Hydrothermal pretreatment enhanced enzymatic hydrolysis and glucose production from oil palm biomass

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ABSTRACT

The present works investigate hydrothermal pretreatment of oil palm empty fruit bunch and oil palm frond fiber in a batch tube reactor system with temperature and time range from 170 to 250 °C and 10 to 20 min, respectively. The behavior of soluble sugars, acids, furans, and phenols dramatically changed over treatment severities as determined by HPLC. The cellulose-rich treated solids were analyzed by SEM, WAXD, and BET surface area. Enzymatic hydrolysis was performed from both pretreated slurries and washed solid, and data obtained suggested that tannic acid derived from lignin degradation was a potential cellulase inhibitor. Both partial removal of hemicellulose and migration of lignin during hydrothermal pretreatment caused structural changes on the cellulose-hemicellulose-lignin matrix, resulting in the opening and expansion of specific surface area and pore volume. The current results provided important factors that maximize conversion of cellulose to glucose from oil palm biomass by hydrothermal process.

Keywords: Oil palm biomass, hydrothermal pretreatment, tannic acid, specific surface area, enzymatic hydrolysis
1. Introduction

Oil palm frond fiber (OPFF) and oil palm empty fruit bunch (OPEFB) have been reported as suitable biomass for biochemistries and biomaterials production since these materials are rich in carbohydrate and lignin, available throughout a year, renewable and sustainable (Ho et al., 2014; Zahari et al., 2014). Better management and maximal utilization of these biomass will increase the value of the by-products generated and promote sustainability to the oil palm industry. However, the natural recalcitrance of lignocellulosic biomass has hindered its potential applications such as biochemical and biofuel production if no pretreatment is performed. Pretreatment is necessary to remove barriers such as hemicellulose and lignin that limit the penetration of enzyme to cellulose. Hydrothermal pretreatment has advantages over other pretreatments since the system only use water and the hydronium ion from water ionization act as catalyst in the reaction medium (Möller et al., 2011; Sabiha-Hanim et al., 2011). It was reported that the breakdown and loosening of the lignocellulosic structure in biomass was achieved by hydrothermal pretreatment and dilute acid at chosen treatment conditions. The removal of hemicellulose and migration of lignin loosened the intact structures of cellulose-hemicellulose-lignin matrix, thus improved adsorption of cellulase on cellulose-rich solids (Hsu et al., 2010; Pu et al., 2013; Xiao et al., 2014).
Successful fractionation of lignocellulosic biomass and the production of soluble sugar derived-carbohydrate and phenol-derived from lignin by hydrothermal pretreatments were greatly influenced by the combined reaction temperature and time normally expressed as treatment severity, log $R_0$ (Overend and Chornet, 1989).

Under hydrothermal pretreatment process, several soluble inhibitors were produced, which hampered the efficiency of enzymatic hydrolysis and fermentation. Soluble sugars both in oligomeric and monomeric forms were produced from degradation of hemicellulose and cellulose components. Xylooligomers (XOS) and xylose were the most detectable soluble sugars generated by hydrothermal pretreatment and studies have found that XOS were strong cellulase inhibitors (Qing et al., 2010; Kont et al., 2013). Acetyl groups in hemicellulose will degrade into acetic acid while pentose and hexose sugars will degrade into furfural and 5-HMF. Both furfural and 5-HMF can be further degraded into formic acids under acidic condition when higher treatment severities is applied (Möller et al., 2011; Nitsos et al., 2013). Phenolic compounds which originate from degradation of lignin and from phenolic ester groups linked with hemicellulose showed inhibition and deactivation of $\beta$-glucosidase from Trichoderma reesei and Aspergillus niger (Ximenes et al., 2010; Ximenes et al., 2011). Recently, Kim and co-workers (2011) found that both XOS and phenolic compounds
were the most important factors caused decreased cellulase activity. Total phenolic compounds as low as 1.3 g/L which strongly inhibited cellulase by precipitation and deactivation of β-glucosidase. The removal of soluble inhibitors prior enzymatic hydrolysis is necessary in order to maximize enzyme activity and reduction of enzyme dosage, in order to promote low cost cellulose to bioethanol production (Ximenes et al., 2010; Kim et al., 2011; Kim et al., 2013).

The cellulose-rich solids are more amenable to enzymatic attack since both hemicellulose and lignin had been partially solubilized and removed during the separation and washing steps. Physical and morphological changes of treated solids can be clearly distinguished from untreated sample with severe damages on the biomass surface, creation of pores/holes and fibrillation of fibers/woods. Removal of hemicellulose and lignin also resulted in increased crystallinity index (CrI) of cellulose, specific surface area (SSA) and pore volume (PV), thus improved the enzymatic digestibility of cellulose to glucose (Hsu et al., 2010; Xiao et al., 2013; Pu et al., 2013).

The aim of the current study was to investigate the chemical and physical changes behavior of OPFF and OPEFB in the process of hydrothermal pretreatment. Factors affecting maximal conversion of cellulose to glucose was highlighted and discussed.
2. Materials and methods

2.1 Raw materials and componential analysis

The OPEFB was collected from Seri Ulu Langat Palm Oil Mill, Dengkil, Selangor, Malaysia, and the OPFF was obtained from the oil palm plantation at Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The samples were prepared as discussed earlier (Zakaria et al., 2014a) prior to component analysis. The oil palm biomass components were determined using a method recommended by Teramoto et al. (2008).

2.2 Hydrothermal pretreatment

Unless otherwise stated, hydrothermal pretreatment of OPFF and OPEFB were conducted in a 35 mL stainless steel tube reactor as reported earlier (Zakaria et al., 2014b). 3 g (oven dried) of lignocellulosic biomass (OPFF and OPEFB) and 30 mL of distilled water was used to fill the reactor (equivalent to 1:10, solid: liquid ratio). The working volume employed in this study was 30 mL. The tightened reactor filled with biomass sample and water was then carefully emerged into a sand bath, which was maintained at temperature range from 170 - 250°C governed by automatic temperature controller. The reactor was agitated at 60 rpm in order to provide homogenize mixing of
the sample in the tube reactor. After 10-20 min of residence time, the reactor was transferred from the sand bath into water reservoir and cooled down to 30°C. The pH values of the raw materials and hydrothermally treated samples were measured using a digital pH meter (D-53, Horiba, Japan). The treated samples were filtered using filter paper No. 2 with a pore size of 5.0 µm (Advantec, Japan) under gravity flow and washed with distilled water until neutral pH. The neutralized solid was oven dried at 90°C for 24 h prior to enzymatic hydrolysis. The intensity of the hydrothermal treatment was expressed as severity factor (log $R_0$). The severity parameters corresponding to different hydrothermal pretreatment conditions are calculated as in Eq. (1).

$$R_0 = t \exp \left[ \frac{(T-100)}{14.75} \right]$$

In which $t$ is the reaction time (min), and $T$ is the hydrolysis temperature (°C) (Overend and Chornet, 1989).

**2.3 Enzymatic hydrolysis**

Unless otherwise stated, enzymatic hydrolysis was performed using an enzyme cocktail constituting 40 FPU/ mL *Acremonium* cellulase (Meiji Seika Co, Japan), and 10% Optimash BG (Genencor International, California, USA). In a standard assay, 0.75
mL (10 FPU/g substrate) of *Acremonium* cellulase, 2.5 mL of 1.0 M acetate buffer, pH 5.0, and 0.6 mL of 10% Optimash BG were added to treated samples (3g of dry weight) in 65 mL tube (NEG, Japan). The reaction mixture was added with distilled water to a total volume of 50 mL. In a smaller scale reaction, about 0.05 g of treated solids with total hydrolysis mixtures (1 mL) were placed in 2 mL Eppendorf tubes. The enzymatic hydrolysis was performed at 50°C for 72 h with stirring/shaking. The experiment was performed in triplicate and the results are presented as the average values. The enzymatic digestibility was represented by the sugars obtained (g sugars/ g materials) or sugar yield as calculated as in Eq. (2):

\[
Sugar \ yield \ (\%) = \frac{weight \ of \ monomeric \ sugars \ after \ enzymatic \ hydrolysis}{weight \ of \ potential \ total \ monomeric \ sugars \ after \ hydrolysis \ using \ H_2SO_4} \times 100 \quad (2)
\]

### 2.4 Analytical procedures

The untreated and treated OPEFB and OPFF samples that were lyophilized with t-butyl alcohol were further dried at 105°C for 6 h with degassing (BELPREP; Bel Japan, Japan) before setting up the measuring device. The measurement was performed using BELSORP-max (Bel Japan, Japan) at a temperature of -196°C. The specific
surface area of the sample was determined from the Brunauer-Emmett-Teller plot of nitrogen adsorption-desorption isotherms (Ishiguro and Endo, 2014). The total pore volume was determined at $P/P_0 = 0.99$.

Detection of hydrolyzed sugars, acetic acid, furfural, 5-HMF, and formic acid from HPT and enzymatic hydrolysis were performed using high-performance liquid chromatography (HPLC) according to the analytical method (NREL/TP-510–42623) (Sluiter et al., 2008). Tannic acid and gallic acid were determined by HPLC equipped with a UV detector (UV-2070 Plus, Jasco, Japan) using an Aminex 87H column (7.8 mm I.D. × 30 cm, BioRad, USA) with a Carbo-P micro-guard cartridge. The column oven was set at 60°C and samples were eluted at 0.60 mL/min with 8.0 mM H$_2$SO$_4$.

SEM and WAXD patterns analysis of the untreated and hydrothermally treated oil palm biomass samples were determined as reported earlier (Zakaria et al., 2014a). The crystallinity index (CrI) was calculated using Eq. (3) based on the method of Segal et al. (1959).

$$\text{Crystallinity index (\%)} = \left(\frac{I_{002} - I_{am}}{I_{002}}\right) \times 100 \quad (3)$$

$I_{002}$: The intensity at about $2\theta = 22.2^\circ$

$I_{am}$: The intensity at $2\theta = 17.6^\circ$
3. Results and discussion

3.1 Chemical compositions and properties of oil palm biomass

As shown in Table 1 the major component of untreated OPFF and OPEFB is cellulose, followed by hemicellulose and lignin. The chemical compositions of oil palm biomass reported in this study were in the ranges of earlier reports (Sabiha-Hanim et al., 2011; Goh et al., 2012; Hong et al., 2013; Ho et al., 2014). OPFF and OPEFB were pretreated by hydrothermal process at 170-250°C for 10-20 min. Solid yield, pH of the treated slurries and chemical compositions changed over treatment severities. The dissolution of soluble components of oil palm biomass occurred and correlated well with treatment severities resulting in decreased solid recovery yield. About 52.2-52.7% of solids yield were obtained from OPFF and OPEFB samples, at the highest treatment severity. pH decreased from neutral to acidic towards higher treatment severities due to accumulation of acetic acid from de-acetylation of hemicellulose degradation (Sabiha-Hanim et al., 2011, Ho et al., 2014).

The chemical composition of oil palm biomass was greatly affected by the hydrothermal pretreatment especially hemicellulose which decreased at higher reaction temperature. However, only partial degradation was obtained from OPFF instead of complete removal of hemicellulose component by dilute acid and hydrothermal
pretreatment observed from rice straw and *Tamarix ramosissima*, respectively (Hsu et al., 2010; Xiao et al., 2013). This might be due to moderate treatment severities and types of biomass used. About 2-fold increase of Klason lignin content was recorded towards higher treatment severities from both OPFF and OPEFB. This can be explained by the fact that most of the lignin was solubilized at selected treatment temperature range and re-condensation of this material took place upon cooling process. The current finding is in agreement with other studies which reported accumulation of Klason lignin under acidic environments (Goh et al., 2010; Sabiha-Hanim et al., 2011; Pu et al., 2013).

The dissolution of hemicellulose and relocation or migration of lignin resulted in higher cellulose content, with the highest at 58.7% and 52.7% from OPFF and OPEFB when hydrothermally treated at log $R_o = 3.94$ and 4.83, respectively. Reduction of cellulose content was recorded when oil palm biomass was pretreated beyond these values indicates `over cooking` and degradation of hexose sugars. The removal of amorphous hemicellulose has increased the crystallinity index (CrI) of cellulose towards higher treatment condition, and the increase in CrI of cellulose indirectly showed higher exposure of cellulose components amenable to cellulase attack.

Untreated and hydrothermally pretreated samples were subjected to SEM and BET analysis for their morphological structure, specific surface area (SSA) and pore
volume (PV) properties to understand the effect of hydrothermal pretreatment on oil palm biomass at structural level. Fig. S1 presents the morphological changes of OPFF and OPEFB samples over hydrothermal pretreatment at different treatment severities. At log $R_o$ = 3.06 to 4.24, OPFF had fibrillated (Fig. S1b-f) and can be distinguished clearly from its untreated form (Fig. S1a). Meanwhile, deposition of spherical Klason lignin droplets were obviously observed on the surface of OPEFB-treated solids (Fig. S1i-l) at log $R_o$ = 4.83 to 5.72, indicating dissolution and degradation of hemicellulose taking place compared to untreated OPEFB with solid and intact surface (Fig. S1g).

The removal of hemicellulose, migration and re-deposition of lignin upon cooling process on the surface of treated solids has been reported to increase the SSA and PV respectively thus substantially improved adsorption of cellulase to cellulose (Hsu et al., 2010; Pu et al., 2013; Xiao et al., 2013). The SSA and PV of untreated OPFF and OPEFB were recorded at 0.98 m$^2$/g, 0.0072 cm$^3$/g and 0.95 m$^2$/g, 0.0056 cm$^3$/g, respectively (Table 1). Increments of SSA and PV were observed when the oil palm biomass was imposed to higher treatment severities. The highest SSA and PV obtained were 7.2 m$^2$/g, 0.045 cm$^3$/g and 5.5 m$^2$/g, 0.042 cm$^3$/g, respectively from OPFF and OPEFB at the harshest treatment condition. Inconsistent values of SSA and PV from OPEFB samples at higher treatment severities may be due to severe disruption of
biomass particles and adherent of spherical lignin droplets on the surface of treated
OPEFB solids, as supported by SEM image. Similar result was reported when *Tamarix*  
*ramossissima* was imposed at severe treatment condition which caused the lignocellulose
damage and resulted in the reduction of SSA (Xiao et al., 2013). Nano pore size of
treated beech wood obtained by hydrothermal treatment and expansion in SSA had
improved interactions between biomass and enzymes leading to saccharification
enhancement (Nitsos et al., 2013). Recently, Ishiguro and Endo (2014) found that
fibrillation of *Eucalyptus* chips by addition of 20% NaOH in hydrothermal-
mechanochemical treatment improved the SSA by 76% (232 m\(^2\)/g) and resulted in
100% glucose yield. The impact of hydrothermal pretreatment on glucose conversion
yield from OPFF and OPEFB will be discussed in a later section.

3.2 Composition of the liquid fraction from hydrothermal pretreatment

Table 2 presents the formation of total and monomeric sugars from liquid
fraction of OPFF and OPEFB as functions of pretreatment severities. The oligomeric
sugars can be estimated by the difference between the total and monomeric sugar
concentrations. Xylooligosaccharides (XOS) and glucooligosaccharides (GlcOS) were
the major products obtained by hydrothermal pretreatment of OPFF and OPEFB with
maximum production recorded at treatment severity, log $Ro = 3.65$ and $5.42$, respectively. OPFF has shown higher production of XOS and GlcOS with concentrations of $97.9$ mg/g and $54.2$ mg/g, respectively at optimal pretreatment condition, log $Ro = 3.65$ compared to OPEFB. This can be explained by higher composition of hemicellulose in OPFF compared to OPEFB (Table 1). Other oligomeric and monomeric galactose, arabinose and mannose were detected in lesser amounts and concentration of sugars were decreased at higher treatment severities, suggesting further degradation of pentose and hexose sugars into fermentation inhibitors. Sabiha-Hanim et al., (2011) reported that about $4.3\%$ of xylose was detected in the hydrolysate of treated OPFF samples when autohydrolyzed at log $Ro = 2.40$ ($121^\circ$C, 60 min). On the other hand, low XOS and xylose was obtained from OPEFB in this study was due to the high treatment severity applied in the hydrothermal pretreatment process that further converted those sugars into degradation by-products. Recently, a higher XOS concentration of $17.6$ g/L was reported from solubilisation of $63$ g/100 g xylan at $210^\circ$C (log $Ro = 3.91$) from OPEFB samples (Ho et al., 2014). This indicates that reaction temperature and time are crucial parameters in decomposition of hemicellulose component and selective generation of oligomeric and monomeric sugars.

In general acetyl groups in hemicellulose will degrade into acetic acid while
pentose and hexose sugars will degrade into furfural and 5-HMF. Fig. 1 shows degradation by-products produced from OPFF and OPEFB hydrolysate as a function of treatment severities. Acetic acid, furfural, 5-HMF and formic acid are the four major by-products produced. The concentrations increased at increasing treatment severities and reached the maximum at the harshest treatment condition, log $R_0 = 4.24$, with production of 71.7 mg/g (acetic), 18.4 mg/g (5-HMF), 37.4 mg/g (furfural) and 48.2 mg/g (formic acid) from OPFF samples (Fig. 1a). Meanwhile, OPEFB showed lower concentration and different profiles of degradation by-products formation than OPFF. 5-HMF produced from OPFF and OPEFB samples were detected at moderate concentrations, indicating minimal impact on hexose-base polysaccharides, suggesting that both oil palm biomass were pretreated in the suitable reaction temperatures and time range. Formic acid production may contributed from further degradation of furfural and 5-HMF at severe treatment condition (Nitsos et al., 2013). The highest formic acid accumulation from hydrothermally treated OPEFB was 68.6 mg/g at log $R_0 = 5.1$, and decreased towards higher treatment severities. The reduction of formic acid at log $R_0 = 5.42-5.72$ probably due to either decomposition of formic acid through decarbonylation and decarboxylation in a water-gas-shift (WGS) reaction (Yasaka et al., 2006). Yoshida and co-workers (2004) reported that the reversibility of decarbonylation has been
evidenced by the direct conversion of carbon monoxide to form formic acid in hot water. Overall, the generation of acetic acid, furfural, 5-HMF and formic acid from both OPFF and OPEFB samples were below the threshold limit of fermentation inhibitory level (Gong et al., 1999).

An attempt to characterize phenolic compounds from hydrothermally-treated oil palm biomass was performed in the present study, since little or no information regarding these compounds are available in the literature. Phenolic compound identified as tannic acid (TA) was characterized from pretreated oil palm biomass’s slurries and the profiles of TA over treatment severities are depicted in Fig. 1c. Tannic acid concentration was detected in the range from 0.0 to 0.47 g/L from OPFF and 0.19 to 0.28 g/L from OPEFB at log $R_o = 3.06$ to 4.24 and 4.54 to 5.72, respectively. Recently, total phenolic compounds were detected about 3.42 g/L from hydrothermally treated OPEFB samples under non-isothermal process at log $R_o = 4.22$ (Ho et al., 2014).

Several researchers extensively reviewed the inhibitory level of phenolic compounds derived from liquid fraction of pretreated biomass by decreasing the rate and extent of cellulose hydrolysis (Kim et al., 2011; Ximenes et al., 2011; Tejirian and Xu, 2011). Full enzyme activity was observed after removal of those compounds (Kim et al., 2011). In another study, TA was reported as the single and the most damaging aromatic compound
compared to gallic acid, hydroxyl-cinnamix, 4-hydroxybenzoic acids, and vanillin,
which caused 20-80% deactivation and loss of cellulase and or β-glucosidase activities
(Ximenes et al., 2011). Furthermore, Tejirian and Xu (2011) found that TA as low as 1
mM caused substantial inhibition with 70-80% decrease in cellulose hydrolysis and
initial hydrolysis rate. Thus removal of these compounds prior to enzymatic hydrolysis
is necessary in order to avoid cellulase inhibition and to obtain maximum conversion of
sugars.

3.3 Enzymatic hydrolysis

Fig. 2 shows glucose yield obtained from OPFF and OPEFB samples at
different treatment severities. Both treated slurries (solid and liquid) and washed treated
solids were subjected to enzymatic hydrolysis for 72 h at 50°C. It was clearly shown
that cellulose rich-solids from OPFF and OPEFB achieved greater glucose conversion
yield compared to pretreated slurries. This was probably due to removal of inhibitory
compounds in the separation and washing steps. Glucose yield increased with treatment
severities, and the highest glucose yield obtained was about 87.1% and 100% from
OPFF and OPEFB, respectively at log \( R_o = 4.54 \) and 5.13. On the other hand,
enzymatic hydrolysis of pretreated OPFF and OPEFB slurries achieved maximum at
61.5% and 73.6%, respectively at moderate treatment severities and decreased towards higher treatment severities ($\log R_0 > 3.94$ and $\log R_0 > 5.13$ for OPFF and OPEFB).

This indicated higher concentrations of inhibitory compounds were released, as shown in Fig. 1 resulting in more detrimental effects to cellulase and other enzyme activities.

Several studies have shown that oligomers such as XOS, xylose (Qing et al., 2010; Kont et al., 2013) and phenolic compounds (Kim et al., 2011; Ximenes et al., 2011; Tejirian and Xu, 2011) were potential inhibitors to cellulase and fermentative microorganisms. Since moderate concentration of XOS and xylose resulted in reduction of enzyme activities towards higher treatment severities (Table 2), we postulate that the inhibition of cellulase and enzymatic hydrolysis from treated slurries may be probably derived from phenolic compounds, especially TA that was recorded about 0.47 g/L at $\log R_0 = 4.24$. This value was high enough to deactivate and destroy cellulase activities as compared to 1mM (Tejirian and Xu, 2011; Ximenes et al., 2011). Even though factors of inhibition may vary from types of enzymes and phenolic compounds present, further investigation in reducing and removal of phenolic compounds in reducing and removal of phenolic compounds will be carried out in the near future.

Maximal glucose conversion yield can be obtained from cellulose-rich solids with removal of hemicellulose and phenolic compounds that inhibited cellulase activity.
Hsu and co-workers (2010) reported that, even though nearly complete removal of xylan was achieved at higher treatment severity, only 70% of glucose yield was obtained and it was found that the cellulose structure was wrapped by the lignin. Interestingly, in contrast, hemicellulose was not completely removed in our study even at the harshest treatment severity, therefore resulting in 87-100% of glucose conversion from OPFF and OPEFB samples. Hence, we suggest that the hydrothermal pretreatment of OPFF and OPEFB at selected treatment severities efficiently dissolved and partially removed the hemicellulose. The migration and re-condensation of acid insoluble lignin component onto OPFF and OPEFB-treated solids during hydrothermal pretreatment did not give any substantial effect on the enzymatic hydrolysis. Furthermore there is no direct correlation of CrI of cellulose with enzymatic digestibility and these studies are in agreement with other studies (Hsu et al., 2010; Xiao et al., 2013). The present results suggest that both partial removal of hemicellulose and migration of Klason lignin during hydrothermal pretreatment gave substantial effects on the structural changes of cellulose-hemicellulose-lignin matrix opening and expansion of SSA and PV. Thus we conclude that the most important factor for obtaining maximal enzymatic digestibility from oil palm biomass is the expansion of the SSA and PV and this is the first report elucidating factors limiting enzymatic hydrolysis of oil palm biomass highlighting
phenolic compounds, SSA and PV.

4. Conclusion

Hydrothermal pretreatment and enzymatic hydrolysis of oil palm biomass demonstrated high conversion of cellulose to glucose. Partial removal of hemicellulose and migration of lignin of treated solids has substantial effect on expanding the surface area and creation of pores for cellulase adsorption. Tannic acid production increased at increasing treatment severities and identified as potential inhibitor to enzymatic hydrolysis. There is no direct correlation between CrI of cellulose and lignin accumulation on the enzymatic digestibility. The most critical factor for the highest adsorption of cellulase on cellulose is specific surface area and pore volume.

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pretreatment of rice straw on structural properties and enzymatic hydrolysis.


Determination of sugars, byproducts, and degradation products in liquid fraction process samples. NREL/TP-510-42623, Laboratory Analytical Procedure (LAPs). National Renewable Energy Laboratory, Golden, CO.


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Table 1 Solid yield, pH, chemical compositions and properties of untreated and hydrothermally treated OPFF and OPEFB.
Table 2 Formation of hydrolyzed sugars from OPFF and OPEFB at different treatment severities, log Ro.

*Oligomeric sugar concentrations can be estimated by the difference between the total (T) and monomeric (M) sugar concentrations determined by HPLC.

**FIGURE CAPTIONS**

Fig. 1 Formation of major degradation by-products from hydrothermal pretreatment of a) OPEFB and b) OPFF at different treatment severities, log Ro.  Symbols represent acetic acid (triangle), HMF (circle), furfural (square) and formic acid (diamond).  c) Generation of phenolic compounds, tannic acid from OPFF and OPEFB as functions of treatment severities, log Ro.

Fig. 2 Yield of glucose from a) OPEFB and b) OPFF at different treatment severities.  The samples (5%) were enzymatically hydrolyzed at cellulase loading 10 FPU/g-substrate, incubate at 50°C for 72 h.
Table 1 Solid yield, pH, chemical compositions and properties of untreated and hydrothermally treated OPFF and OPEFB.

<table>
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<tr>
<th>T (°C)</th>
<th>t (min)</th>
<th>Severity (log Ro)</th>
<th>Solid yield (%)</th>
<th>pH</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Klasson lignin (%)</th>
<th>Crystallinity index, CrI (%)</th>
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<td>40.4 ± 2.4</td>
<td>20.2 ± 2.3</td>
<td>23.1 ± 0.5</td>
<td>57.6</td>
<td>0.91</td>
<td>0.006</td>
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<tr>
<td>210</td>
<td>20</td>
<td>4.54</td>
<td>67.8</td>
<td>3.86</td>
<td>50.6 ± 1.9</td>
<td>0.0 ± 0.0</td>
<td>37.5 ± 6.6</td>
<td>68.8</td>
<td>5.02</td>
<td>0.029</td>
</tr>
<tr>
<td>220</td>
<td>20</td>
<td>4.83</td>
<td>65.1</td>
<td>3.82</td>
<td>52.7 ± 1.8</td>
<td>0.0 ± 0.0</td>
<td>38.1 ± 3.1</td>
<td>72.9</td>
<td>4.64</td>
<td>0.028</td>
</tr>
<tr>
<td>230</td>
<td>20</td>
<td>5.13</td>
<td>62.6</td>
<td>3.80</td>
<td>42.1 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>43.0 ± 3.8</td>
<td>73.7</td>
<td>5.50</td>
<td>0.033</td>
</tr>
<tr>
<td>240</td>
<td>20</td>
<td>5.42</td>
<td>57.7</td>
<td>3.77</td>
<td>39.0 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>47.0 ± 5.5</td>
<td>74.4</td>
<td>5.14</td>
<td>0.033</td>
</tr>
<tr>
<td>250</td>
<td>20</td>
<td>5.72</td>
<td>52.7</td>
<td>3.73</td>
<td>33.6 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>55.4 ± 6.3</td>
<td>72.1</td>
<td>6.21</td>
<td>0.042</td>
</tr>
</tbody>
</table>
Table 2 Formation of hydrolyzed sugars from OPFF and OPEFB at different treatment severities, log Ro.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Arabinose</th>
<th>Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(T)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(M)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(T)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(M)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(T)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>170°C, 10 min</td>
<td>76.4±3.3</td>
<td>70.9±2.1</td>
<td>44.0±2.7</td>
<td>3.4±4.9</td>
<td>29.4±7.7</td>
</tr>
<tr>
<td>180°C, 10 min</td>
<td>54.3±2.2</td>
<td>52.1±3.0</td>
<td>66.0±4.8</td>
<td>4.9±0.4</td>
<td>18.6±0.9</td>
</tr>
<tr>
<td>190°C, 10 min</td>
<td>54.2±0.4</td>
<td>31.7±8.9</td>
<td>97.9±5.9</td>
<td>12.3±1.1</td>
<td>24.2±3.2</td>
</tr>
<tr>
<td>200°C, 10 min</td>
<td>82.6±3.4</td>
<td>23.9±3.5</td>
<td>92.7±2.4</td>
<td>20.2±1.6</td>
<td>24.4±10.8</td>
</tr>
<tr>
<td>210°C, 10 min</td>
<td>29.8±1.6</td>
<td>11.7±2.2</td>
<td>41.3±2.0</td>
<td>20.2±2.2</td>
<td>13.3±12.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Arabinose</th>
<th>Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(T)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(M)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(T)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(M)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(T)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>OPFF</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>210°C, 20 min</td>
<td>7.9±3.6</td>
<td>3.1±0.2</td>
<td>8.4±1.9</td>
<td>2.3±2.9</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>220°C, 20 min</td>
<td>7.5±1.9</td>
<td>4.0±1.6</td>
<td>7.9±1.4</td>
<td>1.7±2.4</td>
<td>4.8±0.7</td>
</tr>
<tr>
<td>230°C, 20 min</td>
<td>5.6±1.8</td>
<td>3.2±1.3</td>
<td>3.2±4.6</td>
<td>1.7±2.4</td>
<td>5.3±1.1</td>
</tr>
<tr>
<td>240°C, 20 min</td>
<td>6.0±3.4</td>
<td>2.5±0.5</td>
<td>4.1±5.7</td>
<td>1.6±1.7</td>
<td>5.0±0.2</td>
</tr>
<tr>
<td>250°C, 20 min</td>
<td>5.7±5.3</td>
<td>1.6±0.6</td>
<td>4.3±6.0</td>
<td>1.8±2.3</td>
<td>13.3±1.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Oligomeric sugar concentrations can be estimated by the difference between the total (T) and monomeric (M) sugar concentrations determined by HPLC.
(A) By-products formation (mg/g-substrate) vs. Severity (log $R_o$)

(Acetic) △
(HMF) ○
(Furfural) □
(Formic acid) ◇

(B) By-products formation (mg/g-substrate) vs. Severity (log $R_o$)

(Acetic acid) △
(HMF) ○
(Furfural) ■
(Formic acid) ◇

Severity (log $R_o$)
Fig. 1 Formation of major degradation by-products from hydrothermal pretreatment of

a) OPFF and b) OPEFB at different treatment severities, log $R_o$. c) Generation of

phenolic compounds, tannic acid from OPFF and OPEFB as functions of treatment

severities, log $R_o$. 
Untreated 3.06 3.36 3.65 3.94 4.24

Glucose yield, % (g/ g- substrate)

Severity (log Ro)

(A)
Fig. 2 Yield of glucose from a) OPFF and b) OPEFB at different treatment severities. The samples (5%) were enzymatically hydrolyzed at cellulase loading 10 FPU/g-substrate, incubate at 50°C for 72 h with stirring.