests that superheated steam treatment could enhance OPEFB structure degradation for the preparation of a suitable substrate in order to produce a higher glucose yield in the enzymatic hydrolysis process.

*Contact information: a: Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor,Malaysia; b: Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, SerdangSelangor,Malaysia; c: Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; d: Department of Biological Functions and Engineering, Graduate School of Life Science and System Engineering, Kyushu Institute Technology,2-4 Hibikino, Wakamatsu-ku, Kitakyushu, Fukuoka 808-0916, Japan.\* Corresponding author: azharis@eng.upm.edu.my*

## INTRODUCTION

Currently, oil palm plantations contribute to the largest solid biomass generation in Malaysia (Baharuddin et al. 2010). From 55.73 million tons of oil palm biomass generated in Malaysia, oil palm empty fruit bunches (OPEFB) contributed 17.0 million tons, or 30.5 percent, and this is increasing year by year (Shuit et al. 2009). Currently OPEFB is the largest source of lignocellulosic material that can be obtained from the palm oil industry. OPEFB is a renewable material and can be obtained at a very low cost directly from the mill, thus making it a very suitable candidate as a potential feedstock for various applications such as biosugar, compost, and bioethanol production (Baharuddin et

al. 2010; Sanchez and Cardona 2007). Bioconversion of OPEFB has many environmental and socioeconomic benefits because it can reduce the adverse impacts of the current inefficient disposal treatment, such as incineration and mulching (Baharuddin et al. 2011). Hubbe et al. (2010) reviewed work showing that composting is an economical way to handle the bulky cellulosic waste and thus reduce the greenhouse gas emission in the environment.

Despite its advantages, the use of OPEFB is hindered due to the low efficiency of the current pre-treatment methods. As reported in many studies, the pre-treatment of OPEFB can be achieved with physical, mechanical, chemical, or biological methods to enhance its digestibility in the subsequent bioconversion process. It is crucial to identify the pre-treatment conditions that not only allow for maximum recovery of hemicelluloses-derived sugar for subsequent fermentation process, but also produce sufficiently accessible cellulose for efficient enzymatic digestion. The bioconversion of lignocellulosic material into hexose and pentose sugars is difficult to achieve, as pretreatment is required prior to depolymerization (Ohgren et al. 2006). In terms of cost, a pre-treatment step is the most significant contributing factor to the production cost of the cellulosic-bioethanol process (Balat et al. 2008).

Among the pretreatment methods, steam explosion is the most studied physical treatment for lignocellulosic materials, especially for hardwoods such as aspen, poplar, and oak (Sanchez and Cardona 2007). However, this method is unreliable and not cost-effective for softwood material. In terms of the solubilization effect, the steam explosion also disrupts the chemical structure of lignin. Thus, this process contributes to the soluble fermentable sugars and phenolic substances in hydrolysate and is, therefore, unfavorable. On the other hand, chemical treatment methods have been reported to be widely used for the pre-treatment of OPEFB (Baharuddin et al. 2009; Hamisan et al. 2009; Hamzah et al. 2011) for fermentable sugars production. Although this technique has been shown to be successful in many laboratory applications, it does not support the green technology concept and minimization of chemical use that should be made priority in order to eliminate the release of chemical wastes into the environment. Moreover, Sánchez and Cardona (2007) revealed that chemical treatments are not competitive for large-scale purposes, and other alternative lignocellulosic treatments need to be considered; one such promising option is superheated steam treatment.

Superheated steam is a form of unsaturated steam produced by the addition of heat to the saturated steam. The heat helps to raise the saturated steam temperature so that it exceeds the boiling point of the liquid at a certain pressure value. The advantages of superheated steam over the other treatments include improved energy efficiency, low risk on environmental impact when condensate is reused, and safe operation (Head et al. 2010). Superheated steam has been extensively applied to treat lignocellulosic materials for various applications for instance, bioabsorbents, biofuel production, activated carbon, and others (Pach et al. 2002; Sagehashi et al. 2006). Based on our knowledge, superheated steam treatment of OPEFB for fermentable sugars production is the first to be reported. Although many studies have been conducted to identify the best pre-treatment for biosugar production, it is a great challenge to acquire the most reliable one when embarking large scale processing of OPEFB in the palm oil industry.

Therefore, the main objective of this study was to investigate the effects of superheated steam temperature and treatment time on the physicochemical properties of OPEFB. In addition, enzymatic hydrolysis of the superheated steam treated OPEFB was conducted to determine the maximum fermentable sugar yield.

## EXPERIMENTAL

### Raw Materials
Oil palm empty fruit bunches (OPEFB) were obtained from Seri Ulu Langat Palm Oil Mill (Dengkil Selangor, Malaysia). Prior to use the materials were physically pressed to remove the oil and moisture and shredded to the size of 15 to 20 cm and kept in a chiller at 4°C for storage.

### Pre-treatment Using Superheated Steam
The pre-treatment of OPEFB was performed using superheated steam equipment (9F-5200C, Naomoto Corporation, Japan). Normal tap water was used to produce the superheated steam. A built-in fan inside the equipment helps to circulate the injected superheated steam thoroughly in the heating chamber. Prior to the treatment process, the pressed-shredded OPEFB were placed on aluminum foil and oven dried overnight at 105°C. In each treatment process, exactly 10 g of OPEFB were treated at different superheated steam temperatures (140, 180, and 210°C) and times (20, 40, 60, and 90 min), respectively. Once completed, the steam injection was stopped and the heating chamber was left to reach the ambient temperature. Then, the treated OPEFB samples were collected, oven dried at 105°C for 24 hrs, and weighed before further analysis.

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

### Particle Size Distribution
Firstly, the treated OPEFB samples were ground using a hammer mill prior to the particle size analysis at various sizes of 500, 250, 150, 106, and 63 µm. The sieving process was operated for about 3 min for each size. Then, the sieve samples obtained were collected and weighed. The particle geometry of all samples was examined by using a light microscope (Keyence, USA).

### Thermal Analysis
A thermogravimetric analyzer (TGA/DTA 6200, SII NanoTechnology Inc, Japan) was used to investigate the mass loss of the samples. The treated samples were ground into powdered form and weighed (5 to 7 mg) for each analysis 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 10 K/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.

## Fourier Transform Infrared (FTIR)

FTIR experiments were performed using Spectrum™ GX, 2000R (Perkin Elmer) from wave-numbers at 500 to 4000 cm$^{-1}$. The instrument was operated at 4 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 OPEFB samples.

## Lignicellulosic Composition Analyses

The cellulose, hemicellulose, and lignin contents were assayed using acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) (Goering and Van Soest 1970). The percentages of cellulose, hemicelluloses, and lignin were computed using the equations below:

$$\text{Cellullose}(\%) = \text{ADF-ADL} \tag{1}$$

$$\text{Hemicellulose }(\%) = \text{NDF-ADF} \tag{2}$$

$$\text{Lignin }(\%) = \text{ADL} \tag{3}$$

## Enzymatic Saccharification

Saccharification process was carried out in duplicate with 5% (w/v) of substrate in 50 mM of sodium acetate buffer with pH 4.8. Enzymes, 10 FPU of Celluclast 1.5 L added with 50 U/mL β-glucosidase of Novozymes, were used for saccharification. The process was performed for 48 hours in an orbital shaking incubator (ZHWY-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 reducing sugar concentration using dinitrosalicylic acid (DNS) based on Miller's (1959) method. The hydrolysis percentage was calculated according to the equation described by Latif et al. (1994) as follows:

$$\text{Hydrolysis }(\%) = \frac{\text{Total reducing sugar (g/L)} \times 0.9 \times 100}{\text{Substrate (g/L)} \times [\text{Hemicellulose (g/g) + Cellulose (g/g)}]} \tag{4}$$

## RESULTS AND DISCUSSION

## Physicochemical Properties of Treated OPEFB

Figure 1 shows the physical appearance of the untreated OPEFB (a), treated OPEFB at 140°C/60 min, treated OPEFB at 180°C/60 min, and treated OPEFB at 210°C/ 60 min. It can be seen that the color of the treated OPEFB at 180°C/60 min changed from light brown to dark brown, and the smell of the sample was similar to the "burnt sugar". For the sample that was treated at 210°C/ 60 min, the color changed to darker brown and

the smell of the "burnt sugar" was more evident. This phenomenon must be attributed to the photochemical reaction of carbohydrate and lignin substance by the increase of superheated steam temperature (Bledzki et al. 2010). From the carbohydrate analysis, it was found that the total carbohydrate of OPEFB was reduced from 71.3% (cellulose and hemicelluloses in the untreated sample) to 63.7% once the OPEFB was treated at 210°C/ 60 min. The reduction of carbohydrate compound was possibly due to the elimination of the waxy layer on the outer surface of the OPEFB structure, as described by Bledzki et al. (2010). In the superheated steam treatment process, hemicellulose was reported to be the easiest component to decompose compared to the two other primary components: cellulose and lignin (Hendriks and Zeeman 2009). This is because the side chain (branched) and backbone of hemicellulose is very sensitive to any thermal treatment process; this is one of the distinctive properties of hemicellulose. This finding is consistent with lignin analysis. Lignin is a well known component that provides mechanical strength, impermeability, and restricts the enzymatic saccharification and; therefore it is well protected during any pre-treatments of lignocellulosic material (Hendriks and Zeeman 2009). From the lignin analysis, it was found that the percentage of lignin composition in the treated OPEFB increased from 14.2% (in the untreated OPEFB) to 20.9% (in the treated OPEFB at 210°C for 60 min). The superheated steam treated OPEFB sample at 210°C/60 min also became brittle and easily broken once milled compared to the untreated OPEFB. This indicated that by increasing the super-heated steam temperature at 210°C for 60 min, the mechanical strength of OPEFB material was reduced.
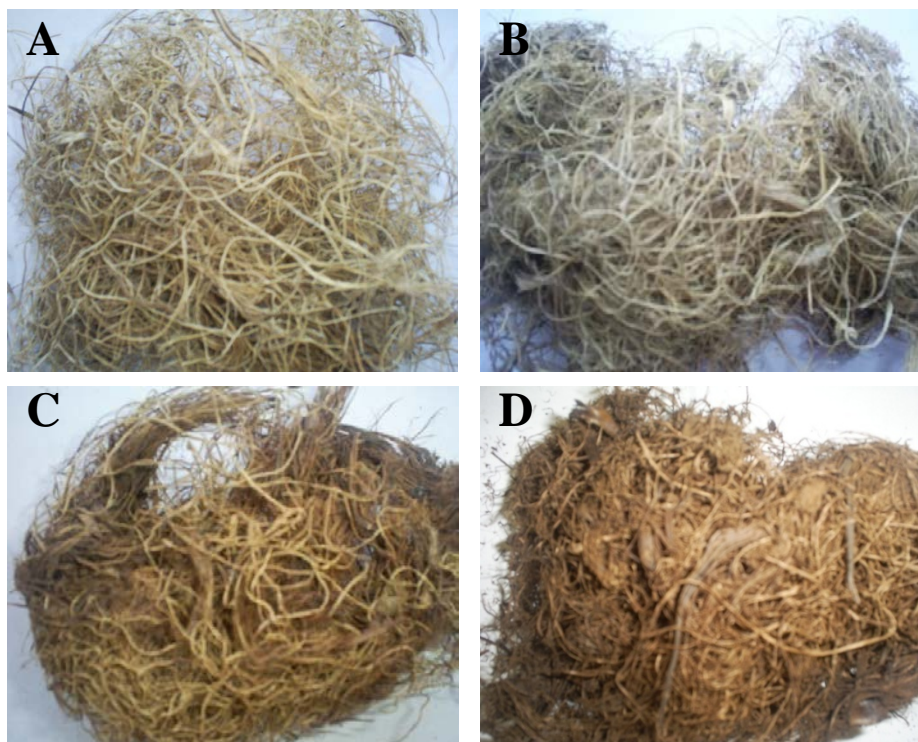


**Fig. 1.** Physical appearance of superheated steam treatment (A) Raw OPEFB, (B) 140°C, (C)180°C, (D) 210°C at a 60min treatment time

## Scanning Electron Microscope (SEM) Observations

Scanning electron micrographs for the untreated and superheated steam treated OPEFB samples at different temperature are shown in Fig. 2. For the untreated OPEFB, it can be clearly seen that silica bodies were found embedded along the OPEFB structure. This is consistent with the previous findings by Baharuddin et al. (2011). A similar observation (Fig. 2b) was also recorded for the OPEFB sample treated at 140°C, which suggested that at that temperature there was still insufficient energy to remove the silica bodies from the material.
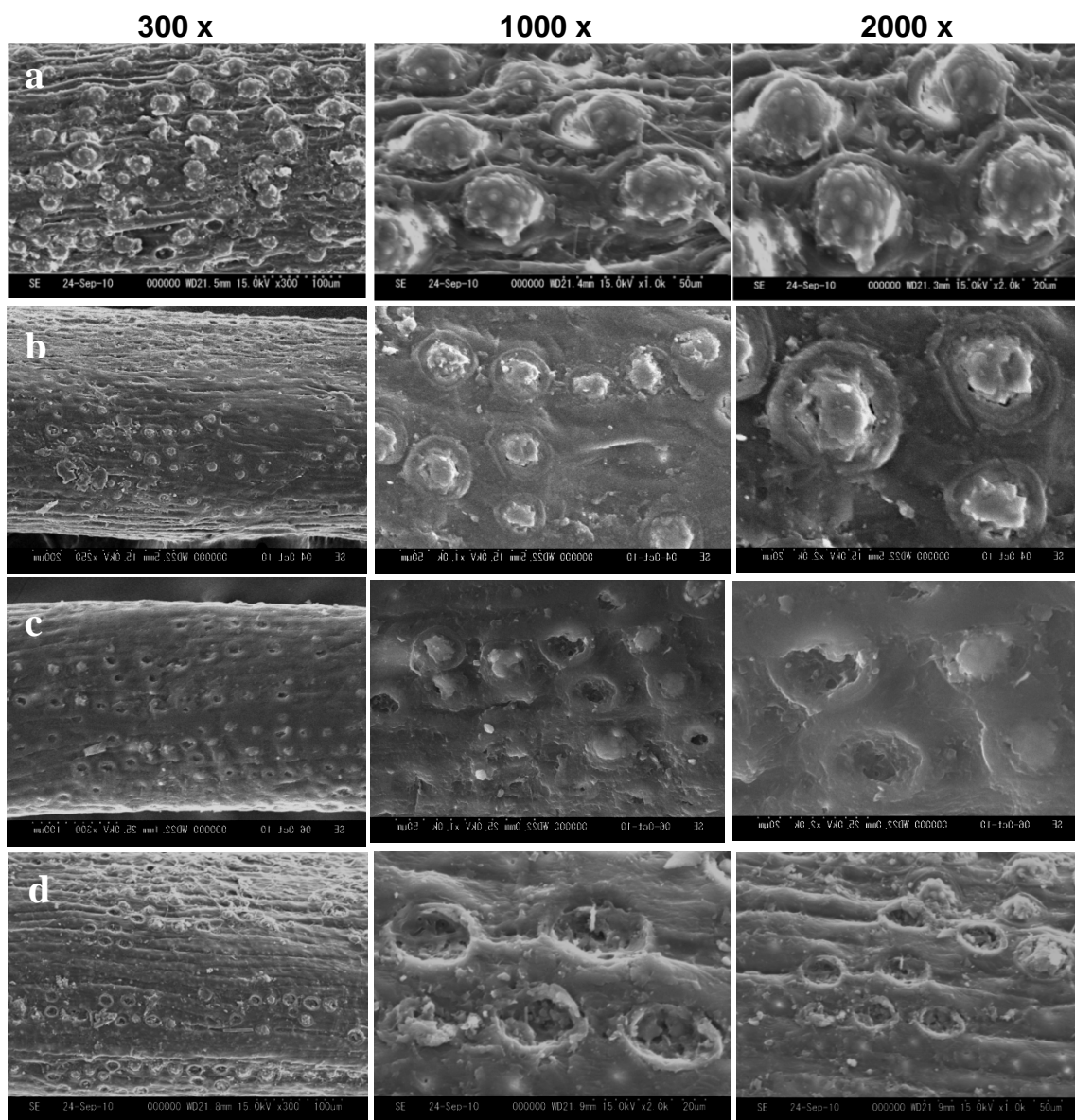


**Fig. 2.** SEM micrograph of raw OPEFB (a), superheated steam treated OPEFB at 140°C (b), 180°C (c), 210°C, (d) for 60 min

**_bioresources.com_**

For the treated OPEFB at 180°C/60 min (Fig. 2c), some of the silica bodies had been removed from the OPEFB structure. It has been reported that the removal of silica bodies from the OPEFB is very important because the presence of silica bodies might restrain the accessibility of enzymes into the internal layer of the OPEFB structure during saccharification process. When the superheated steam temperature was increased to 210°C/60 min, many empty craters can be clearly observed from the micrographs, indicating that the available energy from the superheated steam was able to remove most of the silica bodies in the OPEFB structure.

Upon further extension of the treatment time to 90 min, it can be seen that the weight loss of the OPEFB structure was significantly increased to 17.8%, as shown Table 1. In addition, some cracks and micropores (0.5 to 0.7µm) can also be clearly seen on the strand of the OPEFB structure. In nature, silica bodies act as a shield against fungal attack; they also give the plant structural support (Neethirajan et al. 2009). However, these characteristics have been found to be unfavorable for the enzymatic hydrolysis reaction and become a hindrance to the cellulose to break-down the cellulosic polymer (Hamzah et al. 2011). Based on this finding, it is suggested that the superheated steam would enhance the accessibility of enzymatic hydrolysis of OPEFB, especially when shifted to steam temperatures above 180°C.

## Thermogravimetric (TG) Analysis

The thermal decomposition results of the untreated and treated OPEFB (60 and 90 mins) are shown in Figs. 3 and 4. The results suggested that the OPEFB materials exhibited a higher residual weight ratio after the superheated steam treatment. The lignin component and residual of hemicellulose and cellulose in the OPEFB contribute to the high residual weight in TGA results (Yang et al. 2007). All the profiles of the treated OPEFB samples shifted to a higher temperature range compared to the untreated OPEFB, indicating that the thermal stability occurred after the steam treatment. Treated samples of superheated steam were comprised of modified structure of lignin and other components. Lignin, with its content of aromatic groups and its highly branched structure, is very difficult to decompose by treatment at low temperature. Thus, this phenomenon resulted in increasing thermal stability of the treated sample.

The increase of steam temperature and treatment time caused increases in the main peak height for cellulose degradation in DTG profiles, and shifted them to a higher temperature region of 320 to 390°C. The degradation peak of the cellulose component was slightly shifted to a lower temperature when treated at 210°C for both treatment times. The DTG graph showed a distinguishable peak of preliminary loss of moisture at 100°C, whereafter it was followed by other peak at around 220 to 315°C, which corresponded to the depolymerisation of hemicellulose, a process that mainly occurred from the xylan chain. The treatment time of 20 minutes was insufficient to decompose the hemicellulose component, except at 210°C. However, a hemicellulose shoulder was found to have disappeared after the treatment condition was increased to 210°C for 60 and 90 min. Table 1 shows significant increment of weight loss (%) when temperature and treatment time are increased. Hemicellulose decomposition, silica body removal, and volatilization of some components (CO, $CO_2$, $CH_4$ and some hydrocarbons) contribute to

the biomass weight loss (Yang et al. 2007). This finding also corresponded well with TG-DTG curve and SEM results.

Generally, hemicellulose is enriched with many polyoses chains and branch structures that are susceptible to decomposition at low temperatures (Sagehashi et al. 2006). Palonen et al. (2004) found that the elimination of hemicellulose component tremendously increased the opening of the substrate's pore size and thereby amplified the hydrolysability of the lignocellulosic fiber. Based on the thermal decomposition trend, the cellulose-rich fraction was increased after the superheated steam treatment.

**Table 1.** Weight Loss of OPEFB after Superheated Steam Treatment

| Temperature (°C) | Treatment Time (min) | Weight | | Weight Loss (%) |
|---|---|---|---|---|
| | | Before (g) | After(g) | |
| **140** | 20 | 7.2853 | 7.1994 | 1.18 |
| | 40 | 7.5563 | 7.3161 | 3.18 |
| | 60 | 7.4985 | 7.2475 | 3.35 |
| | 90 | 7.3737 | 7.0638 | 4.20 |
| **180** | 20 | 7.6878 | 7.2851 | 5.24 |
| | 40 | 7.4349 | 6.9752 | 6.18 |
| | 60 | 7.3095 | 6.7627 | 7.48 |
| | 90 | 7.8005 | 7.2383 | 7.21 |
| **210** | 20 | 7.7984 | 7.0984 | 8.98 |
| | 40 | 7.3253 | 6.4922 | 11.37 |
| | 60 | 7.3075 | 6.2782 | 14.09 |
| | 90 | 7.5228 | 6.1783 | 17.87 |

## FTIR Spectra Analysis

Figure 5 shows the results of the Fourier Transform Infrared (FTIR) analysis of the untreated and treated OPEFB samples under different superheated steam temperature for 60 min of treatment time. The results of FTIR spectroscopy of all samples showed a major pattern of a broad adsorption band in the range of 3000 to 3600 $cm^{-1}$ (OH stretching vibration), which can be attributed to the hydrogen bond or hydroxyl group in the phenolic and aliphatic compounds. Absorptions at 2850 and 2910 $cm^{-1}$, which were attributed to the C-H stretching, were gradually diminished with an increase in the treatment temperature. It was reported that the C-H band corresponds to the aliphatic moieties mainly in polysaccharides of cellulose and hemicelluloses (Wang et al. 2009). Ktistensen et al. (2008) reported that a band 1749 $cm^{-1}$ corresponds to the carbonyl groups of hemicelluose. In Fig. 5, a band at1749 $cm^{-1}$ was observed to gradually decrease when the steam temperature increased to 210°C. This result corresponded well with the DTG curve, which showed the removal of the hemicellulose component from OPEFB structure after the superheated steam treatment.
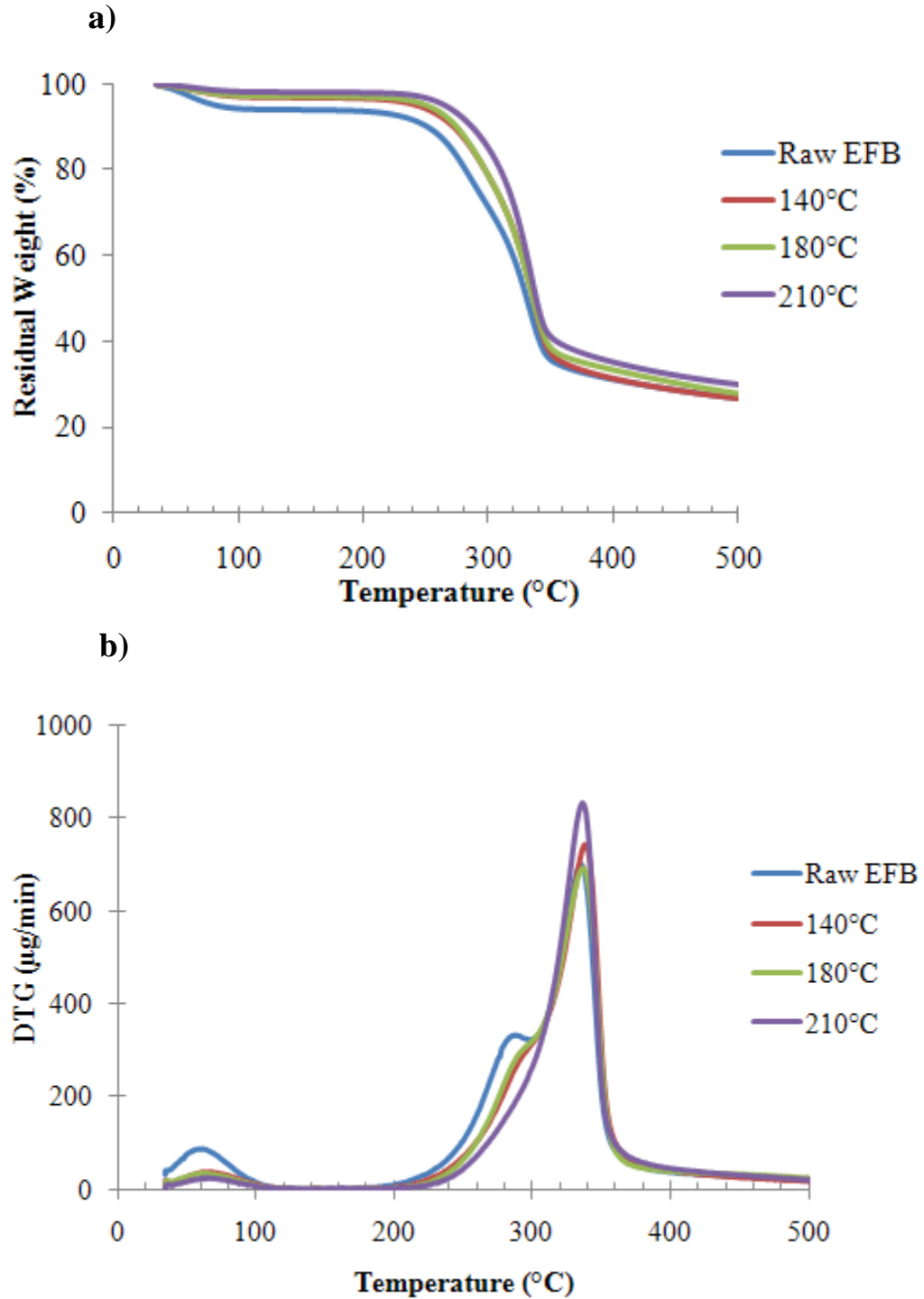
a)



b)



**Fig. 3.** TGA (a) and DTG (b) curve of OPEFB according to the superheated steam temperature for 60 minutes of treatment time
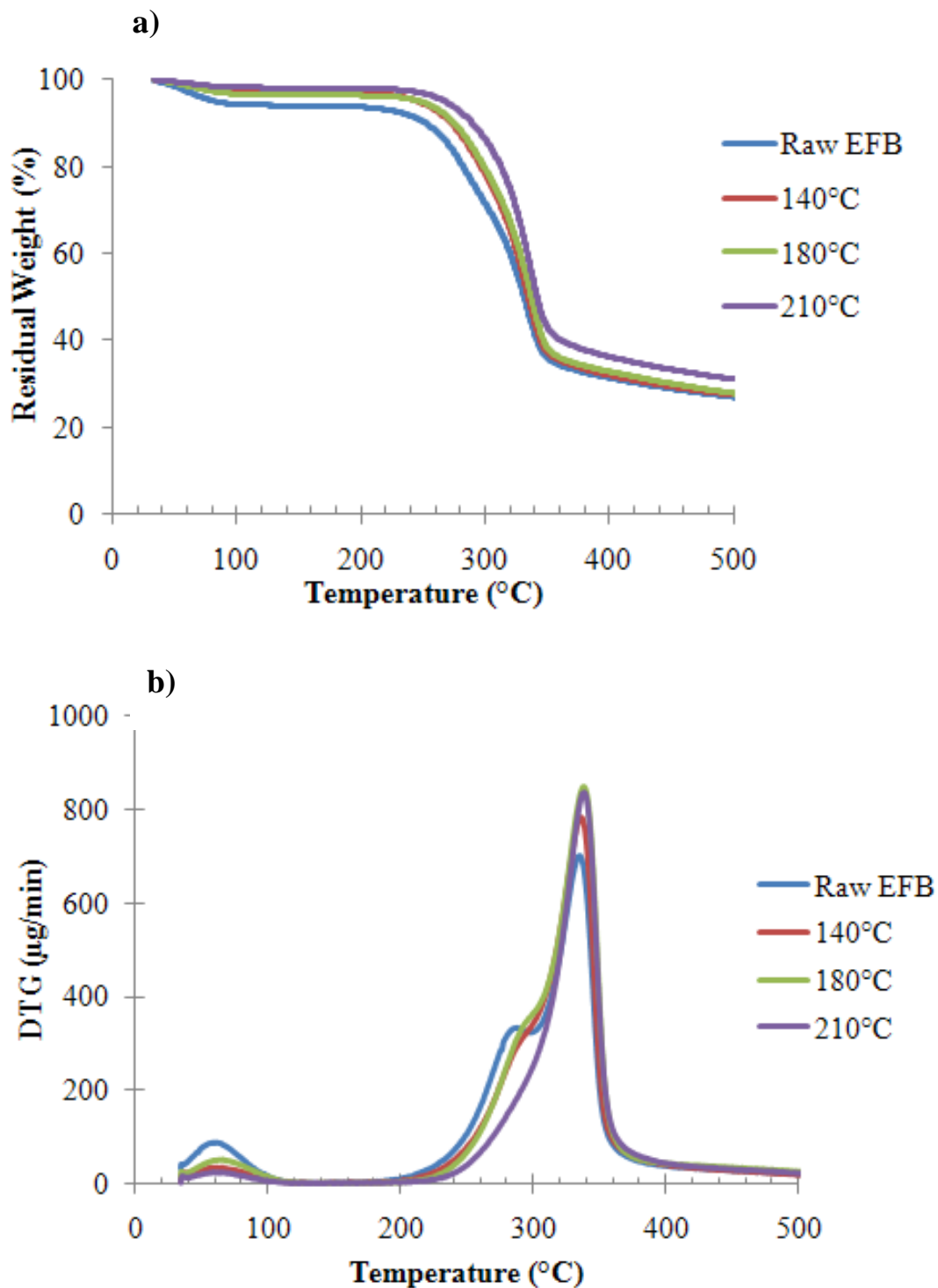
a)



b)



**Fig. 4.** TGA (a) and DTG (b) curve of OPEFB according to the superheated steam temperature for 90 minutes of treatment time

The bending mode of the absorbed water was recorded at 1645 cm$^{-1}$. Important bands to identify the cellulose component were 1420 and 1430 cm$^{-1}$, which were attributed to the amorphous/crystallize cellulose structure. In this study, in general, it was found that the band at 1420 cm$^{-1}$ increased when shifted to the higher steam temperature. The absorption band 1516 cm$^{-1}$ corresponds to the aromatic skeletal vibration (Wang et al. 2009). The range of frequency at 1000 to 1200 cm$^{-1}$ portrayed the C-O-C stretching, covalent bond of C-O, and C-OH linkage, which were reported to be dominant in the cellulose and lignin components (Yang et al. 2007).

In this study, it was found that the increase of steam temperature led to lignin disassociation from the aromatic hydrogen bond. This has been proven by the increasing proportion of aromatic hydrogen from lignin, as indicated by the presence of sharp peaks at 811 cm$^{-1}$. According to Javed et al. (2010), bands of high stretching vibration modes at 811 cm$^{-1}$ (790 cm$^{-1}$ to 815 cm$^{-1}$) indicated that the bonds in O-Si-O became vulnerable due to the high temperature treatment process. On the other hand, lower stretching and absorption band indicates that the symmetric vibrations of silicon atoms in silixane linkages (O-Si-O) are strong and not disrupted. In this study, only slight changes can be seen at the intensity of ca.1100 cm$^{-1}$, which can be assigned as the antisymmetric motion of silicon atoms in siloxane bonds (Swann and Patwardhan 2011).
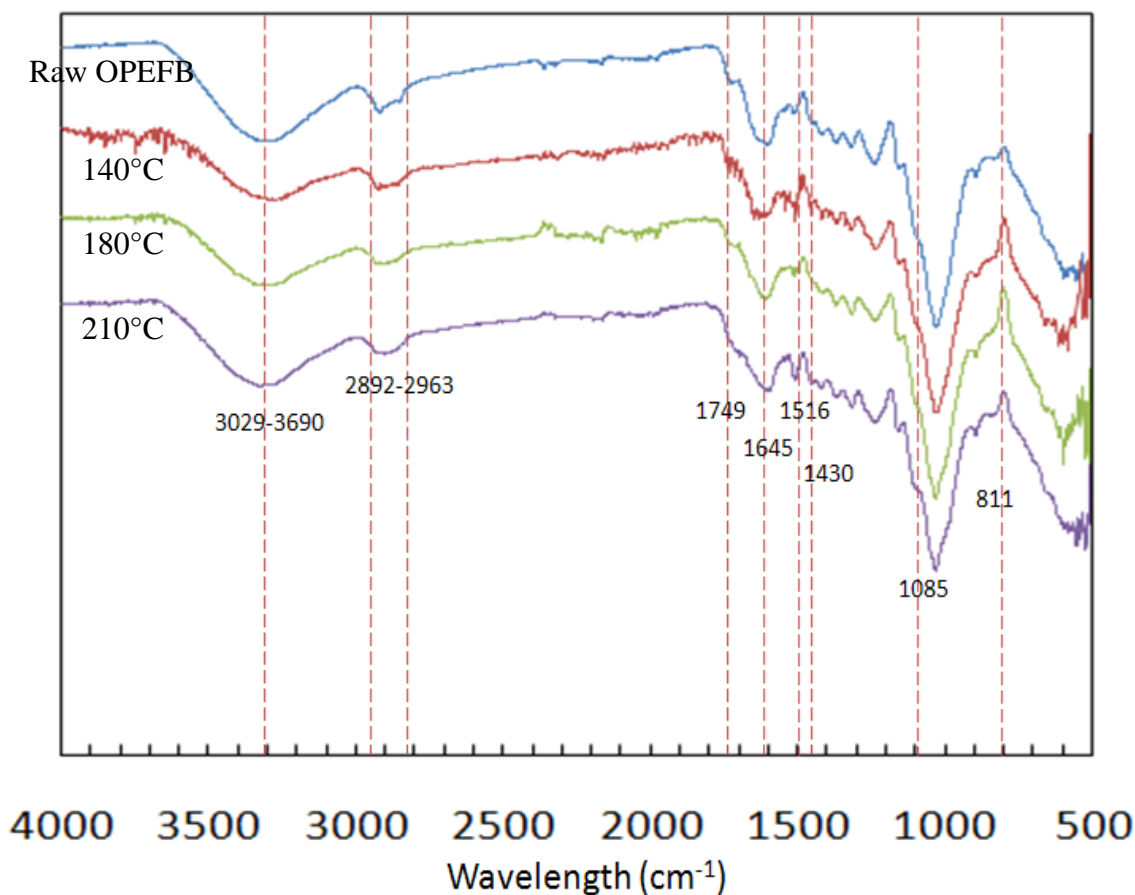


**Fig. 5.** FTIR spectra of raw OPEFB and superheated steam treated OPEFB samples at 140°C, 180°C and 210°C for 60 min of treatment time

## Particle Size Distribution

The particle size distribution of OPEFB at different superheated steam temperatures (140°C, 180°C and 210°C) is illustrated in Fig. 6. Unimodal distributions were vividly apparent for all temperatures after superheated steam treatment. It can be clearly seen that the dominant group of particle size was the group of higher than 500 µm, whereas the least dominant group of particle size was the group of less than 63 µm. This finding was in agreement with Vidal et al. (2011), who elucidated that most of lignocellulosic pretreatments contribute to the biomass particle size reduction.

Interestingly it was also found that as the treatment temperature was increased, the percentage of the small particles also increased, as clearly exhibited in Fig. 6. For example, the particle size of group 150 to 250 µm had increased rapidly as the treatment temperature was increased to 210°C (Fig. 6c). This could be explained based on the structure degradation of OPEFB once treated with superheated steam, as clearly shown in the SEM micro-graph.

The advantage of smaller particles is that they helped to liberate a higher amount of product. For example, it was reported that smaller particles (250 µm) liberated more glucose after 12 hours of saccharification compared to bigger particles (425 µm) (Simarani et al. 2010). In the pyrolysis process, smaller particles (250 to 355 µm) also helped to expedite the pyrolysis reaction, as reported by Abdullah et al. (2011). Therefore, with the higher amount of smaller particles produced as a result of superheated steam treatment, it was expected that it could enhance the enzymatic hydrolysis process as well.

The distribution in the aspect ratio (length/breadth) of treated OPEFB with superheated steam was observed under a light microscope. It was found that the aspect ratio increased with temperature and time of treatment. The aspect ratio at 140°C was recorded to be in the range of 1.23 to 6.22, whereas at 180°C and 210°C they were 1.71-12.24 and 3.5-32.04, respectively. Particles having flake-like and near spherical geometry were commonly found in OPEFB after treatment at 140°C and 180°C, respectively. It was observed that most of the OPEFB fibers were mixtures of various sizes and shapes when treated at 210°C. Interestingly, at this temperature, many of the OPEFB fiber shapes were observed as cylinder-like structures, and this has resulted in the highest aspect ratio (Fig. 7).

According to Lu et al. (2010), cylinder-like structures are more favorable in the bioconversion process because they have a higher surface area to volume ratio compared to the spherical particles, and this may contribute to higher conversion efficiency. Lamsal et al. (2010) reported an attractive outcome of lignocellulosic content in wheat straw after grinding.

The lignin and hemicellulose constituents in the OPEFB fibers were reduced and thereby increased the ratio of cellulose percentage. Indeed, the segregation of OPEFB components occurred during the sieving process.
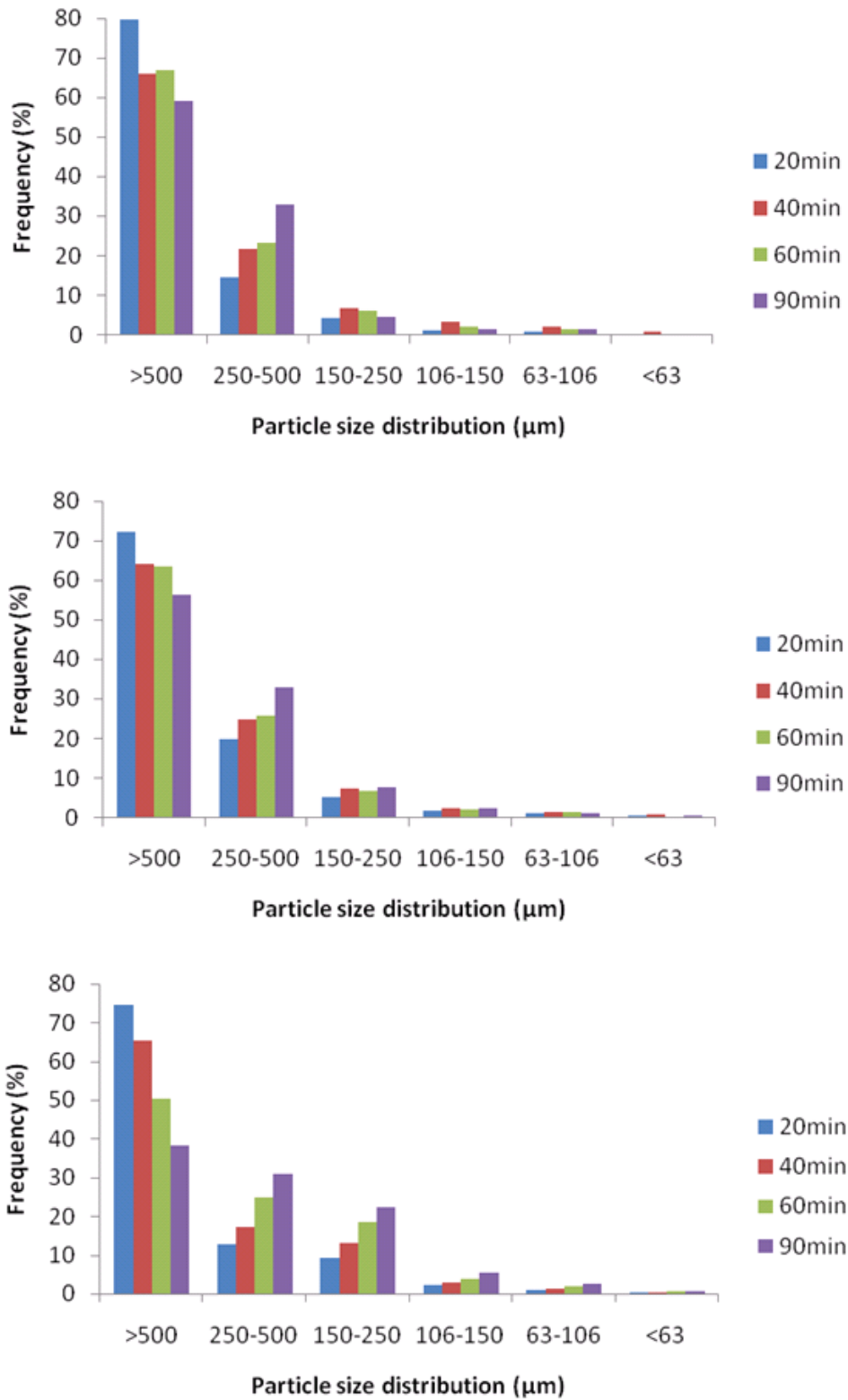
**Fig. 3.** Particle size distribution of OPEFB treated with superheated steam for 140°C (a), 180°C (b),and 210°C (c)
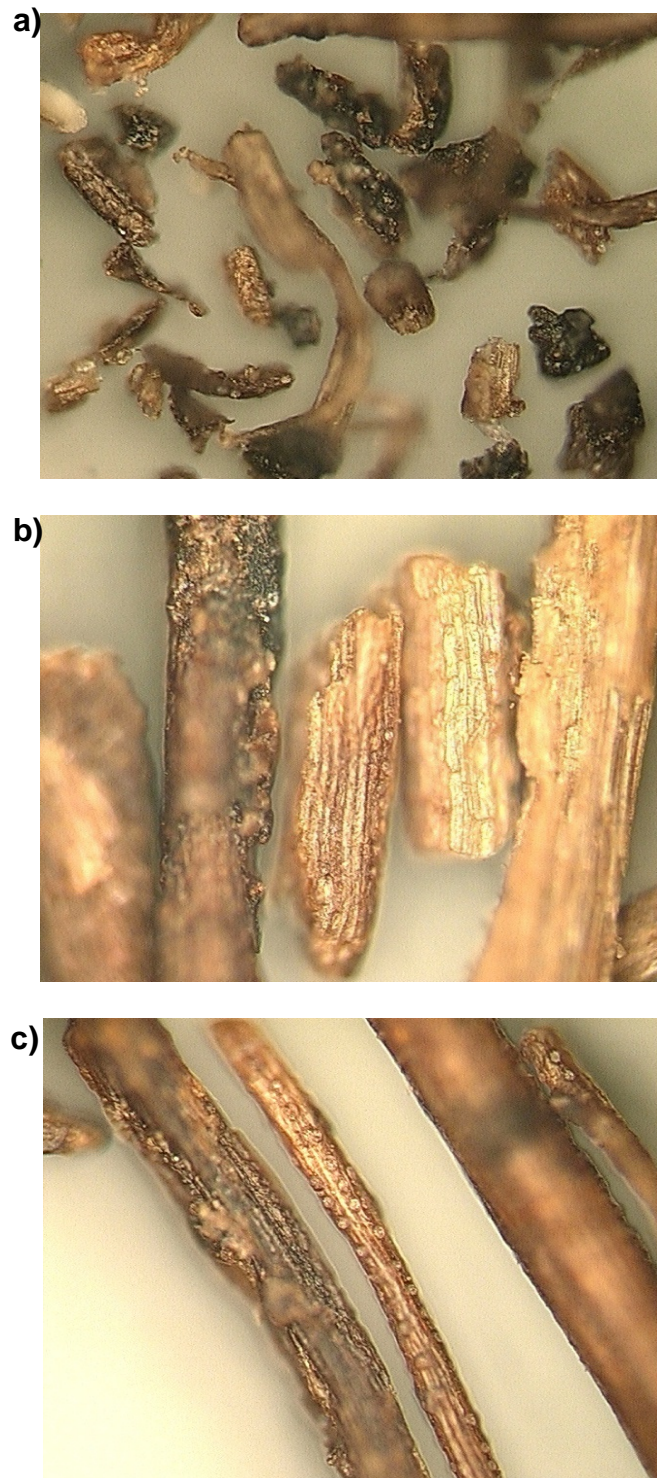
**Fig. 7.** Observation of OPEFB particle geometry; spherical shape (a), flake-like (b), and cylinder-like (c), after treated with 210°C of superheated treatment

### Enhancement of Enzymatic Saccharification

Enzymatic saccharification of the untreated and treated OPEFB at different temperatures is presented in Table 2. It was found that the hydrolysis rate of the untreated OPEFB exhibited the lowest bioconversion of sugars with glucose yield only 15.6%. The enzymatic hydrolysis of OPEFB was found to have increased when enzymatic saccharification was performed using the OPEFB samples that had been treated at 140°C and 180°C. The total reducing sugar results with different temperature treatment showed very significant differences with p-values of less than 0.0001. It can be seen that the treated OPEFB at 180°C for 60 min of treatment gave the highest production of total reducing sugars ($26.5 \pm 2.2$ mg/L), as compared to other temperatures. Based on this result, the treated OPEFB gave 4.3 times higher of sugars yield. The maximum glucose concentration recorded was $504.641 \pm 0.0276$ mg/g, which corresponds to 66.3% of glucose conversion yield.

In this study, it was also found that the hydrolysis rate of OPEFB for the total reducing sugars was only $13.6 \pm 5.7$ mg/L when enzymatic saccharification was performed using the OPEFB sample that had been treated at 210°C. Although this result is slightly awkward, it is consistent with the results of physicochemical and thermal properties of the untreated and steam treated OPEFB. Due to the disruption of silica bodies and degradation of hemicelluloses component after superheated steam treatment, the efficiency of enzymatic reaction in the saccharification process improved and resulted in higher glucose yield. However once the OPEFB sample was treated at 210°C, a portion of cellulose component was deteriorated and could not be converted into glucose.

**Table 2.** Enzyme Saccharification of OPEFB after 48 Hours Incubation for 60 min

| Superheated steam condition | Total Reducing Sugar (mg/L) | Glucose/initial sample (mg/g) | Hydrolysis Rate (%) |
|---|---|---|---|
| Raw EFB | 6.154 ± 1.419 | 149.367 ± 0.0197 | 15.601 |
| 140°C | 20.643 ± 6.00 | 470.042 ± 0.0375 | 50.337 |
| 180°C | 26.532 ± 2.210 | 504.641 ± 0.0276 | 66.329 |
| 210°C | 13.604 ± 5.790 | 482.700 ± 0.0104 | 38.260 |

### CONCLUSIONS

1. In this study, superheated steam treatment was employed to alter the interfacial properties of Oil Palm Empty Fruit Bunch (OPEFB). Morphological and particle distribution studies revealed the alteration of OPEFB, especially the removal of silica bodies along the structure.
2. FTIR and TG analyses clearly showed the changes of chemical structure such as hemicelluloses degradation after the treatment.
3. The optimum temperature/time for the treatment for OPEFB was determined to be 180°C/60 min, which was later used to produce the highest hydrolysis rate of glucose production at 66.3%. Thus, it can be concluded that the superheated steam treatment is a promising method to enhance the enzymatic hydrolysis for biosugar production.

## ACKNOWLEDGMENTS

## REFERENCES CITED

Abdullah, N., Sulaiman, F., and Gerhauser, H. (2011). "Characterisation of oil palm empty fruit bunches for fuel application," *J. Phys. Sci.* 22(1), 1-24.

Baharuddin, A. S., Abd Razak, N. A., Abdul Rahman, N. A., Budiatman, S., Shirai, Y., and Hassan, M. A. (2009). "Biocoversion of oil palm empty fruit bunch by *Aspergillusniger* EB4 under solid-state fermentation," *Pertanika J. Trop. Agric. Sci.* 32 (2), 143 -151.

Baharuddin, A. S., Lim, S. H., Md.Yusof, M. Z., Abdul Rahman, N. A., Md. Shah., U. K., Hassan, M. A., Wakisaka, M., Sakai, K., and Shirai, Y. (2010). "Effects of palm oil mill effluent (POME) anaerobic sludge from 500 m$^3$ of closed anaerobic methane digested tank on pressed-shredded empty fruit bunch (EFB) composting process," *Afr. J. Biotechnol.* 9(16), 2427-2436.

Baharuddin, A. S., Abdul Rahman, N. A., Md. Shah, U. K., Hassan, M. A., Wakisaka, M., and Shirai, Y. (2011). "Evaluation of pressed shredded empty fruit bunch (EFB)-palm oil mill effluent (POME) anaerobic sludge based compost using Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analysis," *Afr. J. Biotechnol.* 10(41), 8082-8089.

Balat, M., Balat, H., and Öz, C. (2008). "Progress in bioethanol processing," *Progress in Energ. Combustion Sci.*34(5), 551-573.

Barbieri, S., Elustondo, M., and Urbicain, M. (2004). "Retention of aroma compounds in basil dried with low pressure superheated steam," *J. Food Eng.* 65(2004), 109-115.

Bledzki, A. K., Mamun, A. A., and Volk, J. (2010). "Physical, chemical and surface properties of wheat husk, rye husk and soft wood and their polypropylene composites," *Composites: Part A* 41,480-488.

Gani, A., and Naruse, I. (2007). "Effect of cellulose and lignin content on pyrolysis and combustion characteristics for several types of biomass," *Renew. Energ.* 32(4), 649-661.

Goh, C. S., Tan, K. T., Lee, K. T., and Bhatia, S. (2010). "Bio-ethanol from lignocellulose: Status, perspectives and challenges in Malaysia,"*Bioresour.Technol.* 101, 4834-4841.

Goering, H. K., and Van Soest, P. J. (1970). "Forage fiber analyses (apparatus, reagents, procedures, and some application)," USDA Handbook 379, U.S. Gov. Print. Office, Washington, DC.

Hamisan, A. F., Abd-Aziz, S., Kamaruddin, K., Md. Shah, U. K., Shahab, N., and Hassan, M. A. (2009). "Delignification of oil palm empty fruit bunch using chemical and microbial pretreatment methods," *Int. J. Agric. Res.* 4(8), 250-256.

Hamzah, F., Idris, A., and Shuan,T. K. (2011). "Preliminary study on enzymatic hydrolysis of treated oil palm (Elaeis) empty fruit bunches fibre (EFB) by using combination of cellulase and β 1-4 glucosidase," *Biomass Bioenerg.* 35, 1055-1059.

Head, D. S., Cenkowski, S., Arntfield, S., and Henderson, K. (2010)."Superheated steam processing of oat groats," *LWT - Food Sci. Technol.* 43, 690-694.

Hendriks, A. T. W. M., and Zeeman, G. (2009). "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresour. Technol.*100, 10-18.

Hubbe, M. A., Nazhad, M., and Sánchez, C. (2010)."Composting as a way to convert cellulosic biomass and organic waste into high-value soil amendments: A review," *BioResources* 5(4), 2808-2854.

Javed, S. H., Naveed, S., Feroze, N., Zafar, M., and Shafaq, M. (2010). "Crystal and amorphous silica from $KMnO_4$ treated and untreated rice husk," *J. Qual. Technol. Manage.* 6(1), 81-90.

Kelly-Yong, T. L., Lee, K. T., Mohamed, A. R., and Bhatia, S. (2007). "Potential of hydrogen from oil palm biomass as a source of renewable energy worldwide," *Energy Policy* 35, 5692-5701.

Kristensen, J. B., Thygesen, L. G., Felby C., Jørgensen, H., and Elder, T. (2008). "Cell-wall structural changes in wheat straw pretreated for bioethanol production," *Biotechnology for Biofuels*, BioMed Central, http://www.biotechnologyforbiofuels.com/1754-6834/1/5

Latif, F., Ibrahim, M. R., and Kauser, A. M. (1994). "Saccharification of *Leptochoa fusca* (Kallar grass straw) using thermostable cellulases," *Bioresour. Technol.* 50(2), 107-111.

Law, K. N., Wan Daud, W. R., and Ghazali, . (2007). "Morphological and chemical nature of fiber strands of oil palm empty-fruit-bunch (OPEFB)," *BioResources* 2(3), 351-362.

Lu, H., Ip, E., Scott, J., Foster, P., Vickers, M., and Baxter, L. L. (2010). "Effects of particle shape and size on devolatilization of biomass particle," *Fuel* 89(2010), 1156-1168.

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem.* 31(3), 426-428.

Misson, M., Haron, R., Kamaroddin, M. F. A., and Amin, N. A. S. (2009). "Pretreatment of empty palm fruit bunch for production of chemicals via catalytic pyrolysis," *Bioresour. Technol.* 100 (11), 2867-2873.

Neethirajan, S., Gordon, R., and Wang, L. (2009). "Potential of silica bodies (phytoliths) for nanotechnology," *Trends Biotechnol.* 27(8), 461-467.

Ohgren, K., Rudolf, A., Galbe, M., and Zacchi, G. (2006)."Fuel ethanol production from steam-pretreated corn stover using SSF at higher dry matter content," *Biomass Bioenerg.* 30(10), 863-869.

Omar, R., Idris, A., Yunus, R., Khalid, K., and Aida Isma, M. I. (2011). "Characterization of empty fruit bunch for microwave-assisted pyrolysis," *Fuel* 90(4), 1536-1544.

Pach, M., Zanzi, R., and Bjornbom, E. (2002). "Torrefied biomass a substitute for wood and charcoal," 6th Asia-Pacific International Symposium on Combustion and Energy Utilization, 20-22 May 2002, Kuala Lumpur.

Palonen, H., Thomsen, A. B., Tenkanen, M., Schmidt, A. S., and Viikari, L. (2004). "Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood," *Appl. Biochem.Biotechnol.* 117(1), 1-17.

Prachayawarakorn, S., Prachayawasin, P., and Soponronnarit, S. (2006). "Heating process of soybean using hot-air and superheated-steam fluidized-bed dryers," *Lebensmittel-Wissenschaft Und-Technologie* 39(7), 770-778.

Sagehashi, M., Miyasaka, N., Shishido, H., and Sakoda, A. (2006). "Superheated steam pyrolysis of biomass elemental components and Sugi (Japanese cedar) for fuels and chemicals," *Bioresour. Technol.* 97, 1272-1283.

Sánchez, O. J., and Cardona, C. A. (2007)."Trends in biotechnical production of fuel ethanol from different feedstocks," *Bioresour. Technol.* 99(13), 5270-5295.

Shen, D. K., Gu, S., and Bridgwater, A. V. (2010). "The thermal performance of the polysaccharides extracted from hardwood: Cellulose and hemicellulose," *Carbohydr. Polym.* 82(1), 39-45.

Shuit, S. H., Tan, K. T., Lee, K. T., and Kamaruddin, A. H. (2009). "Oil palm biomass as a sustainable energy source: A Malaysian case study," *Energy* 34(9), 1225-1235.

Simarani, K., Hassan, M. A., Abd-Aziz, S., Wakisaka, M., and Shirai, Y. (2009). "Effect of palm oil mill sterilization process on the physicochemical characteristics and enzymatic hydrolysis of empty fruit bunch," *Asian J. Biotechnol.* 1(2), 57-66.

Swann, G. E. A., and Patwardhan, S. V. (2011). "Application of Fourier Transform Infrared Spectroscopy (FTIR) for assessing biogenic silica sample purity in geochemical analyses and palaeoenvironmental research," *Clim. Past* 7, 65-74.

Umikalsom, M. S., Ariff, A. B., Zulkifli, H. S., Tong, C. C., Hassan, M. A., and Karim, M. I. A. (1997)."The treatment of oil palm empty fruit bunch fibre for subsequent use as substrate for cellulase production by *Chaetomiumglobosum* Kunze," *Bioresour. Technol.* 62(1-2), 1-9.

Vidal, B. C. J., Dien, B. S., Ting, K. C., and Singh, V. (2011). "Influence of feedstock particle size on lignocellulose conversion. A review," *Applied Biochemistry Biotechnology* 164, 1405-1421.

Wang, K., Jiang, J. X., Xu, F., and Sun, R. C. (2009). "Influence on steaming pressure on steam explosion pretreatment of Lespedza stalks (*Lespedzacryobotrya*): Part 1. Characteristics of degraded cellulose," *Polym. Degrad. Stab.* 94(9), 1379-1388.

Yang, H., Yan, R., Chen, H., Lee, D. H., and Zheng C. (2007). "Characteristics of hemicellulose, cellulose and lignin pyrolysis," *Fuel* 86, 1781-1788.