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To cite this article: Hery Sutanto *et al* 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* **742** 012004

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Modification of Pyrogallol with Palmitic Acid as a Soluble Biodiesel Additive

Hery Sutanto¹, Rosa Belinda¹, Silvy Yusri¹, Akhmad Darmawan², Evita H. Legowo¹, and Mohammad Nasikin³

¹ Department of Chemical Engineering, Faculty of Life Sciences and Technology, Swiss German University, Tangerang, Banten, Indonesia.
(E-mail: hery.sutanto@sgu.ac.id, teresia.rosabelinda@gmail.com, silvy.yusri@sgu.ac.id, evita.legowo@sgu.ac.id)

² Research Center for Chemistry, Indonesian Institute of Sciences, Kawasan PUSPIPTEK Serpong, Tangerang Selatan, Banten, Indonesia.
(E-mail: akhmad.darmawan@lipi.go.id)

³ Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, Jawa Barat, Indonesia.

E-mail: hery.sutanto@sgu.ac.id

Abstract. Biodiesel, as a promising substitute for fossil diesel oil, has nevertheless a main disadvantage, which is oxidized easily. The oxidation degrades its quality and causes disturbances to the machine during operations. Therefore, antioxidant is commonly used in biodiesel. However, the common antioxidant used, namely pyrogallol, has a poor solubility in biodiesel due to the polarity difference. Therefore, this research focused on the modification of pyrogallol by attaching palmitic acid molecule to the benzene ring of pyrogallol molecule through the Friedel-Crafts acylation. The product was characterized by TLC and ¹H NMR spectroscopy. It was found that the reaction produced a new product, 1.1'-(4,5,6-trihydroxy-1,3-phenylene)bis(hexadecane-1-one). The new product was subjected to a solubility test and an antioxidant activity evaluation. The result showed that the solubility limit of the product was at 2500 ppm, which was significantly higher than pyrogallol analyzed using one sample t-test. However, using DPPH method, it was found that the antioxidant activity of the new product was slightly lower than pyrogallol as the IC50 value of the new product was 18.44 ppm, and pyrogallol's was 3.07 ppm.

1. Introduction

Within the next 20 years, the world's population growth and emerging economies would trigger the rise in the energy demand, which would be dominated nevertheless by fossil fuels [1]. On the other hand, with the scarcity issue of fossil fuel and the increasing anthropogenic green house gas (GHG) emission which is caused mostly by the combustion of fossil fuels [2], the use of renewables is growing rapidly [1]. One of the type of renewables that took the largest part in the renewable energy mix in 2016 was bioenergy, that includes biofuels as a substitute of oil, for instance bioethanol and biodiesel [3]. Biodiesel, a fuel made of vegetable oil or animal fats, is a sustainable substitute for fossil diesel fuels. It is renewable, widely available, has a higher cetane number and combustion efficiency, with a lower sulfur content [4]. However, the main disadvantage of biodiesel is its susceptibility to oxidation due to the unsaturated fatty acid content [5]. The oxidation leads to acids and polymer sediments formation,



which cause engine blocking and corrosion. Therefore, antioxidant additive is required to delay the degradation [6].

Commonly used antioxidants are phenolic compounds, such as propyl gallate, pyrogallol (PY), gallic acid, TBHQ, and others. PY is found to be among the best antioxidant for biodiesel [7], [8]. However, as they are polar molecules whereas biodiesel is nonpolar, the phenolic antioxidants have poor solubility in biodiesel [7], [9]. Over 80 years ago it was already developed that the solubility of phenolic compounds in oil could be improved by attaching an alkyl or aryl group to the benzene ring without decreasing their antioxidant activity [10]. In this research, palmitic acid was used to be attached to the PY ring by following Friedel-Crafts acylation. Friedel-Crafts acylation is a very common method of creating a carbon-carbon bond between a benzene and an alkyl or acyl group.

2. Research Method

2.1. Friedel-Crafts acylation

5 mmol of PY was dissolved in 7 mL nitromethane and stirred for 10 minutes. Another mixture of 5 mmol palmitoyl chloride and 0.25 g AlCl₃ was stirred for 10 minutes. A watch glass was used to cover the beaker. The two were combined and stirred for 15 minutes, also covered by a watch glass. [11-12].

2.2. Product identification

The reaction product was analyzed using thin layer chromatography (TLC) to identify the presence of a new product in the reaction product. Then the component of interest was separated by preparative TLC, and further characterized using proton nuclear magnetic resonance (¹H NMR) to identify the molecular structure of the new product.

2.3. Solubility test

Solubility test [13]: The separated product was subjected to solubility test to evaluate the solubility limit of the new product compared to PY in biodiesel. The solubility test was fulfilled by measuring the absorbance value of the antioxidant in biodiesel using UV/Vis spectrophotometer. Four concentrations of antioxidant (1500 ppm, 2000 ppm, 2500 ppm, 3000 ppm) in biodiesel were measured. The absorbance value is expected to increase with the increasing concentration. When the absorbance value is stagnant or decreasing after a certain concentration, then the solubility limit of the antioxidant in biodiesel is reached.

2.4. Antioxidant activity evaluation

Antioxidant activity evaluation [14]–[16]: The antioxidant activity of the new product must also be evaluated. TLC-DPPH method was used for the qualitative analysis. Each PY and the new product with a concentration of 1500 ppm were spotted onto a TLC plate, then the spots were sprayed by 0.2% methanolic DPPH. The color change of the methanolic DPPH would indicate the antioxidant activity. Afterwards, the quantitative analysis was done by using DPPH radical scavenging assay. Five concentrations of PY (1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm) and five concentrations of the new product (10 ppm, 12 ppm, 15 ppm, 17 ppm, 20 ppm) were prepared. After 30 minutes of incubation, the absorbance value was measured using UV/Vis spectrophotometer at 515 nm. Then the % inhibition and IC₅₀ value could be calculated and compared.

2.5. Formula used in the analysis

2.5.1. Retention factor_f

It is a value obtained by dividing the distance traveled with the total distance in TLC analysis.

$$R_f = \frac{\text{distance traveled}}{\text{total distance}}$$

2.5.2. Yield (%)

The amount of the new product contained in the reaction product was obtained by dissolving 25 mg of the reaction product in chloroform, then it was completely spread on a TLC paper. Preparative TLC was done to separate and acquire the new product. So, the yield calculation was:

$$\text{Yield} = \frac{\text{weight of acquired product}}{\text{initial weight of the reaction product}} \times 100\%$$

2.5.3. One sample t-test

One sample t-test is a method for hypothesis testing for a small-sized sample where the sample standard deviation s might not be close to the population standard deviation σ . The solubility limit of the new product was set as the null hypothesis:

$$H_0: \mu = 2500 \text{ ppm.}$$

Nine solubility limit data of pyrogallol was tested to prove whether the true value, which is 2500 ppm, was really different or not. *P-value* represents the occurrence probability of a given event. The higher the *P-value* means the higher the probability of pyrogallol's solubility limit to be 2500 ppm, which supported H_0 to be plausible, that pyrogallol's solubility limit was really no different than 2500 ppm.

As a general rule of thumb, *P-value* = 0.05 was used as the cutoff for significance, so if the *P-value* was smaller than 5%, it was then convincing that the true value, which was 2500 ppm, was really different.

Prior to finding the *P-value*, the t value must be calculated using the equation below:

$$t = \frac{\bar{X} - \mu}{s/\sqrt{n}}$$

where \bar{X} is the sample mean (2167 ppm), μ is the null hypothesis (2500 ppm), s is the sample standard deviation (250), and n is the sample size. The t value is then transformed into a *P-value* by using a t table.

3. Result and discussion

3.1. Friedel-Crafts acylation

Palmitoyl chloride is acid halide derivative of palmitic acid which was used in this experiment to shorten and simplify the reaction to represent palmitic acid molecule. It acts as the R-X reactant in the *Friedel-Crafts* reaction in the presence of AlCl_3 . The acylation reaction produced a product with two phases, liquid and waxy phase. The product mixture had a yellow color because of the use of excess AlCl_3 in nitromethane [17]. After a few hours, the mixture color changed to grey with some visible black spots, indicating that the excess AlCl_3 might have fumed since colorless malodorous gas kept forming. AlCl_3 has a characteristic to fume to form hydrogen chloride when exposed to moist air [18]. The grey color might come from remaining palmitoyl chloride and the product's color, while the black spots might be

the remaining PY that had been oxidized by AlCl_3 -nitromethane, whose combination possessed a strong oxidizing power [19].

3.2. Product identification

The reaction product was analyzed using TLC. The TLC result (Figure 1) of the reaction product showed that PY was still contained in both waxy and liquid product. Moreover, a new spot with an R_f value of 0.77, higher compared to PY's which was 0.34, was identified also in both waxy and liquid product, meaning that the reaction product was more nonpolar compared to PY, because the new spot travelled further with the nonpolar mobile phase, while PY was more attracted to the polar stationary phase, the silica gel TLC plate. The mobile phase was a combination of n-hexane and ethyl acetate with a ratio of 3:2. The widening PY spot in the liquid product (d) indicated a very high PY content.

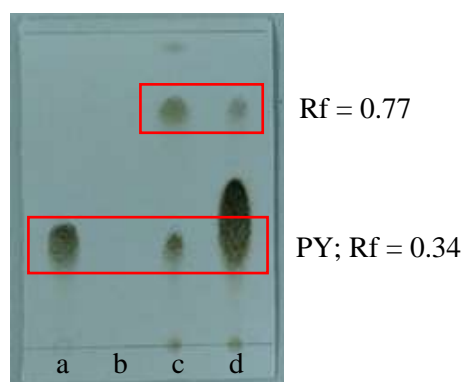


Figure 1. TLC analysis result; (a) PY standard; (b) palmitoyl chloride standard; (c) waxy product; (d) liquid product

The new product ($R_f = 0.77$) was separated and collected by preparative TLC and further characterized using ^1H NMR to identify the molecular structure. The complete chemical shift in the spectrum is shown in Table 1. The spectrum indicated that unreacted PY and palmitoyl chloride were present in the separated mixture. A singlet signal at a chemical shift δ 6.795 suggested that a new product, 1.1'-(4,5,6-trihydroxy-1,3-phenylene)bis(hexadecane-1-one) with a molecular weight of 602.93 g/mol and a chemical formula of $\text{C}_{38}\text{H}_{66}\text{O}_5$ was formed:

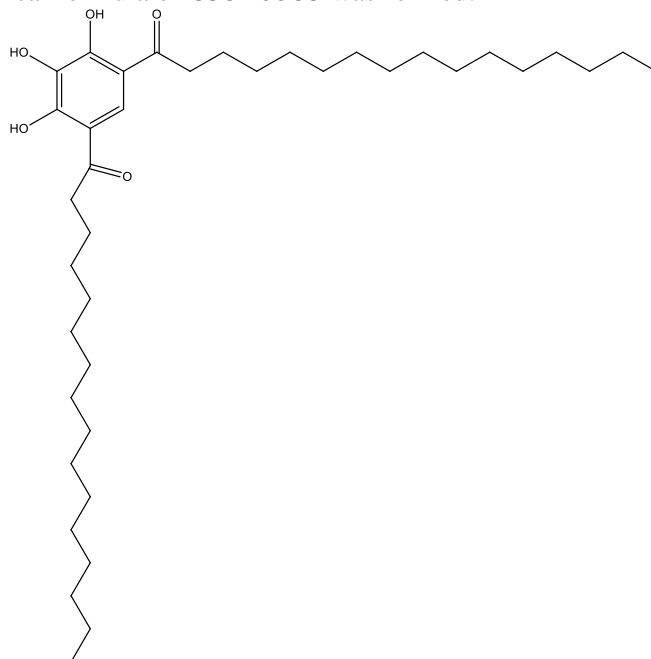


Figure 2. Molecular structure of 1.1'-(4,5,6-trihydroxy-1,3-phenylene)bis(hexadecane-1-one)

Two palmitic groups were substituted to the PY ring instead of only one as initially predicted, due to the strongly activating nature of the existing substituents, the three hydroxyl groups [20].

Table 1. ^1H NMR chemical shift data

δ	integration
0.85-0.88	9
1.2-1.5	72
1.6	4
1.75	2
2.3	2
2.6	4
6.6	2
6.78-6.8	1
6.795	1

The reaction yielded 17.6% of the new product, 1,1'-(4,5,6-trihydroxy-1,3-phenylene)bis(hexadecane-1-one). The yield calculation was done by dissolving 25 mg of the reaction product in chloroform, then the mixture was separated using preparative TLC method. After dried and weighed, 4.4 mg of the separated product was obtained.

3.3. Solubility test

In this research, PY was modified with palmitic acid to increase its solubility in biodiesel. Therefore, the new product of the reaction was subjected to a solubility test to compare the solubility of PY and the new product. According to the hypothesis, the new product has a better solubility in biodiesel compared to PY. The solubility test was carried out by measuring the absorbance value of the antioxidant in biodiesel. This approach was used because absorbance is proportional to concentration. The biodiesel-antioxidant mixture was diluted to 1:300 with methanol. The absorbance was measured at 260 nm, referring to the scanning result of PY, palmitoyl chloride, and new product which showed a maximum absorbance at the respective wavelength. The measurement was repeated twice. The result showed that the solubility limit of PY was at 2000 ppm (Figure 3) since the absorbance value reached its highest at the respective concentration, while the new product's solubility limit was at 2500 ppm (Figure 4). This result confirmed that the solubility of the new product in biodiesel was better than PY's.

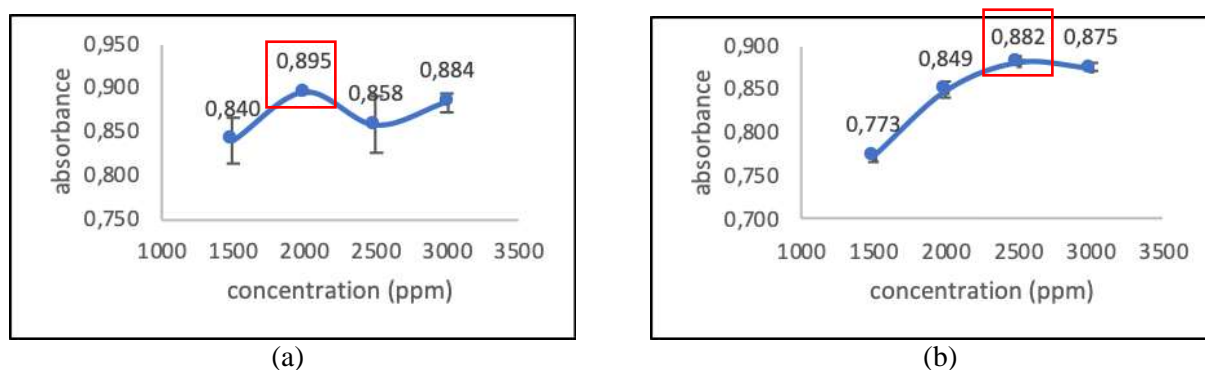


Figure 3. Solubility limit of PY (a) and new product (b)

However, the solubility limit data set must be further analyzed using one sample t-test hypothesis testing. The purpose was to conclude whether the new product's solubility limit was significantly different from PY's solubility limit. Statistical approach is important in a scientific research to draw a reliable conclusion from repetitive measurements, which often come out differently each time.

Nine PY solubility limit data sample with a mean of 2250 ppm was tested against the solubility limit of the new product, 2500 ppm, as the reference value. Theoretically, when PY is modified by attaching a long chain hydrocarbon to its molecule, the modified PY molecule becomes more nonpolar, thus becoming more soluble in nonpolar solvents, for instance biodiesel. Therefore, a one-tailed test was chosen, because the data set could not have a higher value than the reference value. The test concluded that the solubility limit data of the new product was statistically significant with a P-value of 0.00275.

3.4. Antioxidant activity evaluation

Although the main focus of this research was on improving the solubility of PY in biodiesel, the antioxidant activity of the modified PY must be evaluated as well, to ensure that the new product did not lose its function as an antioxidant.

The principle of the qualitative evaluation was the color change of DPPH. At room temperature, DPPH is a stable free radical which has a violet color in a methanolic solution. In the presence of antioxidants, the free radicals react with a hydrogen so DPPH is losing its free radical property, changing its color to yellow [21-22].

When the 0.2% methanolic DPPH solution was dropped onto the PY and the new product on the TLC paper, both spots immediately turned bright yellow, as seen in Figure 5 below:

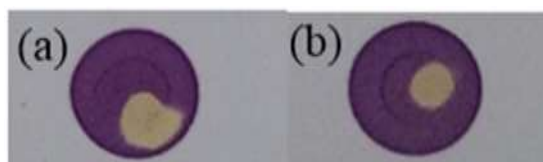


Figure 4. Qualitative TLC-DPPH method;
(a) PY; (b) new product

PY is among the best antioxidant [7], [8], so the result above was expected. As for the new product, from the result above it could be qualitatively concluded that the new product had an antioxidant activity, as it changed the color of the methanolic DPPH from violet to yellow.

After qualitatively proved that the new product also had an antioxidant activity, the quantitative evaluation was performed to compare the antioxidant activity of PY and the new product. The working principle of DPPH radical scavenging assay was the same as explained above, which was based on the color change of DPPH radical due to its reaction with antioxidant, thus changing its color from violet to yellow. However, in the quantitative evaluation, the ability of the antioxidant to inhibit the DPPH free radical, which was evident by the color change, was measured using a UV/Vis spectrophotometer. The absorbance of each sample was measured at 515 nm. The absorbance of DPPH is commonly measured at 515 – 520 nm [15].

Upon DPPH addition, the samples were incubated in a dark container for 30 minutes to allow the DPPH to completely react. They must be kept in the dark because light exposure decreases the absorbance of methanolic DPPH [22]. From the measured absorbance value, the inhibiting percentage was calculated and made into a linear regression plot (Figure 6 and 7). From each graph, the linear regression was used to find the IC_{50} value, which was the amount of antioxidant required to reduce the DPPH radical concentration up to 50% [16].

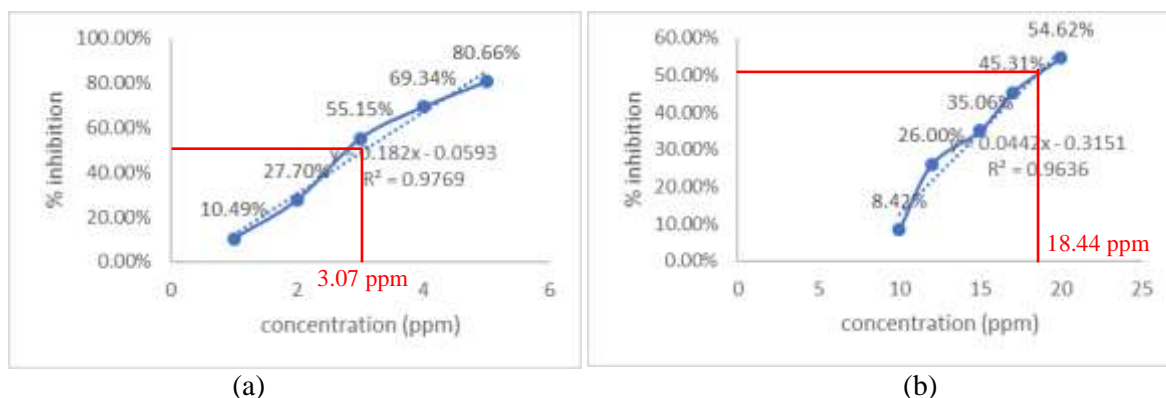


Figure 5. Inhibiting percentage of PY (a) and new product (b)

The IC_{50} value of PY was 3.07 ppm and for the new product was 18.44 ppm. The IC_{50} value of both PY and the new product was calculated from each linear regression to be 3.07 ppm and 18.44 ppm, suggesting that the antioxidant activity of the new product is significantly lower than PY. However, since the molecular weight of the new product, 602 g/mol, is notably heavier than PY, 126 g/mol, if the IC_{50} value is converted to molar concentration, it will show that the antioxidant activity of PY is only slightly higher than the new product, which are 24 μ M and 30 μ M, respectively.

The decreasing antioxidant activity may be caused by some factors. First, since the purification of the new product was only done by preparative TLC, the purity of the product was probably low. Furthermore, the preparative TLC also included a drying process, either by leaving the sample openly to dry or by heating the sample in a vacuum oven. These factors might reduce the antioxidant activity of the sample, which was already 2.5 months old by the time of the antioxidant activity evaluation.

4. Conclusions

Friedel-Crafts acylation reaction successfully modified PY with palmitic acid, producing a new product 1.1'-(4,5,6-trihydroxy-1,3-phenylene)bis(hexadecane-1-one), which was identified using TLC and 1H NMR. The new product has a significantly higher solubility limit at 2500 ppm compared to biodiesel analyzed using one sample t-test, and a slightly lower antioxidant activity than PY with an IC_{50} value of 18.44 ppm.

It is recommended to improve the yield of the reaction product, for instance by finding another alternative solvent for the Friedel-Crafts acylation, considering that palmitoyl chloride is not completely soluble in nitromethane. The product's purity improvement for further characterization by preparative HPLC could give a more accurate characterization result. Moreover, to have a better confirmation of the new product, a liquid chromatography tandem mass spectroscopy (LC-MS/MS) analysis could also be performed.

5. Acknowledgment

The authors would like to thank The Ministry of Research, Technology and Higher Education Republic of Indonesia for financial support through Penelitian Terapan Unggulan Perguruan Tinggi (PTUPT) research grant (contract no. 2639/L4/PP/2019).

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