Hydrothermal pre-treatment of oil palm empty fruit bunch into fermentable sugars

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Abstract. Presently oil palm empty fruit bunch (OPEFB) is one of the solid waste which is produced daily whereby it is usually left at plantation site to act as organic fertilizer for the plants to ensure the sustainability of fresh fruit bunch. The major drawback in biomass conversion technology is the difficulty of degrading the material in a short period of time. A pre-treatment step is required to break the lignocellulosic biomass to easily accessible carbon sources for further use in the production of fuels and fine chemicals. Therefore, this study investigated the effect of hydrothermal pre-treatment under different reaction temperatures (100 - 250°C), reaction time (10 - 40 min), solid to solvent ratio of (1:10 - 1:20 w/v) and particle size (0.15 – 1.00 mm) on the solubilization of OPEFB to produce soluble fermentable sugars. The maximum soluble sugars of 68.18 mg glucose per gram of OPEFB were achieved at 175°C of reaction temperature, 20 min of reaction time, 1:15 w/v of solid to solvent ratio for 0.30 mm of particle size. Results suggest that reaction temperature, reaction time, the amount of solid to solvent ratio and size of the particle are crucial parameters for hydrothermal pre-treatment, in achieving a high yield of soluble fermentable sugars.

1. Introduction
The palm oil industry has brought excessive economic benefits and act as biggest exchange earner to Malaysia till today. Now, Malaysia accounts for 39% of world palm oil production and 44% of world exports [1]. Consequently, the waste biomass generated from this industry is also high. There are six types of biomass produced from this industry include oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (OPEFB) and palm pressed fibers (PPF), palm kernel shells (PKS) and palm oil mill effluent palm (POME).

Hence, oil palm wastes have enormous potential as sustainable biomass resource for the production of alternative fuel and valuable chemicals. Out of these waste biomass, OPEFB is the most...
underutilized biomass that is generated almost every day in palm oil mills as it is obtained after stripping off the fresh fruit bunch. The amount of OPEFB was estimated about 22.43 MMT in 2014 [2]. Compared to shells and fibers, OPEFB is not incinerated in mills to produce energy. Several plants using OPEFB have experienced low electricity production, high wear and tear (fouling, sintering, and corrosion) and incomplete combustion [3]. Therefore, it is generally left aside to naturally decompose or used minimally for mulching. At times they are burnt to avoid storage. Therefore, large amounts of OPEFB are still available in the mills for further conversion.

The major drawback in biomass technology is the difficulty of degrading the material in a short period of time. A pre-treatment step is required to break the lignocellulosic compound to easily accessible carbon sources for further use in the production of fuels and fine chemicals. Pre-treatment is a significant stage of fuels and chemical production from lignocellulosic biomass. The purpose of pre-treatment is to alter or remove the hemicelluloses and lignin in order to increase the pore volume and internal surface area as well as decrease the degree of polymerization and crystallinity of the cellulose [4]. Pre-treatment must meet the following requirements: (1) to improve the formation of sugars or the ability to subsequently produce sugars by enzymatic hydrolysis; (2) to avoid the degradation or loss of carbohydrates; (3) to prevent the formation of by-products which are inhibitory to the subsequent enzymatic hydrolysis and fermentation processes; and (4) to be cost-effective.

One renowned green pre-treatment method applied for conversion of lignocellulosic biomass is hydrothermal pre-treatment. The method of hydrothermal pre-treatment is popular due to the usage of water as a solvent and low operation cost. This method has the following advantages, non-chemicals addition and able to open up the plant cell wall structure, and hence, efficient solubilization and degradation of lignocellulosic biomass [5,6]. This method can be a prospective alternative for the pre-treatment of OPEFB resulting in the efficient hydrolysis of the organic matter contained to obtain valuable products for future energy needs.

In this study, the solubilization of fermentable sugars was carried out at reaction temperatures from 100°C to 250°C and reaction time from 10 to 40 min for several of solid to solvent ratio from 1:10 w/v to 1:20 w/v. At optimum conditions, the effect of particle size from 0.15 mm to 1.00 mm was studied. The effects of these parameters towards the solubilization of fermentable sugars from OPEFB were mainly discussed.

2. Material and methods

2.1. Preparation of Sample
The raw materials for this study, which is mill-processed OPEFB was collected from Palm Oil Mill Seri Ulu Langat, Dengkil, Selangor, Malaysia (GPS Coordinates: 02.8507°N, 101.5501°E). It was obtained after oil palm fruits are removed from the fresh fruit bunch during the milling process (threshing). After the threshing process, the OPEFB was shredded into loose fibers using a shredder. The shredded OPEFB was further milled using Crusher (SY-50, China) at Wood Composite Laboratory, Forest Research Institute of Malaysia (FRIM), Kepong, Selangor, Malaysia (GPS Coordinates: 3.2419°N, 101.6370°E) and sieved between 0.15 and 0.50 mm using Laboratory Test Sieve (Endecotts Ltd., UK). Then, it was dried at 60°C overnight and kept in an air-tight container till their prior use. All chemicals and standards used in this study (D(+)-Glucose and acetic acid) was purchased from Wako, Japan.

2.2. Hydrothermal Pre-treatment
The study on the pre-treatment process of OPEFB was conducted using a hot-compressed water system. The shredded OPEFB were placed inside an enclosed stainless steel reactor (OM LabTech Co., Ltd, Japan) with a working volume of 200 ml. The steam pressure inside the reactor is dependent on the temperatures being set. The reactor was loaded with a mixture of 2.5 g of dried sample and distilled water to obtain 1:10, 1:15 and 1:20 w/v of solid to solvent ratio. The reaction temperatures were valued from 100 to 250°C for 10 to 40 min of reaction time to determine optimum soluble sugar
content. For these experiments, 0.30 mm of particle size was used. After achieving optimum conditions, the optimum particle size was determined.

2.2. Analytical Methods
Total sugar in soluble products (hydrolysate) was determined by phenol-sulphuric method with some modification [7]. The hydrolysate (0.1 ml) was mixed with 1.9 ml of distilled water, 1 ml of 5 % w/v phenol and 5 ml of concentrated sulphuric acid and was vortex for 30 s. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 490 nm against distilled water as blank using UV-1800 Spectrophotometer (Shimadzu, Japan). Glucose was selected as a standard considering the major component of biomass. The pH of the liquid portion was determined using a digital pH meter (Mettler Toledo, USA). The concentration of acetic acid was also quantified using a gas chromatograph (GC-8A, Shimadzu, Japan) coupled with a flame ionization detector (FID) and a Unisole F-200 30/60 column (3.0 mm in diameter and 2.0 m in length). 1 µl of sample was injected with nitrogen gas, N₂ as carrier. The injector, detector and column temperatures were kept at 180°C, 180°C and 150°C, respectively.

3. Results and discussion
The preliminary study was done to determine the optimum reaction temperature. For this purpose, the reaction time of 20 minutes, 1:15 solid to solvent ratio and 0.30 mm particles were used. The profile of total soluble sugar obtained is shown in figure 1. It can be seen that amount of total sugar compounds increased gradually with increasing temperature from 100°C to 175°C at 20 minutes reaction time achieving the optimum value of 68.18 mg glucose per gram of OPEFB. The subcritical reactions are recognized to increase the ionization constant of water at high temperature, in which the concentrations of H⁺ and OH⁻ are increased, thereby assisting the hydrolysis of cellulose and hemicellulose into monosaccharides as mentioned by Lin et al. [8]. The increase in concentration of ions, not only helps in destroying the glycosidic bonds in the cellulose but also provides a shorter time for the conversion of oligosaccharides to monomers [9]. However, further increase in temperature to 250°C reduced the total sugar yield to less than 10 mg glucose per gram of OPEFB.

![Figure 1. Total sugars at various reaction temperatures for 20 min of reaction time and 1:15 w/v of solid to solvent ratio](image-url)
Increase in reaction temperature showed a decrease in total soluble sugar yield. This suggests that the sugars are further converted or decomposed at higher temperatures to organic acids, 5-HMF, furfural and other decomposed compounds. The samples were tested and acetic acid was found as a dominant component as shown in figure 2. The decreased total sugar yield after 175°C was attributed to the enhanced degradation of the monosaccharides into by-products and proved by the pH of the liquid as seen in figure 2. The hydrolysate turned from neutral into acidic toward higher reaction temperature treatment due to the accumulation of organic acids. The pH value at optimum reaction temperature (175°C) was 4 and it decreased significantly as temperature increased. This phenomenon can be correlated with the increase in acetic acid concentration. The highest amount of acetic acid was 72.78 mg/g of dry matter at 250°C. Acetic acid is a well-known valuable by-product from the degradation of complex hemicellulose compound. At elevated temperature will bring about lower pH in solvent, enabling the release of O-acetyl, acetic and uronic acids from hemicellulose [10]. However, this compound is known as a toxic compound that could inhibit the microbial growth in subsequent biological conversion process as stated by Chong et al. [11].

To further verify the optimum reaction temperature, a ±5°C from 175°C was studied. For this study, the hydrolysis time was varied between 10 to 40 min and the solid to solvent ratio of 1:10, 1:15 and 1:20 was used to simultaneously study the effect of reaction time and solid to solvent ratio. The results as shown in figure 3 showed that the total sugar yields increased from 170°C to 175°C and dropped at 180°C for all reaction time and solid to solvent ratio. The highest total sugar obtained after the hydrothermal pre-treatment at various parameters remained at 68.18 mg glucose for 1:15 solid to solvent ratio at 20 min, and then decreased somewhat by increasing reaction time. The trends were similar at all solid to solvent ratio, establishing the optimum conditions at 175°C and 20 min reaction time. However, the significant sugar yield was achieved at 1:15 w/v solid to solvent ratio as shown in figure 3 (B). This result proved that amount of solid to solvent ratio is one of important parameter to be evaluated.

However, as a whole, the results showed that the degradation of lignocellulosic material through hydrothermal pre-treatment is fast and relatively efficient to produce fermentable sugars. As to compare with common hydrolysis method (acid hydrolysis), it requires longer reaction time between one to two hours to degrade the lignocellulosic material [12,13]. Zakaria and co-workers [6] also
reported finding in hydrolysis of oil palm empty fruit bunch under batch water treatment at sub-critical temperature and pressure. It was found that, the maximum total sugars 60.1 mg per g of OPEFB were obtained at 210°C in a batch treatment at reaction time of 20 min.

![Graph showing total sugars at different temperatures](image1)

**Figure 3.** Effect of total sugars at different solid to solvent ratio; (A) 1:10 w/v, (B) 1:15 w/v and (C) 1:20 w/v for reaction temperatures of 170°C, 175°C and 180°C and reaction times from 10 to 40 mins

Generally, a high solid to solvent ratio could promote an increasing concentration gradient, resulting in an increase of diffusion rate that allows greater extraction of solids by solvent [14]. Therefore, at optimum conditions of 175°C of reaction temperature, 20 min reaction time and 1:15 solid to solvent ratio, the size of particle was varied and investigated. The values of solubilized total sugar, ranging from particle size 0.15 mm to 1.00 mm are presented in figure 4.

![Graph showing total sugars at different particle sizes](image2)

**Figure 4.** Total sugars obtained at various particle sizes for 1:15 w/v of solid to solvent ratio, 20 min of reaction time and 175°C of reaction temperature
It was found that the highest solubilized total sugar was 68.18 mg glucose/g of dry matter at 0.30 mm of particle size. Meanwhile, at the finest particle size of 0.15 mm, the solubilized total sugar was found as 55.74 mg glucose/g of dry matter. Theoretically, smaller particle size should give more yield because of the higher surface area to volume ratio which enhances the contact between the solvent molecules and plant material during the extraction process [15]. However, this finding was different from the theory as observed, that when the particle size was too fine, the sample in the reactor was compacted and agglomerated when in contact with the solvent at high pressure and temperature.

4. Conclusion
Hydrolysis of OPEFB substrates showed that the hydrothermal pre-treatment method provided an accelerated pre-hydrolysis of OPEFB during the treatment process. The maximum total soluble sugars of 68.18 mg glucose per gram of OPEFB were achieved at 175°C of reaction temperature, 1:15 w/v of solid to solvent ratio and 0.30 mm of particle size at 20 min of reaction time. A significant drop of the soluble sugars in the OPEFB hydrolysate was due to the generation of acetic acid. Results suggest that reaction temperature, time, solid to solvent ratio and particle size are crucial parameters for hydrothermal pre-treatment, in order to achieve a high yield of soluble fermentable sugars. The soluble sugars obtained can be used as feedstock for conversion to biofuels and fine chemicals.

5. References
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