# Characterization of pyroligneous acid produced from microwave-assisted treatment of palm kernel shell

**Raja Safazliana Raja Sulong[1], Seri Elyanie Zulkifli[1], Rosnani Hasham[1] and Zainul Akmar Zakaria[2*]**

[1]Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310, Johor Bahru, Johor, Malaysia
[2]School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310, Johor Bahru, Johor, Malaysia

*zainul@ibd.utm.my

**Abstract**. Palm oil plantation is one of the largest cultivation in Malaysia. The massive production of palm oil has abandoned huge palm oil biomass such as palm kernel shell that has become increasingly threatening environmental issue. Conversion of biomass through microwave pyrolysis has become one of the solutions to manage the abundance of biomass. Therefore, the aim of this study is to utilize the palm kernel shell for the production of pyroligneous acid (PA) by using microwave-assisted pyrolysis which would then be evaluated for its total phenolic content and scavenging DPPH free radicals. Pyroligneous acid of palm kernel shell will be produced from the condensation of smoke generated during pyrolysis process. From this study, the TPC observed was 49.96 mg GAE/g, whereas for the IC50, DPPH value obtained was, 66.19 µg/mL. Pyroligneous acid produced during the pyrolysis process has the potential to apply in various applications and could serve as an alternative eco-friendly source of natural antioxidant.

## 1. Introduction

*Elaeis guineesis* or known as oil palm tree is one of the most important fruit crops in the world. Nowadays, Malaysia has reached approximately 5.8 million hectares of oil palm planted area that cover the area of peninsular Malaysia, Sabah and Sarawak which accounted for 36% of the world's production (Table 1) which also put Malaysia as the world's second largest producers of palm oil after Indonesia.

Unfortunately, the massive production of palm oil has resulted in the abundance of oil palm biomass. Most of these oil palm biomasses were disposed poorly and it has become increasingly threatening environmental issue. Oil palm biomass includes oil palm trunk (OPT), oil palm fronds

(OPF), empty fruit bunch (EFB) and palm kernel shell (PKS) (Figure 1). According to Onoja et al (2019), collection of palm oil biomass is higher about 90% from the palm oil processing. While, only 10% of palm oil yield produced from palm oil processing [1]. In this study, PKS biomass was selected as starting material because of the high lignin content [2]. PKS are the shell fractions left after the nut has been removed after crushing in the palm oil mill. Kernel shells are a fibrous material and can be easily handled in bulk directly from the product line to the end use. Moisture content in kernel shells is low compared to other biomass residues with different sources suggesting values between 11% and 13%.

Table 1. Production of palm oil in 2016

| Country | Production (in metric tons) |
|---|---|
| Indonesia | 36,000,000 |
| Malaysia | 21,000,000 |
| Thailand | 2,200,000 |
| Colombia | 1,320,000 |
| Nigeria | 970,000 |
| World | 58,800,000 |

The main routes for biomass conversion are via thermochemical and biochemical technologies. Recently, pyrolysis process has become an attractive thermochemical conversion for converting the plant biomass. This is because pyrolysis is more flexible in scale-up and the process is not complicated than other technologies [3]. Pyroligneous acid is one of the by-products produced from pyrolysis process. Its main component includes lignin, hemicelluloses and cellulose. Further breakdown of these heteropolymers would yield compex mixture of phenols, furans, syringols, alcohols, vanilins, acetic acid, catechols and other carboxylic acids [4]. Pyroligneous acid has been reported to be used as plant enhancer, antioxidant [5], antibacterial [2], [6], anti-inflammatory [7], and antifungus. These properties can be attributed to the presence of organic compounds such as organic acids and phenol.

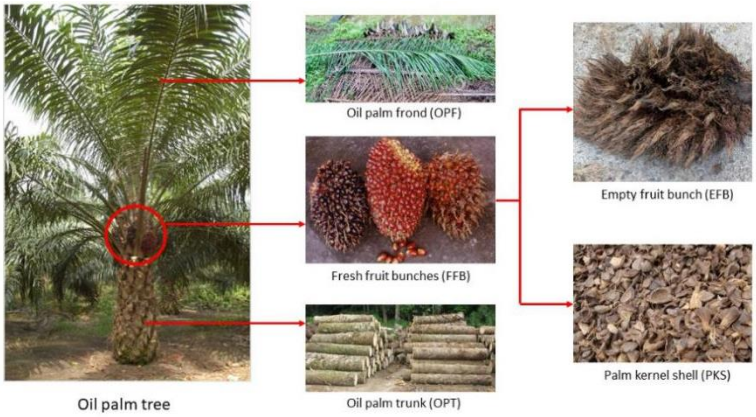


**Figure 1.** Oil palm biomass

## 2. Material and method

### *2.1. Sample preparation*

Palm kernel shell (PKS) was collected from a local palm oil mill located at Felda Kulai, Johor, Malaysia. PKS was washed and dried at temperature of 105℃ in oven drying. Then the sample was cutted into the small pieces to obtain particles sizes of 1-3mm, approximately. The PKS sample was characterized for chemical component analysis of biomass focuses on cellulose, hemicellulose and

lignin. Also, PKS was analyzed for ultimate analysis (consists of the determination carbon, hydrogen, nitrogen and sulphur elements) and proximate analysis (consists of the determination of moisture, ash, volatile matter and fixed carbon contents), respectively. Also, the sample was analyzed for thermogravimetric analysis represent the decomposition of biomass component which are hemicellulose, cellulose and lignin.

*2.2. Microwave-assisted pyrolysis*

Microwave-assisted pyrolysis system as used in this study consists of modified microwave oven, temperature controller, quartz glass reactor, thermocouple type-R, series of condenser and water cooling circulator system (Figure 2). 100 g of PKS and 75 g of activated carbon (AC) were dried overnight at 105℃ prior used. After that, both sample of PKS and AC were loaded into quartz glass reactor. Thermocouple was inserted inside the quartz glass reactor to monitor the temperature using data logger system (PicoLog Recorder software, version 5.23.0). Nitrogen gas connected to the pyrolysis system was allowed to pass through the system and nitrogen flow rate was controlled at 3 liter per minute using nitrogen flow rate controller. Temperature of pyrolysis process was controlled at 480℃ using temperature controller, while condensing temperature was set at 6℃ using water cooling circulator system. Liquid and gaseous products of pyrolysis process were passed through column condenser before liquid products (pyroligneous acid and bio oil) were collected using round bottom flask (receiving flask) at the bottom and excess gases was released to the environment. Biochar remained inside the quartz glass reactor. Upon completion pyrolysis process, liquid and solid products were collected and weighted to determine the percentage of solid, liquid and gases product of pyrolysis process.


**Figure 2.** Microwave-assisted pyrolysis

*2.3. Extraction of pyroligneous acid*

Pyroligneous acid (PA) is a liquid fraction obtained from liquid product of pyrolysis experiment. Liquid product was collected and filtered using Whatman No. 4 filter paper to separate PA from bio

oil and any impurities. It was then extracted using 99.5 % ethyl acetate (EA) AR grade using method as suggested by Loo et al., (2008) with slight modification as follows; PA was extracted using EA at 1:1 ratio in a 250 mL separatory funnel where the mixtures were homogenously shaken for 3 minutes under ambient condition and let to stand for 30 minutes for phase separation [8]. The top organic layer (EA) was collected while the bottom aqueous layer was extracted for a second time using fresh EA. The PA extract was then subjected to rotary evaporation (Heidolph, Germany) under reduced pressure (120 mBar) at 80 ºC until 1/3 of the initial volume was collected inside the flask. Then concentrated PA will be placed in a desiccator for a week to remove excess water.

### *2.4. Total phenolic content*

The total phenolic content of the samples was determined using the Folin-Ciocalteu's reagent as described by Ma et al. (2014) [9]. Gallic acid at different concentration (10 - 100 µg/mL) was used as a reference standard for plotting calibration curve. 1 mL of respective samples (100 µg/mL) was mixed with 1 mL of 50% (v/v) Folin-Ciocalteu phenol reagent in a falcon tube. The mixture was vortexed for 30 s and left at room temperature for 2 min. After 2 min, 1 mL of 10% (w/v) sodium carbonate ($Na_2CO_3$) solution was added to neutralize the mixture. The mixture was vortexed immediately for 10 s and allowed to stand in the dark for 2 h at room temperature. The assay was done in the dark due to light sensitivity of the reagent and to reduce the UV light disruption towards redox reaction. Absorbance measurement of the resulting blue color mixture was recorded at 765 nm by using UV-Vis spectrophotometer (Shimadzu, Japan). The result was reported as mean values expressed as microgram of gallic acid equivalents (GAE) per milligram of sample (µg GAE/mg).

### *2.5. DPPH free radical scavenging activity*

The scavenging capability of polyphenol extract towards DPPH free radical will be performed based on the method investigated by Brand-Williams et al. (1995) with some modification [10]. 60 µM of a radical stock solution will be prepared by dissolving DPPH in methanol (MeOH) (24 µg/ml) to form a purple color solution. A pyroligneous acid sample as well as ascorbic acid and BHA standard at different concentration (10 - 30 µg/ml) will be prepared by using MeOH as a solvent. 0.2 ml of pyroligneous acid or ascorbic acid at different concentration will be mixed with 1.8 ml of the methanolic DPPH• solution to measure the absorbance for sample or standard. The mixture will be shaken and allow to stand for 30 min in the dark place at room temperature. After that, the absorbance will be obtained by using UV-Vis spectrophotometer at 517 nm. The reaction mixture with the higher absorbance shows a lower free radical scavenging activity. The ability of samples or standard to scavenge DPPH free radical will be calculated by the following equation (1):

DPPH radical scavenging (%) = $(Abs_{control} - Abs_{sample\ or\ standard})/ Abs_{control} \times 100$

$IC_{50}$ value will be measured from the graph plotted between DPPH radical scavenging capabilities (%) against samples concentration. $IC_{50}$ define as the required amount of antioxidant for 50% decreases of the initial DPPH concentration. $IC_{50}$ with the low value shows strong antioxidant activity of the sample.

## 3. Result and discussion

### *3.1. Characterization of PKS*

Table 2 shows the proximate analysis, ultimate analysis and lignocellulosic content of PKS. In general, ash content in range between 5 - 6% observed with no slagging problem whereas ash content of 12% and above expected to have severe slagging problem. Typically, the composition of oxygen element in biomass range between 30 - 40 wt% of the dry basis, in line with the principal constituent of biomass carbon, making up from 30 - 60 wt% of dry basis depending on ash content. Hydrogen is third major component in biomass, comprising typically 5 - 6 % dry basis. The nitrogen was slightly

high component in biomass. The sulphur can also be found in low quantity, usually less than 1% dry basis. These components can lead in the formation of pollutant emissions and sulphur in certain ash reactions leading to fouling and slagging. Typically, agricultural lignocellulosic biomass includes 10-25% lignin, 20 - 30% hemicellulose and 40 - 50% cellulose [11]. However, in this study PKS was found in higher lignin which is 48.52%, while cellulose is 33.61% and hemicellulose in low quantity, 17.87%.

**Table 2.** Characterization of PKS

| A. Proximate analysis | Content (%) |
|---|---|
| Moisture content | 7.00 |
| Ash content | 0.68 |
| Volatile matter | 88.45 |
| Fixed carbon | 3.87 |
| **B. Ultimate analysis** | **Content (%)** |
| Carbon, C | 51.60 |
| Oxygen, O | 41.14 |
| Nitrogen, N | 0.39 |
| Hydrogen, H | 6.65 |
| Sulphur, S | 0.22 |
| **C. Lignocellulosic content** | **Content (%)** |
| Lignin | 48.52 |
| Cellulose | 33.61 |
| Hemicellulose | 17.87 |
| Extractive | 4.26 |

Thermogravimetric analysis represents the decomposition of the lignin, cellulose and hemicellulose. From the previous study reported hemicellulose decomposes at a lower temperature range (200 to 320 °C), while cellulose undergoes degradation at 280 to 360 °C and lignin from 140 to 600 °C [4]. The TGA profile for PKS is shown in figure 3 where the temperature used was between 30 to 850 ºC.

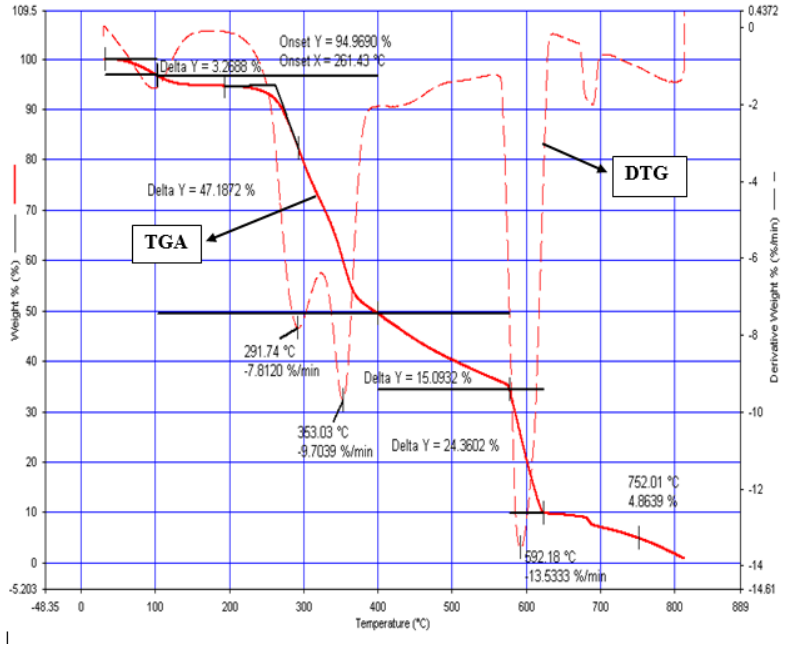


**Figure 3.** TGA-DTG curve profile of PKS

The TGA curve shows three different regions. At temperature below 200ºC, about 3.27% weight loss of PKS sample due to the removal of moisture content. The TGA results show the decomposition of hemicellulose, cellulose and some of lignin content at temperature between 250 – 380 ºC and with a total mass loss of 47.19 wt%. The weight loss of PKS sample recorded was 15.09 wt% in stage three due the remaining decomposition of heavy components mainly from lignin.

### *3.2. Characteristic of pyroligneous acid*

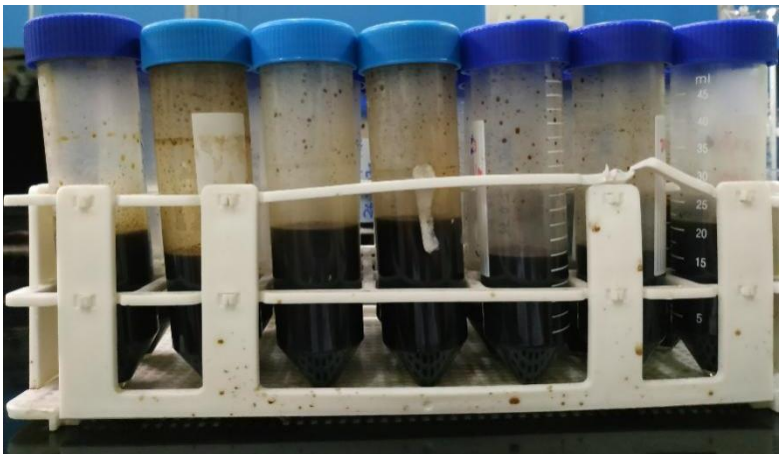

**Figure 4.** Pyroligneous acid

The percentage of solid, liquid and gases product of pyrolysis process were determined. About 33 to 43% of char was obtained, while, for PA and gasses product recorded was 17 to 20% and 30 to 40%, respectively. PA was found to be a reddish brown color (figure 4) and has low pH value ranging from 2 -3. The PA happen in acidic form due to its high amount of volatile acids mainly formic acid and acetic acid [12]. PA can appears in color range from dark green or dark red through black [13]. It happens depends on different type of biomass and method of pyrolysis.

*3.3. Total phenolic content*

The total phenolic content was determined from the linear equation of gallic acid calibration curve (figure 5) and expressed as microgram of gallic acid equivalents per milligram of sample (µg GAE/mg). The absorbance of mixture containing gallic acid, Folin Ciocalteu's reagent and sodium carbonate increased in proportion with increasing gallic acid concentration, where the colour change from yellow to blue.

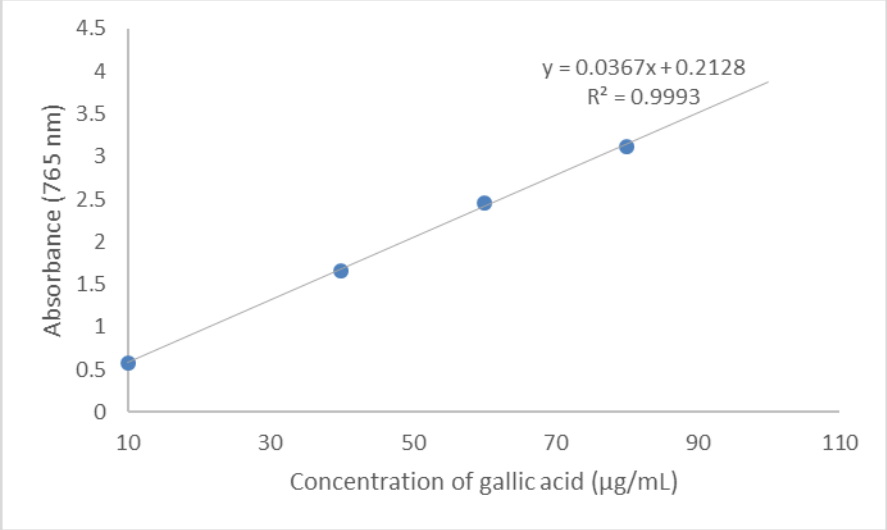


**Figure 5.** Gallic acid calibration curve

Table 3 shows the percentage of DPPH free radical inhibition for different concentrations (10 – 30 µg/mL) of the PA as well as ascorbic acid and butylated hydroxyanisole (BHA), both as standard antioxidant compounds.

From this study, the TPC observed was 49.96 mg GAE/g, whereas for the $IC_{50}$, DPPH value obtained was, 66.19 µg/mL. From the previous study reported that PA of pineapple biomass showed the TPC was 2.67 ± 0.14 mg GAE/g [14]. It shows that TPC content of PA from PKS is higher compared to pineapple biomass, probably due to the higher lignin content. There are various available evidences from previous studies to support the efficacy claims of PA as DPPH free radical scavenger. The $SC_{50}$ value (half maximal sample concentration) of dichloromethane extract PA from *Rosmarinus officinalis* leaves was ranged from 15 ± 0.18 to 23 ± 0.23 µg/mL, meanwhile the ethyl acetate extract PA was ranged from 16 ± 0.20 to 31 ± 0.26 µg/mL [15]. The $SC_{50}$ value of methanol extract PA from *Schisandra chinensis* fruit was 41 ± 1.2 µg/mL [9]. Similar finding was also reported by other researchers such as Ma et al. (2013) who work with the PA obtained from *Rosmarinus officinalis* leaves [15] and Ma et al. (2011) who worked with walnut shell [16].

**Table 3.** Percentage of DPPH inhibition of standard and sample

| Standard/PA sample | Concentration (µg/mL) | DPPH inhibition (%) | DPPH, IC50 (µg/mL) |
|---|---|---|---|
| Ascorbic acid | 10 | 22.18 ± 0.35 | |
| | 15 | 35.54 ± 0.34 | |
| | 20 | 44.59 ± 0.26 | 22.34 |
| | 25 | 54.36 ± 3.97 | |
| | 30 | 67.60 ± 1.42 | |
| BHA | 10 | 13.65 ± 0.26 | |
| | 15 | 20.31 ± 1.05 | |
| | 20 | 23.49 ± 0.02 | 39.94 |
| | 25 | 30.77 ± 1.56 | |
| | 30 | 39.16 ± 1.85 | |
| PA | 10 | 6.70 ± 0.08 | |
| | 15 | 10.78 ± 0.04 | |
| | 20 | 14.05 ± 0.02 | 66.19 |
| | 25 | 18.99 ± 0.06 | |
| | 30 | 21.83 ± 0.04 | |

## 4. Conclusion

From this study, PA from PKS showed great antioxidant activity. However, more studies need to be carried out prior to any commercialization attempts for this compound.

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## Acknowledgements

Authors would like to acknowledge Ministry of Education, Malaysia for the financial assistance through the FRGS research grant (4F994). Also to Universiti Teknologi Malaysia for the Research University grant (07G78) as well as the Zamalah Scholarship to Raja Safazliana Raja Sulong.