EFFECT OF DIFFERENT ALKALINE TREATMENT ON THE RELEASE OF FERULIC ACID FROM OIL PALM EMPTY FRUIT BUNCH FIBRES

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ABSTRACT

Serious thought on sustainability of palm oil industry triggers ways to minimise the impacts caused to the environment by recycling oil palm empty fruit bunch (OPEFB). An alkaline treatment strategy was developed for ferulic acid (FA) release from OPEFB fibres. The selected treatment of autoclaving OPEFB (120°C, 3 hr) and hydrolysing it with 20 g kg^{-1} NaOH (90°C, 3 hr) yielded 62.52 ± 6.24 mg litre^{-1} FA. Based on alkali selection (5 to 50 g kg^{-1} of NaOH, KOH and K$_2$CO$_3$), 20 g kg^{-1} KOH showed almost similar FA release to 20 g kg^{-1} of NaOH, as it solubilised 56.94 ± 3.52 mg litre^{-1} FA. The addition of sodium bisulphite (NaHSO$_3$) resulted in an increase of 4.23 mg litre^{-1} FA using KOH treatment. FA decreased when subjected to prolonged reaction times at high temperature, while at ambient temperature, only about 30 to 35 mg litre^{-1} FA was released. Fourier transform infrared (FTIR) analysis showed evidence of decrease in aromatic groups, lignin and ester linkage stretching. The selected treatment method using 20 g kg^{-1} NaOH and KOH solubilised 4.24 mg and 3.84 mg FA from 1 g of OPEFB lignin, respectively. From this finding, OPEFB exhibited a great potential as a feedstock for FA production. FA obtained from this treatment is very useful precursor for vanilic acid, vanillin and other value-added products formation through microbial conversion.

INTRODUCTION

South-east Asian countries with a tropical climate combined with consistent high humidity and temperature can become prosperous with oil palm plantation. Malaysia, Indonesia and Thailand are the world leading palm oil producers (Mukerjee and Sovacool, 2014). The oil palm planted in Malaysia, *E. guineensis* Jacq. originated in West Africa was introduced to Malaysia in the 1860s. Oil palm in Malaysia has developed into a major economical crop. Palm oil production resulted in plenty of biomass being produced such as oil palm empty fruit bunch (OPEFB), mesocarp fibres, palm kernel shells, oil palm fronds, trunks and palm oil mill effluent. Sustainability is a crucial factor to be considered in order for the palm oil industry to survive long-term without causing adverse effects to the environment. Fortunately, these biomass which are renewable and in continuous supply have significant potential for the development of bio-based value-added products.

Among oil palm biomass, OPEFB is the main by-product with about 18 million tonnes (Goh *et*

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al., 2010) being generated annually. This biomass is utilised for mulching in oil palm plantations as it has the properties of recycling nutrients, conserving moisture and reducing surface runoffs. In addition, Akhtar et al. (2010) mentioned that OPEFB is also burned in the mill boilers with pressed fibres and shells in order to generate steam to operate power plants that produces electricity for the mill. Despite this, there are still surplus quantities available. Therefore, due to its availability and low cost, it would be a great alternative, especially in Malaysia, for OPEFB to be the starting material for the generation of bio-products like bio-compost (Baharuddin et al., 2011), enzymes (Umikalsom et al., 1997), lignophenol (Abdullah et al., 2009), biofuels like bioethanol (Piarpuzán et al., 2011) and biobutanol as a mixture of acetone-butanol-ethanol (Ibrahim et al., 2012). OPEFB is a lignocellulosic fibre containing cellulose, hemicellulose and lignin in the respective percentages of 50.3, 26.1 and 18 (Baharuddin et al., 2011).

Lignin is a polymer of phenylpropanes that could be characterised based on the relative abundance of syringyl, guaiacyl and p-hydroxyphenyl units (Fukushima, 2001). These units are derived from primary monolignols, i.e, sinapyl, coniferyl and p-coumaryl alcohols, respectively (Pandey and Kim, 2010). OPEFB fibre is of SGH-lignin units (Sun et al., 1999a), given that it is more of S- and G-lignin with fewer H-lignin. Ferulic acid (FA) (4-hydroxy-3-methoxycinnamic acid) is of guaiacyl lignin and exists naturally in almost all plant cell walls, ranging from vegetables, spices and medicinal plants to agricultural residues. According to earlier researches by Sun et al. (1999a, b), OPEFB contained FA in a smaller proportion compared to other phenolic acids. FA is mainly esterified and also etherified to lignin and other polysaccharides of OPEFB (Sun et al., 1999b). FA is esterified to polysaccharides (Barberousse et al., 2008) and lignin (Pan et al., 1998) through its carboxylic bonding site. Ether bonds occur where lignin attach to the phenolic bonding site of FA, while ester-ether bridge cross-link polysaccharides and lignin through bonding at the phenolic and the carboxylic site of FA, respectively (Pan et al., 1998).

The demands for FA are in the medical, pharmaceutical and cosmetic industries because of its anti-microbial, anti-inflammatory and antioxidant properties (Ou and Kwok, 2004). The other renowned function is in the production of biovanillin (bio-flavour), the active ingredient of vanilla aroma as FA serves as the precursor for microbial conversion into biovanillin (Torres et al., 2009). FA because of its phenol structure can be biosynthesised into the aromatic compound, biovanillin. Owing to the fact that biovanillin can be produced from FA through the biotechnological route, we could possibly reduce the market reliability on the vanilla plants as the natural vanilla source by providing this universal flavour at a competitive and affordable price.

Various agricultural wastes have been studied to determine their FA content and different methods of chemical hydrolysis have been developed to release the hydroxycinnamic acid from biomass lignin such as wheat straw (Pan et al., 1998; Billa et al., 1996), corn cob (Torre et al., 2008), maize bran (Tilay et al., 2008), brewer's spent grain (Mussatto et al., 2007) and vine shoot prunings (Max et al., 2009). All these studies focused on alkaline hydrolysis to release FA from the biomass. Alkali saponify intermolecular ester bonds that cross-link lignin and hemicellulose (Misson et al., 2009), suggesting that only FA esterified in the biomass was released. Through the alkaline hydrolysis condition reported by Tilay et al. (2008), 1.487% (w/w) esterified FA was solubilised from maize bran, 1.022% (w/w) from rice husk, 0.972% (w/w) from rice bran, 0.893% (w/w) from sugar-cane bagasse, 0.867% (w/w) from wheat straw and 0.346% (w/w) from wheat bran. Black liquor waste, which is equivalent to the produced alkaline hydrolysate, could be utilised as biovanillin precursor in order to reduce environmental impact caused by the pulping processes. This also included black liquor generated from the OPEFB pulping process (Jiménez et al., 2009).

In this study, OPEFB was alkali-treated for the release of FA. A few treatment strategies were carried out to select the best treatment that release significant amounts of FA from OPEFB. The effects of different alkalis concentrations, sodium bisulphite concentrations, reaction times at high and ambient temperatures on FA release were also studied. The raw (untreated) and treated OPEFB fibres were subjected to characterisation of lignocellulosic compositions and Fourier transform infrared (FTIR) analysis was used to examine the compositional changes of OPFEB fibres before and after treatment. Mild alkaline treatment conducted was targeted to release a significant amount of free FA that was esterified to lignin and hemicellulose of OPEFB.

MATERIALS AND METHODS

Substrate

Shredded OPEFB was obtained from Seri Ulu Langat Palm Oil Mill in Dengkil, Selangor, Malaysia. The fibres were soaked in commercial detergent prior to oil and dirt removal and then were sun dried. The dried OPEFB fibres were ground and sieved to approximately 2 to 10 mm length fibres.

Effects of Different Treatment Strategies, Different Alkalis and Concentrations on FA Release

Table 1 summarises the four types of treatment strategies performed. All the experiments were conducted in the dark. Alkali screening was conducted using three types of alkali, namely NaOH, KOH and K_2CO_3 (Akhtar *et al.*, 2010) with the concentrations of 5, 10 (Mussatto *et al.*, 2007), 20 (Hamisan *et al.*, 2009; Mussatto *et al.*, 2007), 30, 40 (Torre *et al.*, 2008) and 50 g kg^{-1} of each by simple autoclaving of OPEFB fibres in different alkalis at 121°C, 0.1 MPa for 15 min. The intermediate concentration of 30 g kg^{-1} and higher concentration of 50 g kg^{-1} were studied for comparison. The alkaline hydrolysate produced was adjusted to pH 2 and centrifuged at 12 000 rpm for 10 min.

Effects of $NaHSO_3$ Concentrations; Reaction Times at High and Ambient Temperatures on FA Release

$NaHSO_3$ was added into 20 g kg^{-1} KOH solution for hydrolysing OPEFB fibres. The range of volumes applied were 98 (Tilay *et al.*, 2008), 196 (twice the volumes) and 392 μl (four times the volumes) of $NaHSO_3$ in order to observe the appropriate volumes that could preserve FA in the OPEFB hydrolysate produced. The volumes of $NaHSO_3$ mentioned were used in 15 ml KOH solution for hydrolysing 1.5 g OPEFB fibres (ratio of 10:1). The mixture was autoclaved at 121°C, 0.1 MPa for 15 min. Using the selected alkali and ($NaHSO_3$) concentrations, the effects of reaction times at high temperature on FA release were determined by increasing the autoclaving time to 20, 30 and 60 min (Mussatto *et al.*, 2007), while ambient temperature treatment was conducted at 37°C with agitation speed of 200 rpm for 16 hr.

Determination of FA

FA was detected by HPLC (model RI-1530, Jasco) using Chromolith Performance RP-18 End-capped (100 x 4.6 mm) column from Merck. Separation was achieved using isocratic gradient of water/acetonitrile/acetic acid (88:10:2, v/v/v) at room temperature with flow rate of 1 ml min^{-1} and detected by UV at 280 nm (modified from Muchuweti *et al.*, 2005).

FTIR Analysis of OPEFB Fibres

Samples [raw (ground OPEFB without any thermal or chemical treatment), autoclaved, KOH-treated and NaOH-treated OPEFB] were sent for attenuated total reflectance (ATR) - FTIR analysis at the Faculty of Science, Universiti Putra Malaysia, Selangor, Malaysia.

Lignocellulosic Characterisation of OPEFB Fibres

The lignin, cellulose and hemicellulose contents of raw, autoclaved, KOH-treated and NaOH-treated OPEFB fibres were characterised using acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL) analyses at the Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia.

TABLE 1. TREATMENT STRATEGIES CONDUCTED ON OIL PALM EMPTY FRUIT BUNCH (OPEFB) FIBRES FOR FERULIC ACID RELEASE

Treatment strategies	References
Treatment A OPEFB soaked in 20 g kg^{-1} NaOH (4 hr) and autoclaved at 121°C, 5 min	Umikalsom *et al.* (1997)
Treatment B1 OPEFB autoclaved at 120°C, 3 hr Autoclaved OPEFB was NaOH-treated (20 g kg^{-1}) at 90°C, 3 hr (agitated at 120 rpm in water bath shaker)	Ju *et al.* (2010) Hamisan *et al.* (2009)
Treatment B2 OPEFB (1 g: 10 ml water) autoclaved at 120°C, 3 hr Dry autoclaved OPEFB was NaOH-treated (20 g kg^{-1}) at 90°C, 3 hr (agitated at 120 rpm in water bath shaker)	Modified Ju *et al.* (2010) Hamisan *et al.* (2009)
Treatment C OPEFB treated with 20 g kg^{-1} NaOH at 90°C, 3 hr (agitated at 120 rpm in water bath shaker)	Hamisan *et al.* (2009)

RESULTS AND DISCUSSION

Effects of Different Treatment Strategies on OPEFB Fibres for FA Release

Table 2 shows that Treatment B1 released higher FA of 62.52 ± 6.24 mg litre^{-1} compared to Treatments A, B2 and C. Different thermal treatments and alkaline hydrolysis methods conducted on OPEFB fibres resulted in various amounts of free and esterified FA being released into OPEFB alkaline hydrolysate with other smaller fractions of the complex strand of lignocellulosic material. Integration of thermal treatment (Treatments A, B1 and B2) was conducted in order to overcome the relatively low effect of alkali hydrolysis alone on the release of lignin-derived products from OPEFB. Thermal treatment was done using an autoclave, which provided saturated steam under 0.1 MPa that increased the liberation of low molecular weight phenolic compounds (Ju *et al.*, 2010). Sudden pressure release in the autoclave caused breakage of the fibres and accessibility for further alkali attack. Moreover, the high resistance of the hydroxyl functional group of phenols ensured that they remained in the liquid portion and biomass without being evaporated (Akhtar *et al.*, 2010).

Treatment A showed that the FA release was just slightly lower than Treatment B1. It was suggested that the FA released through Treatment A was almost the same as through Treatment B1; however, the higher temperature and pressure of the autoclave degraded the fragile FA that was released into the alkaline hydrolysate. The alkaline hydrolysis conducted in Treatment B1 was at a lower temperature (90ºC) which was less harsh on the FA and reduced the possibility of it re-polymerising. This is because the temperature range of 120ºC to 160ºC partially oxidised FA (Iiyama and Lam, 1990). On the other hand, Treatment B2 gave lower FA release than Treatment B1. In Treatment B2, autoclaving OPEFB with water was considered a mild hydrothermal, by which hemicelluloses were mainly degraded (Duff and Murray, 1996). When further treated with alkali at a lower temperature, the undegraded lignin portion released a smaller amount of FA into the hydrolysate.

Water added to the OPEFB prior to autoclaving in Treatment B2 was subjected to FA determination in order to verify that no FA loss had occurred. It was observed that the liquid did not contain FA. However, there were trace amounts of vanillic acid (1.92 ± 0.27 mg litre^{-1}) and vanillin (1.55 ± 0.13 mg litre^{-1}) detected. It was suggested that the small amount of FA released was oxidised to vanillic acid and vanillin. In this case, FA loss could be negligible as the amount was small. On the other hand, Treatment C showed that without the thermal treatment, lower FA concentration was solubilised. This suggested that integration of the thermal treatment for fibrous structure was necessary in order for the alkali to easily disrupt the plant cell walls.

Selection of treatment strategy (Treatment B1) was based on one type of alkali, *i.e* NaOH. In term of determining the appropriate treatment conditions for OPEFB fibres to release significant amount of FA, different types of alkali, concentrations and suitable NaHSO$_3$ concentration were studied for FA release, however using a simpler treatment condition. Alkaline treatment was conducted due to the presence of hydroxyl anions that hydrolysed the ester linkages (Max *et al.*, 2009). The selection of NaOH and KOH were based on the availability of hydroxyl ions. Meanwhile, K$_2$CO$_3$ provides carbonate ions which was reported by Akhtar *et al.* (2010) as a good delignifying agent and it was observed that 1 M K$_2$CO$_3$ degraded more than half the lignin composition and produced liquid containing mainly phenols and esters.

TABLE 2. FERULIC ACID RELEASE THROUGH DIFFERENT TREATMENT STRATEGIES USING 20 g kg^{-1} NAOH

Treatment strategies	FA release (mg litre^{-1})[a]
Treatment A OPEFB soaked in NaOH for 4 hr and autoclaved at 121°C, 5 min	47.66 ± 1.18
Treatment B1 OPEFB autoclaved at 120°C, 3 hr and NaOH-treated at 90°C, 3 hr	62.52 ± 6.24
Treatment B2 OPEFB (with water) autoclaved at 120°C, 3 hr and NaOH-treated at 90°C, 3 hr	33.16 ± 3.20
Treatment C OPEFB treated with NaOH at 90°C, 3 hr	15.81 ± 1.69
Water from Treatment B2	Not detected

Note: [a] All experiments were conducted in triplicates and the standard deviations are ≤ 6.
　　　OPEFB – oil palm empty fruit bunch.

Effects of Different Alkalis and their Concentrations on FA Release

Figure 1 shows the FA release by each alkali at different concentrations. It was observed that the FA release was almost the same using KOH and NaOH, while K_2CO_3 exhibited a lower FA release. A 20 g kg^{-1} of KOH and NaOH released 32.98 ± 2.66 mg litre^{-1} FA and 32.01 ± 5.38 mg litre^{-1} FA, respectively. KOH selection in conducting further experiments in this study was due to its benign effect to the environment as compared to NaOH. Besides the usage of the hydrolysate for biovanillin production, excess potassium-based hydrolysate could be a source of fertiliser as potassium is more beneficial for soil than sodium (Huang *et al.*, 2008). In the perspective of using residual OPEFB from hydrolysis to obtain cellulosic pulps, KOH pulping is a good alternative as the fibre obtained is of higher brightness than the one resulting from soda or Kraft pulping as was shown by Rodríguez *et al.* (2008) using rice straw. Contrarily, NaOH would be more cost-effective in the industrial scale.

Due to the FA release using 20 g kg^{-1} and 30 g kg^{-1} KOH showing no significant difference, the lower concentration of 20 g kg^{-1} KOH was selected for milder condition as proposed by Karagöz *et al.* (2006). A lower concentration of alkali reduced the production of water-soluble hydrocarbons, which contain degraded cellulose and hemicellulose. Based on the chemical compositions, OPEFB is categorised under hardwood (Shibata *et al.*, 2008) which has low lignin content compared to softwood, thus supporting the fact that the low concentration

of alkali used for hydrolysis is sufficient. In most researches for FA solubilisation, 40 g kg^{-1} (1 M) of alkali was used as the minimum concentration (Tilay *et al.*, 2008; Max *et al.*, 2009). However, according to the results obtained, it was suggested that 20 g kg^{-1} of NaOH and KOH released higher FA from OPEFB lignin than 40 g kg^{-1} of the same alkalis.

On the other hand, the lower FA release by K_2CO_3 compared to KOH and NaOH might have occurred as lignin degradation is sensitive to K_2CO_3 (Akhtar *et al.*, 2010) and caused re-polymerisation of lignin's small fragments at increasing concentrations. Akhtar *et al.* (2010) showed that K_2CO_3 is superior to KOH and NaOH in terms of reactivity and efficiency in liquefying and degrading OPEFB lignin. However, in this study, in term of FA release from OPEFB, KOH and NaOH are superior to K_2CO_3. This might be due to FA being re-polymerised into lignin polymer. Their results on the similar effects caused by treatments using both KOH and NaOH were in agreement with those of this study.

Alkaline hydrolysis was conducted in this study instead of hydrolysis using solvent. This was because Miller *et al.* (1999), in their study of lignin depolymerisation by bases in alcohol solvents, reported that side products were formed when alkali in ethanol solvent reacted on lignin. Acetic acid was mainly generated, which was according to Palmqvist and Hahn-Hägerdal (2000), prevented microbial growth. In addition to this, acids produced by alcohols neutralised the reacting alkali and caused the need for higher concentration of alkali. Therefore, the alkaline hydrolysate produced in this study favoured the subsequent fermentation
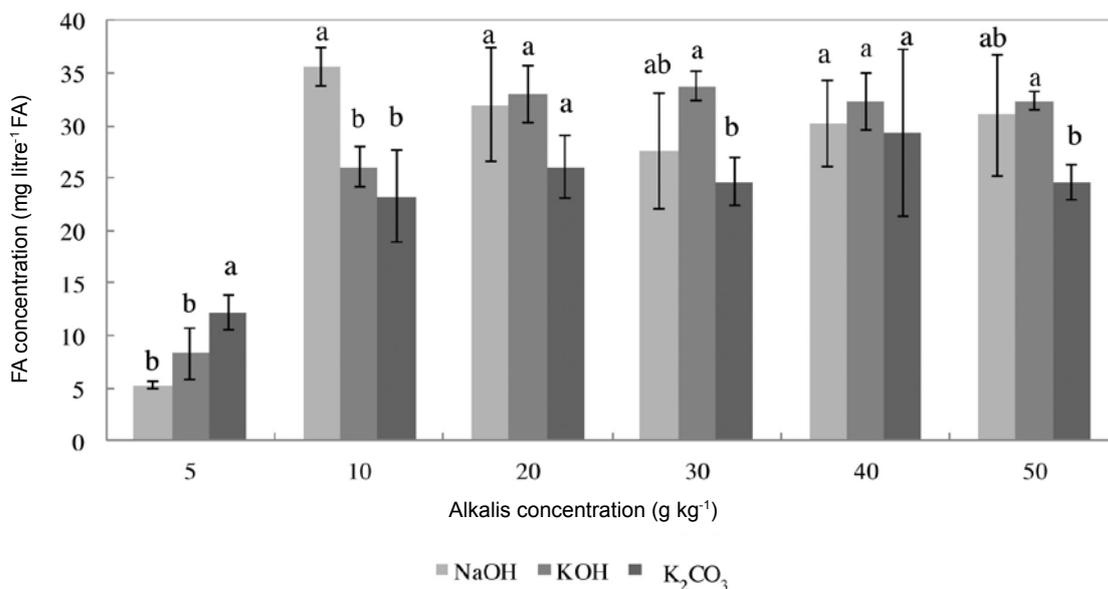
Note: a/b/ab - The data shown with different letters were significantly different (P<0.05) for each of the concentration.

Figure 1. Ferulic acid (FA) release from oil palm empty fruit bunch fibres using NaOH, KOH and K_2CO_3 of different concentrations. All experiments were conducted in triplicates and the standard deviation is ≤ 8.

to produce biovanillin. In contrast, if the acid pre-hydrolysis step was done, disposal of acidic effluent would be an environmental issue. Besides that, acidic hydrolysis also caused re-polymerisation of lignin-degraded products (Yuan *et al.*, 2010).

Effects of NaHSO₃ Concentrations on FA Release

Figure 2 shows the FA released using 20 g kg⁻¹ KOH with the addition of certain volumes of 0.025 M NaHSO₃. Without the addition of NaHSO₃, 28.58 ± 1.13 mg litre⁻¹ FA was released, meanwhile with the addition of 98 μl of NaHSO₃, a 4.23 mg litre⁻¹ increase of FA was observed. However, addition of two times and four times of 98 μl NaHSO₃ volume did not result in increasing FA content in the hydrolysate. The difference in FA concentrations released using 20 g kg⁻¹ KOH (*Figures 1* and *2*) was probably caused by the oxidation effect. FA in the complex hydrolysate system is extremely susceptible to oxidation and re-polymerisation. The NaHSO₃ (Tilay *et al.*, 2008) which provides sulphite that act as the capping agent for unstable lignin intermediates (Yuan *et al.*, 2010) was used to overcome this problem.

Based on the selected treatment strategy (Treatment B1), alkali concentration (20 g kg⁻¹ KOH) and NaHSO₃ concentration (98 μl in 15 ml KOH solution hydrolysing 1.5 g OPEFB fibres), 56.94 ± 3.52 mg litre⁻¹ FA was solubilised. The same treatment conditions using 20 g kg⁻¹ NaOH, however without the addition of NaHSO₃ released 62.52 ± 6.24 mg litre⁻¹ FA. Comparing both 20 g kg⁻¹ of NaOH and KOH effects on FA solubilisation from OPEFB fibres, it was suggested that both could be utilised to release FA, having the release was in the range of 50 to 70 mg litre⁻¹ FA.

## Effects of Reaction Times at High and Ambient Temperatures on FA Release

Referring to *Figure 3*, it was observed that increasing reaction times when autoclaving at 121°C decreased the release of FA. Billa *et al.* (1996) reported that hydroxycinnamic acids released upon alkaline treatment decreased with the rises in temperature and reaction time. This may be due to the accelerated oxidation of phenolic acids at higher temperatures (Joan *et al.*, 2006) and prolonged times.

Figure 4 shows that even at the first 2 hr of hydrolysis time, about 33.34 ± 1.46 mg litre⁻¹ FA was released and it was almost constant throughout the treatment. This was in accordance to the results obtained by Torre *et al.* (2008), in which, FA concentration increased significantly within 2 hr. They reported that through alkaline hydrolysis (20 g kg⁻¹ NaOH) at room temperature for 6 hr with solid to liquid ratio of 0.084 g g⁻¹, 1171 ± 34 mg litre⁻¹ FA was hydrolysed from corn cob. However, through this study, only 34.55 ± 6.71 mg litre⁻¹ FA was solubilised from OPEFB fibres at 12th hr using 20 g kg⁻¹ KOH and solid to liquid ratio of 0.097 g g⁻¹. Different bases were utilised in both studies, provided that higher amount of alkali was used in study by Torre *et al.* (2008). Furthermore, corn cob contained 20.3% (w/w) lignin and 34.7% (w/w) hemicellulose compared to only 14.85% (w/w) lignin and 26.69% (w/w) hemicellulose in OPEFB (*Table 3*). Therefore, it was suggested that treatment at an ambient temperature was not economically feasible in order to obtain FA from the OPEFB fibres and required longer reaction times compared to biomass of softwood source.
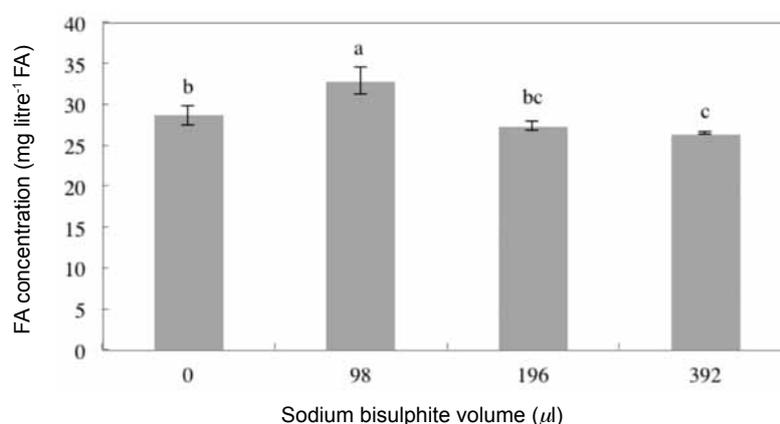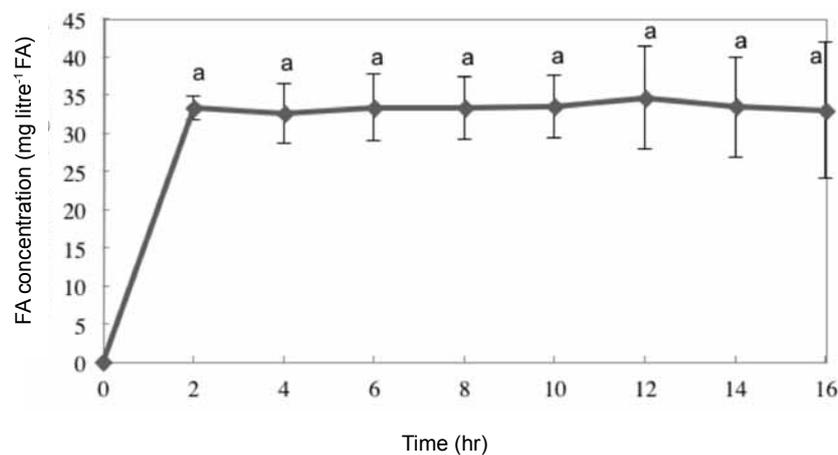

Note: a/b/bc/c - The data shown with different letters were significantly different (P<0.05).

Figure 2. Ferulic acid release from oil palm empty fruit bunch fibres using 20 g kg⁻¹ KOH with different volume of 0.025 M NaHSO₃. All experiments were conducted in triplicates and the standard deviation is < 2.
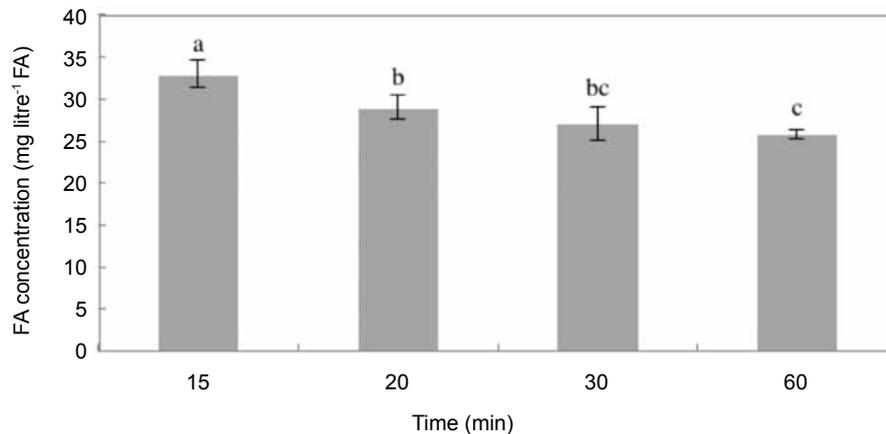
Note: a/b/bc/c - The data shown with different letters were significantly different (P<0.05).

Figure 3. Ferulic acid (FA) release from oil palm empty fruit bunch fibres by autoclaving at 121°C with increasing time. All experiments were conducted in triplicates and the standard deviations are < 2.

Note: a - The data shown with similar letter were not significantly different (P>0.05).

Figure 4. Ferulic acid (FA) release from oil palm empty fruit bunch fibres using 20 g kg^{-1} KOH and 98 μl 0.025 m NaHSO$_3$ at 37°C for 16 hr. All experiments were conducted in triplicates and the standard deviation is ≤ 8.

Lignocellulosic Contents of Treated and Untreated OPEFB Fibres

Table 3 shows the respective chemical contents in raw, autoclaved, KOH-treated and NaOH-treated OPEFB fibres. Lignin of KOH-treated and NaOH-treated fibres were removed by 14.67% and 20.42%, respectively. Moreover, hemicelluloses removal percentages were 30.13% and 35.76% for both alkalis treated fibres. This finding is in line with the study reported by Hamzah *et al.* (2011) where they found that the surface of OPEFB treated with sodium hydroxide was covered with craters as a result of silica removal and partial lignin decomposition. The increase in cellulose contents complemented with decreases in lignin and hemicellulose contents. Release of FA was related to the loss of hemicellulose and lignin as FA was esterified to both

the components. Besides that, the ash content was observed to be increased when OPEFB fibres were chemically treated and this was in agreement with results by Umikalsom *et al.* (1997).

KOH treatment released 3.84 mg FA from 1 g OPEFB lignin and 0.57 mg FA from 1 g OPEFB fibres, while NaOH treatment released 4.24 mg FA from 1 g OPEFB lignin and 0.63 mg FA from 1 g OPEFB fibres. Sun *et al.* (1999a) reported that through treatment conducted using 40 g kg^{-1} NaOH, 0.043% (w/w) of the FA esterified in OPEFB was released. In this study, treatment using 20 g kg^{-1} NaOH resulted in higher liberation of FA from OPEFB fibres, which was 0.063% (w/w), while 20 g kg^{-1} KOH released 0.057 % (w/w) FA that is esterified in OPEFB. Integration of thermal and alkaline hydrolysis in this study was suggested to have better solubilisation of FA from OPEFB fibres.

**TABLE 3. CHARACTERISATION OF LIGNOCELLULOSIC CONTENTS OF
OIL PALM EMPTY FRUIT BUNCH (OPEFB) FIBRES**

OPEFB fibres	Percentage dry weight (% w/w)[a]			
	Lignin	Cellulose	Hemicellulose	Ash
Raw (untreated)	16.16 ± 1.24	43.74 ± 2.40	28.58 ± 1.96	1.06 ± 0.01
Autoclaved	14.85 ± 1.16	48.17 ± 5.02	26.69 ± 0.28	1.10 ± 0.02
KOH-treated	13.79 ± 0.66	61.09 ± 1.47	19.97 ± 1.65	2.92 ± 0.35
NaOH-treated	12.86 ± 0.52	61.37 ± 1.05	18.36 ± 1.71	2.31 ± 0.35

Note: [a] All experiments were conducted in duplicates and the standard deviation is ≤ 5.

The FA release from OPEFB was also compared to FA release from brewer's spent grain. Mussatto *et al.* (2007) reported a yield of 9.65 mg FA from 1 g solubilised lignin of brewer's spent grain hydrolysed by 20 g kg^{-1} NaOH and this study resulted in 4.24 mg FA from 1 g OPEFB lignin using 20 g kg^{-1} NaOH, which was less than half compared to the FA released from brewer's spent grain. This might have been caused by the different treatment methods and acid pre-treated brewer's spent grain, rather than OPEFB, which was only alkali-treated. Moreover, the solid to liquid ratio (1:20 w/w) suggested that Mussatto *et al.* (2007) used a higher volume of alkali than that used in this study (1:10.3 w/w).

KOH treatment (20 g kg^{-1}) showed higher FA release from 1 g of OPEFB fibres (0.57 mg FA) compared to 1 g of vine shoot prunings (0.41 mg FA), even though Max *et al.* (2009) used higher KOH concentration of 120 g kg^{-1} to treat vine shoot prunings. This is caused by thermal treatment in this study that enhanced the FA release during subsequent alkaline hydrolysis. The results suggested that with thermal treatment, lower alkali concentration is adequate for better FA release from biomass compared to low temperature treatment (35°C) that required higher alkali concentration and longer reaction time as conducted by Max *et al.* (2009).

FTIR Analysis of OPEFB Fibres

Figure 5 shows the FTIR spectra of raw (untreated), autoclaved, KOH-treated and NaOH-treated OPEFB fibres. The obtained patterns were similar to the one reported by Baharuddin *et al.* (2011), using shredded and pressed-shredded OPEFB fibres. The percentage of Transmittance (% T) was in increasing order from raw, autoclaved to KOH-treated and NaOH-treated OPEFB fibres for peaks representing the functional groups that were being studied. High % T refers to the decreased in the stretching of certain functional groups, showing that in the previously mentioned order, the studied groups or linkage(s) decreased.

There were several peaks that gave significant difference in the % T amongst all four samples, which were of interest. The peak at 1029 cm^{-1} was attributed to aromatic CH in-plane deformations in the guaiacyl unit of the lignin molecule (Sun *et al.*, 1999a). The FTIR spectrum of the both alkali-treated OPEFB fibres showed reduction in this particular functional group. The region of 3342 to 3337 cm^{-1} corresponded to the intermolecular hydrogen bonding (H-bonded OH groups) that indicated phenolic compounds. Meanwhile, 1727 to 1720 cm^{-1} was attributed to stretching of carbonyl groups that indicated ester linkages. Spectra from 1613 to 1598 cm^{-1} indicated the presence of aromatic C=C groups (Akhtar *et al.*, 2010). In addition, bands at 1600 to 1500 cm^{-1} and 1420 cm^{-1} represented aromatic skeleton vibrations. Besides that, 1263 to 1240 cm^{-1} was typical for lignin components and the shoulder peak of aromatic ring bends was at 1155 to 1146 cm^{-1}. All listed peaks and regions of interest showed higher % T for both the alkali-treated OPEFB fibres, suggesting that the NaOH and KOH treatments released phenolic acids, including FA from OPEFB lignin.

CONCLUSION

Treatment strategy of autoclaving OPEFB at 120°C for 3 hr and followed by alkali hydrolysis at 90°C for 3 hr was the best alternative method in solubilising significant amounts of FA from OPEFB. In addition, $NaHSO_3$ was used to prevent re-polymerisation of FA with lignin monomers. The KOH of 20 g kg^{-1} added with $NaHSO_3$, solubilised 56.94 ± 3.52 mg litres^{-1} FA, while 20 g kg^{-1} NaOH (without the addition of $NaHSO_3$) released 62.52 ± 6.24 mg litres^{-1} FA. About 50 to 70 mg litres^{-1} FA was solubilised from OPEFB and the release was observed to be affected by treatment strategies, types of alkali and its concentrations. Advancement of this study is in term of alternative choice of KOH usage to release FA. Besides the widely used NaOH, KOH usage would favour the environment as a potash fertiliser. The findings of FA release from this treatment may
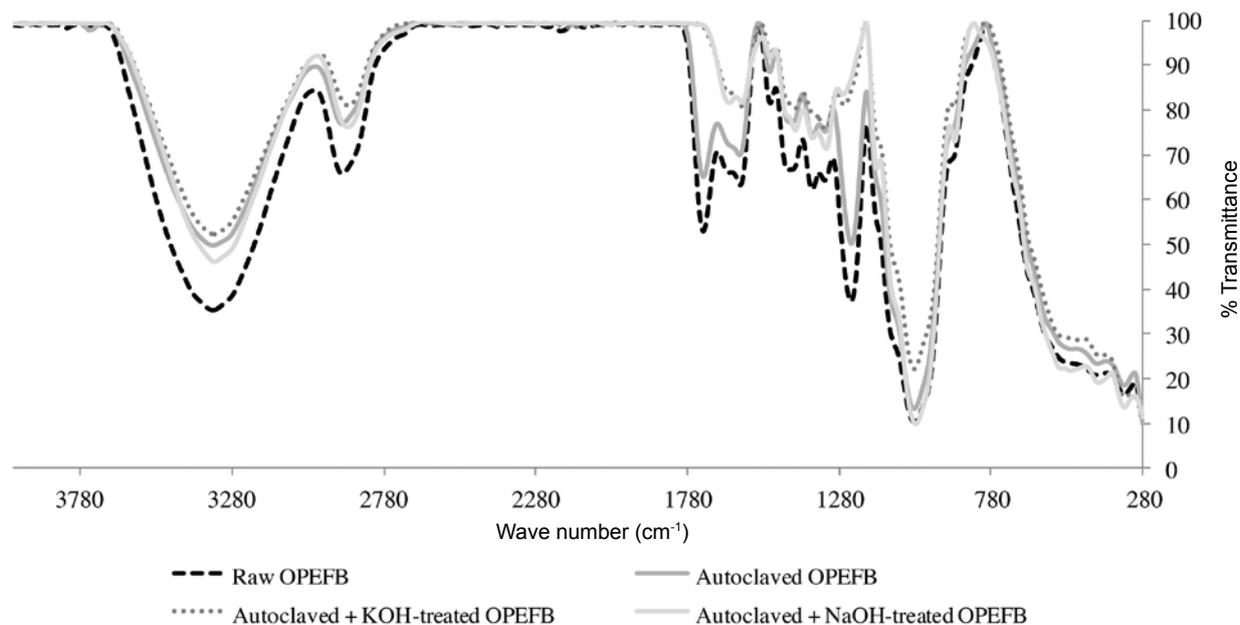
Figure 5. Fourier transform infrared (FTIR) analysis of raw (untreated); autoclaved (120°C, 3 hr); autoclaved then alkali-treated (20 g kg⁻¹ KOH and NaOH, 90°C, 3 hr) oil palm empty fruit bunch (OPEFB) fibres.

further support the microbial conversion of FA into value-added products for instance vanillic acid and vanillin.

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