Antioxidant Extraction from Indonesian Crude palm Oil and It’s Antioxidation Activity

Supriyono¹, WB Sediawan²
¹Engineering Faculty, Setia Budi University - Surakarta – Indonesia
²Chemical Engineering Dept. Gadjah Mada University – Yogyakarta – Indonesia
suprisuwito@gmail.com

Abstract. Crude palm oil (CPO) is a vegetable oil that came from a palm tree bunch. Palm oil tree was known as highest vegetable oil yield. It was grown across Equatorial County, especially in Malaysia and Indonesia. The greenish red color on CPO was came from carotenoid antioxidant, which could be extracted and use widely as functional food and other purposes as antioxidant source on the biodiesel posttreatment to prevent biodiesel oxidation. Another antioxidant that also found in CPO is tocopherol. The aim of the research work is to find antioxidant activity on CPO comparing to the synthetic antioxidant that available in a market. On this research work, antioxidant was extracted by using a mixture of acetone and n. hexane, while activity of the antioxidant extract was determined by DPPH method. Antioxidant activity of the extracted compound has better performance compare to pure tocopherol. While the solvent mixture does not influence on the antioxidant activity.

1. Introduction

Crude Palm Oil (CPO) is one of biodiesel feedstock in Indonesia, which is rich with antioxidants. Biodiesel production through transesterification will destroy the antioxidants content, thus in the end of the process we should introduce antioxidant to the biodiesel product to maintain their stability to the oxidation process. The aim of this research is to remove antioxidants that present in CPO before processing to biodiesel product. Then the antioxidants will reintroduce to the biodiesel product. Simply said, the aim of the research is to find rerouting of biodiesel production through transesterification process.

1.1. Palm Oil Tree

Palm oil tree is part of the Arecacea family. There are two species of oil palm oil trees, namely the African palm oil tree and the American palm oil tree. The African palm oil tree Elaeis guineensis, originates from West Africa between Angola and the Gambia, while the American palm oil tree, Elaeis oleifera, comes from Central and South America. The uniqueness of Palm oil tree in the ability to produce 2 types of oil from two different sources, oil that comes from palm fibre is namely Crude Palm Oil (CPO), while oil derived from palm kernel oil is namely Palm Kernel Oil (PKO). The fatty acid composition of CPO and PKO can be seen in table 1 (Asian Agri, 1994).

| Composition   | Units |
|---------------|-------|
| Free Fatty Acid | 2.8 % mass |
| Water         | 0.08 % mass |
| Impurities    | 0.11 % mass |
| Fe            | 11 ppm |
| Cu            | 0.3 ppm |
Iodine Number | 54 | g iodium/100 g sample
Carotenoid     | 600 | ppm
Tocopherol    | 690 | ppm
Fatty Acid     | 97  | % mass

From the table 1. It was shown that free fatty acid on crude palm oil is relatively low compare to other crude vegetable oil such as olive oil, sunflower oil, corn oil and peanut (ground nut) oil. The main reason on the low free fatty acid content on crude palm oil is the fact that oil composition on crude palm oil is dominated by Fatty Acid fraction, as shown in table 2. The other reason on low FFA on crude palm oil is contribution of carotenoid and tocopherol as primary antioxidant, which could prevent oxidation of the double bond on unsaturated fatty acid which also composed crude palm oil. For this reason, the effort to separate antioxidant from crude palm oil is worth to explore.

Table 2. Fatty Acid Composition on Crude palm Oil

| Fatty Acid | CPO (%) |
|------------|---------|
| Lauric     | -       |
| Miristic   | 1 – 3   |
| Palmitic   | 41 – 43 |
| Stearic    | 4 – 6   |
| Oleic      | 38 – 40 |
| Linoleic   | 10 - 11 |

1.2. Antioxidants
Antioxidants are compounds that added in a small amount to prevent or reduce the oxidation processes in a hydrocarbon compound. According to Larson R.A. and Marley K.A. (2011), there are several types of antioxidants

a. Radical peroxyl Quencher (Chain Breaker) the example is Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Pyrogallol (PG)
b. Peroxide Destroyer (Reducing Agent)
c. Metal Chelating Agent
d. Acid Neutralizer

While in a term of mechanism, there are two types of antioxidants, primary and secondary. In the primary antioxidant or free-radical scavengers, antioxidative activity works by giving atom or electron to the free radical derivative. The examples of antioxidants are the type of amine compound (p-Phenylene diamine, trimethyl dihydroquinolines, alkylated diphenyl amines) and phenol compounds suppose Butylated hydroxytoluene (BHT). The reaction between primary antioxidants with free radicals will stop the autocatalytic cycle in the peroxide oxidation process. While secondary antioxidants or peroxide decomposers referred to mechanism by removing the catalyst that trigger the oxidation process so to prevent initiation of the oxidation reaction. Examples of decomposing peroxide (peroxide decomposer) is a trivalent phosphorous and divalent sulphur, thiodipropionates and organophosphates. Synergetic effect is expected to occur when the primary antioxidant used in conjunction with a secondary antioxidant. However, synergetic effect did not effectively prevent oxidation processes that came from Ultraviolet rays. The oxidation process caused by the presence of metal in the hydrocarbon is prevented by the addition of chelating agent. According Scheumann (1942) the antioxidant of phenol group has strong antioxidant capabilities while the disadvantages is could absorb water contained in the air and then antioxidants distributed between water and hydrocarbons. While according to Dinkov (2009) are generally effective use of antioxidants at concentrations between 200 to 1000 ppm.

According Knothe (2007), the oxidation process was triggered by a small group of fatty acids that are able to act as a catalyst in the oxidation. The oxidation process is accelerated by an increase in temperature, metal content and the absence of light. The existence of free radicals and the
geometry of the molecules also play a role. The primary oxidation process of the double bond will generate hydroperoxide. Because hydroperoxide is unstable, there will be a further reaction which runs parallel to, among other things.

a. The secondary oxidation which results in rearrangement of molecular weight products that remain, molecular geometry changes of cis to trans or vice versa.

b. Reaction breaking (cracking) and formation of compounds with shorter chains (aldehydes and acids)

c. Dimerization, which results in a molecule with greater weight

1.3. Extraction process

Extraction is the process of separating a substance contained in other substances based on differences in solubility. Extraction is divided into two categories based on the phase involved, namely homogeneous extraction and heterogeneous extraction.

1.3.1. Homogeneous extraction

Homogeneous extraction is the process of collecting a substance that is dissolved in the first solvent using a second solvent, wherein the solvent I and solvent II are not mutually dissolve, as shown in Figure 1.

An active substance A is soluble in the solvent I and solvent II. In the beginning A is only present in the solvent I, then solvent II was introduced into the system, then there will be a shifting of A from the solvent I to the solvent II. Shifting will continue to the equilibrium state where A moved from the solvent I to the solvent II is equal to the A switched from solvent II to solvent I. If the concentration of A in the solvent I is \( C_I \) and the concentration of A in the solvent II is \( C_{II} \), then according to González et al (2010) the equilibrium state relations were expressed by equation 1

\[
K = \frac{C_I}{C_{II}}
\]  

While substance A that left in the solvent I were expressed by equation 2.

\[
X_n = X_0 \left( \frac{KV}{KV+S} \right)^n
\]  

Where

- \( X_n \) = Concentration of substance A in a solvent I after \( n \) step of extraction
- \( X_0 \) = Initial Concentration of substance A in a solvent I
- \( K \) = Equilibrium Constant
- \( V \) = Volume of solvent I
- \( S \) = Volume of solvent II in every step of extraction

The same equation also applied to the substance B and C

1.3.2. Maceration

The simplest method on heterogeneous extraction is by maceration, in which the material to be extracted is immersed in a solvent as shown in Figure 2. During the period of immersion, substance will diffuse out from the body of solid into the solvent phase. The next step is separation of the solids with liquids that already contains extracts dissolved in the solvent. The extract is separated by evaporation of the solvents both in ambient pressure with high temperature or in a vacuum pressure with lower temperature. Maceration method is still used because it is cheap and easy to do. Disadvantage of this method is on the selectivity of extraction process. Another disadvantage is the possibility of damage on the extract due to the heating process (Wang, 2010).
According to Chew et al. (2011), diffusion of the substances from the solid phase into a liquid phase is typically slow. For the purpose of accelerated extraction process, maceration carried out at temperatures around the boiling point of the solvent. High temperature process is one of the sources in decomposition of extracted substance.

Furthermore, maceration process was used to develop better extraction methods as Microwave assist extraction (Veggi et al., 2013), Subcritical water extraction (He et al., 2012), Ultrasonic (Wu et al., 2014), Pulse Electric Field (Corrales et al., 2008; Boussseta et al., 2013), High Hydrostatic Pressure (Rendueles et al., 2011; Mathavi et al., Devi, 2013), High Voltage Electric Discharges (Rajha et al., 2014) and Enzyme Assist Extraction (Wu et al., 2014; Martínez et al., 2013) dan supercritical extraction (McHugh & Krukonis, 2013; Darani & Reza Mozafari, 2010; Zuknik, 2012).

1.4. Antioxidant Activity Test Methods 2.7 DPPH (2,2-diphenyl-1-picrylhydrazyl)

DPPH is a stable free radical at room temperature and is often used to assess the antioxidant activity of several compounds or extracts of natural ingredients. DPPH antioxidant interaction with either the transfer of electrons or hydrogen on DPPH radical character will neutralize free radicals from DPPH. DPPH test principle is the removal of colour to measure the antioxidant capacity directly reach DPPH radical by monitoring the absorbance at a wavelength of 517 nm using a spectrophotometer. DPPH radical with organic nitrogen is a centralized stable free radical with a dark purple colour which when reduced to non-radical by antioxidants to a yellow colour (Bendra, 2012).

Because of the unpaired electrons, DPPH provide strong absorption at 517 nm. When the electrons into pairs by the presence of free radical catcher, then the stoichiometric absorbance decreases according to the number of electrons captured. The existence of antioxidant compounds can change the colour of DPPH solution from purple to yellow (Dehpour et al., 2009). Absorbance change as a result of this reaction has been used extensively to test the ability of some molecules as free radical catcher. DPPH method is easy, fast, and sensitive to test the antioxidant activity of certain compounds or plant extracts (Koleva et al., 2002; Prakash et al., 2010). DPPH is a free radical that can react with compounds that can donate a hydrogen atom, it is useful for testing the antioxidant activity in an extract. According to Valentao et al, the antioxidant activity was expressed by Inhibition 50% concentration (IC_{50}) which is the concentration of the sample that could reduce DPPH radicals by as much as 50%. IC_{50} value calculated based on the percentage of inhibition to radical DPPH of each sample solution concentration with the formula

\[
\% \text{ inhibition} = \left( \frac{OD_{\text{control}} - OD_{\text{sample}}}{OD_{\text{control}}} \right) \times 100\%
\]

Where: \( OD_{\text{control}} \) = Inhibition of DPPH radical
\[ OD_{sample} = \text{Inhibition of the sample} \]

2. Materials and methods

2.1. Materials

Crude Palm Oil (CPO) was supplied by PT Indo Sawit Subur, North Sumatra Plantation Indonesia. All materials used in the experiment were pro analysis grade.

2.2. Methods

The commonly used method most used to determine antioxidant activity on natural antioxidants is a test method using DPPH free radicals. The purpose of this method is to determine the equivalent concentration parameter that gives 50% effect of antioxidant activity (IC\textsubscript{50}). This can be achieved by interpreting the experimental data of the method.

Sample collection with random sampling method. Samples are stored in jerry cans. For pretreatment, the sample was melted and homogenized then stored in dark brown bottles glass. Three neck rounded flask was placed in a waterbath which is set to the temperature of 50\degree C. Then 100 ml CPO introduced in the three- rounded neck flask and mixed with the 100 mL solvent, stirred for 5 minutes. Solvent that use in this study was compose by n. hexane and acetone in a different ratio. CPO and solvent mixture are poured into a funnel separator, the oil phase is separated from the solvent. Solvents which already contain antioxidant was further distilled in a vacuum distillation at a temperature of 60\degree C and a pressure of 200 mmHg to separate solvent from the extract of antioxidant. Antioxidant extract then weighing and analysed the antioxidant activity by DPPH method. Extraction and distillation process were repeated for 3 times.

The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute methanol and 0.3 mL of DPPH radical solution 0.5 mM in methanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UVVIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of methanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing methanol (3.5 mL) and DPPH radical solution (0.3 mL).

3. Result and Discussion

3.1. Antioxidant extraction

The equilibrium constant K also known as the distribution ratio, distribution coefficient or partition coefficient. The equation, known as the law of distribution, applied only in the aqueous solutions where the coefficient of activity is negligible. The law of distribution is a method used to determine the activity of a solute in one solvent if the activity of the solute in another solvent is known, provided that the two solvents are not completely mixed with one to another. The law of distribution is widely used in the extraction, analysis and determination of the equilibrium constant.

| Ratio Acetone to n. hexane | Extract concentration (ppm) | K value |
|-----------------------------|----------------------------|---------|
| 90:10                       | 2000                       | 0.91    |
|                             | 1800                       | 0.91    |
|                             | 1600                       | 0.77    |
|                             | 1400                       | 0.77    |
The value of the equilibrium coefficient is calculated by applying percentage of scavenging value to the equilibrium formula. To get the $K_1$ and $K_2$ values, it was calculated from percentage of scavenging value of each concentration in a sequence of processing times from the first to the third. Where $K_1$ is percentage of scavenging value of the first stage of extraction divided by the scavenging value of the second stage of extraction, while $K_2$ is the scavenging value of the second stage divided scavenging value of the third stage. From the table 2 it could be seen that the values for $K$ is almost the same and could concluded that $K_1$ and $K_2$ have a slight difference. Exception is on the data on concentration of 1200 ppm where the value of $K_1$ and $K_2$ has a big difference. It seems that on the low concentration, mostly of the antioxidant will extracted on the first stage and just a leave a little amount to be extracted on the second and third stage. Ratio of solvent mixture also influenced to the extraction processes. Higher acetone ratio implies to the higher antioxidant that could recover on the first stage of extraction.

### 3.2. Antioxidant Activity

The activity of antioxidant was determine using DPPH method, and calculated on the inhibition percentage, which is the value showed effectiveness as antioxidant.

![Figure 4. Correlation between antioxidant concentration to inhibition percentage for the ratio of acetone to n.hexane 9:1](image-url)
Figure 5. Correlation between antioxidant concentration to inhibition percentage for the ratio of acetone to n.hexane 7:3

Figure 6. Correlation between antioxidant concentration to inhibition percentage for the ratio of acetone to n.hexane 5:5

The data in fig. 4 to fig. 6 shown that antioxidant extract from first stage of extraction has better performance than pure tocopherol, this could be explained that there is more than one antioxidant in Crude Palm Oil. At least there are tocopherol and carotenoid antioxidant, in which these antioxidants will work mutualism and resulting on better performance in the antioxidative stability.

From this study it could be proposed to build a new route on the development of biodiesel production process from crude palm oil. Recently biodiesel production from crude palm oil was produce without separation of the antioxidant content. This antioxidant could decompose by catalyst that use in the process, and in the end of the process for the reason to maintain biodiesel quality and to prevent from decomposition by oxidation process, biodiesel should mix with antioxidant. The new route that propose is separation natural antioxidant in CPO on the first step and then the fatty acid could further process to produce biodiesel. After purification step on biodiesel production, the antioxidant could reintroduce into biodiesel to increase the quality of final biodiesel.
4. Conclusion

The antioxidant in the CPO could be extracted using a mixture of n-hexane and acetone. The antioxidant extracted from crude palm oil has better performance than pure tocopherol on the antioxidative performance.

ACKNOWLEDGMENT

Author team would like to thank the Directorate General of Higher Education, Ministry of Higher Education Research and Technology, Republic of Indonesia, for financial support of this work through Hibah Pasca Doktor 2017 Project. Contract number 001/LPPM-USB/PPD/IV/2017

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