Purification of vitamin E from palm fatty acid distillate through neutralization, extraction, and adsorption methods

Anggita Veningtia Sari, Ardiyan Harimawan, and Dianika Lestari*
Department of Chemical Engineering, Institut Teknologi Bandung, Bandung 40132 Indonesia
*corresponding author’s e-mail: dianika@che.itb.ac.id

Abstract. Vitamin E is one of the compounds in palm fatty acid distillate (PFAD) which was carried away due to thermal treatment in the deodorization unit in the refining of crude palm oil (CPO). The process of purifying vitamin E can be done by combining several common processes such as neutralization, extraction, and adsorption. The neutralization process is carried out to remove the content of free fatty acids (FFA), which are the saponifiable compounds and the largest component in PFAD. FFA in PFAD were first neutralized using magnesium oxide and most of FFA removed during process. Vitamin E and the remaining FFA are carried away during the extraction process with hexane due to similar polarity. Vitamin E extracted from neutralized PFAD was then subjected to batch adsorption process using incubator shaker with silica as the adsorbent. The adsorption process in this study resulted in a vitamin E uptake percentage of 98%. Isopropanol is used as solvent in the desorption process and produces a vitamin E recovery percentage of 96.9%. The concentration of final concentrate was obtained with vitamin E purity of 5.6% (12282 ppm) and the amount increased 9.2 times compared to concentrate from neutralization followed by extraction process. The concentrate has strong antioxidant activity as evidenced by the IC50 value of 23.3 ppm.

Keywords: PFAD, vitamin E, neutralization, extraction, adsorption

1. Introduction
Palm fatty acid distillate (PFAD) is a by-product from refining process of crude palm oil (CPO) and widely used for oleochemical industry and a raw material for animal feed. PFAD consists of 81.7% free fatty acids (FFA), 14.4% glycerides, 0.8% squalene, 0.5% vitamin E, 0.4% sterols, and 2.2% other substances [1]. Among these components, vitamin E has been reported to have powerful antioxidant properties and the highest economic value [2]. Vitamin E is a fat-soluble natural antioxidant and has two main structures namely tocopherol and tocotrienol. As the essential vitamin, vitamin E cannot be self-produced by the human body and needs to be obtained from the food supplement [3]. Thus, turning PFAD into value-added products such as vitamin E would seem beneficial.

Since vitamin E in PFAD mixed with various other components, separation of vitamin E from PFAD is difficult task due to relatively low concentration. However, the availability of abundant stocks in Indonesia makes PFAD a potential raw material for producing vitamin E. The method of separating vitamin E from PFAD consists of two stages i.e. the separation of saponifiable components (FFA and glycerides) and unsaponifiable component (squalene, sterols, etc.). Many attempts are made to separate vitamin E from PFAD such as molecular distillation [4], esterification and crystallization [5], and neutralization and adsorption chromatography [6]. Understanding the separation processes is crucial to have products with high vitamin E purity and recovery.
In this study, vitamin E in PFAD were concentrated by removing the extraneous matters, especially FFA. Better removal of the fatty components resulted in better concentration of vitamin E in the final product [6]. There are three stages for vitamin E purification, namely neutralization, extraction, and batch adsorption. FFA in PFAD were first neutralized with alkaline saponification using magnesium oxide. Vitamin E is then extracted using hexane and subjected to batch adsorption using incubator shaker and silica as the adsorbent. To produce the final concentrate, desorption was carried out using isopropanol followed by evaporation. The antioxidant activity of vitamin E is generally evaluated using DPPH spectrophotometry method. The result from this study provide information on the separation of vitamin E in PFAD by combining neutralization, extraction, and adsorption methods and their effect on the purity of vitamin E produced.

2. Materials and methods

2.1. Materials
PFAD with an iodine value of 52.2 g of I$_2$/100 g and slip melting point of 45-50°C was obtained from PT. Tunas Baru Lampung Tbk., Lampung, Indonesia. Column chromatography grade silica gel 60 (diameter size of 0.063-0.200 mm; specific pore volume of 0.74-0.84 ml/g) was purchased from Merck. Magnesium oxide and hexane solvent used were technical grade and other chemicals used for analysis were either of analytical grades.

2.2. Neutralization of PFAD
Neutralization of PFAD was carried out using a modified fusion method (Patent US 2,890,232). PFAD is preliminary heated in neutralization reactor at temperature 60-65°C. After completely melted, magnesium oxide (MgO) is added with a mole ratio PFAD:MgO of 1:1.1. After mixing evenly, then water added as a catalyst until the mixture expands. This reaction lasts for 5-7 minutes with stirring. The metal salt formed is Mg-PFAD salt.

2.3. Extraction
The Mg-PFAD salt produced from neutralization reaction was then extracted using hexane as an organic solvent. The mass ratio Mg-PFAD:hexane of 1:4. The extraction process is carried out in three steps with the same solvent at temperature 60-65°C for 45 minutes for each step. The solids and solvents in the mixture are then followed by filtration. The resulting extract then used to the next stage.

2.4. Batch Adsorption
Batch mode adsorption studies were carried out using a modified version of the method of Chu et al. [7-8]. Weighed amount of silica with silica mass ratio of 0.5 g/50 ml extract of known initial vitamin E concentration in covered Erlenmeyer flasks. The samples were agitated in an incubator shaker at 180 rpm and 25°C for 60 minutes. The contents of the flask were then filtered through 400-micron nylon mesh filter. Adsorption is carried out in two steps on the same extract. Silica saturated with vitamin E then mixed and shaken with isopropanol with silica mass ratio 1 g/50 ml solvent at 180 rpm and 40°C for 60 minutes. After desorption process, the content of the flask was filtered. The solution was collected and then solvents removed by rotary evaporation.

2.5. Analysis

2.5.1. Determination of FFA. The content of FFA was determined by titration method. A sample of 0.1 g was weighed and then mixed with solution containing with 25 ml of ethanol, 25 ml of chloroform, and three drops of phenolphthalein pH indicator in 250 ml Erlenmeyer flask and previously neutralized to pink color. This mixture is titrated with 0.1 N KOH to the same color using 25 ml burette. The resulting titrant volume is used to calculate acid number and FFA content in the sample.
2.5.2. Determination of total tocopherol. The content of tocopherol compounds was determined according to the colorimetric method described by Wong et al. [9]. In volumetric flask, the samples (0.2 g) was dissolved in 5 ml of toluene, then added 3.5 ml of 2,2-bipyridine (0.07%-w/v in ethanol) and 0.5 ml of FeCl₃·6H₂O (0.2%-w/v in ethanol). The solution was made up to 10 ml with ethanol. After standing for one minute, the absorbance at 520 nm was determined by using spectrophotometer (Genesis 10 VIS). A calibration curve of α-tocopherol in toluene was performed in a concentration range of 0-1000 ppm.

2.5.3. DPPH radical scavenging activity. The antioxidant activity in the sample was determined using 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) by the decolorization of methanol solution. DPPH (0.1 mM in methanol) was prepared and then 2 ml of this solution was mixed with 8 ml of sample with different concentration. The solution was then shaken and incubated at 30°C for 30 minutes in the dark. The absorbance was read at 517 against a blank. The radical scavenging activity was calculated as inhibition concentration percentage (%IC) according to the following equation:

\[
%IC = 100\left(\frac{Abs_{\text{blank}} - Abs_{\text{sample}}}{Abs_{\text{blank}}}\right)
\]

(1)

The IC50 values (the concentration of sample required for 50% inhibition of DPPH radicals) were obtained through extrapolation from regression analysis. The antioxidant was evaluated based on IC50 value.

3. Results and discussion

3.1. Effect of neutralization process

The physicochemical characteristic of PFAD and Mg-PFAD are shown in Table 1. Yield of Mg-PFAD produced from neutralization process was 1.16 g Mg-PFAD/g PFAD. Changes in the fatty components were reflected in acid number and FFA content, both of which lower in Mg-PFAD compared to PFAD due to the removal of FFA [6]. Neutralization of PFAD decreased acid number and FFA content in Mg-PFAD was 87.71% and 87.66%, respectively. No degradation of vitamin E during the process. However, there is still 12.34% of FFA content which had not been saponified in Mg-PFAD salt.

| Characteristic       | PFAD          | Mg-PFAD       |
|---------------------|---------------|---------------|
| Acid number (mg KOH/g sample) | 209.87 ± 1.52 | 25.84 ± 0.03  |
| FFA content (%-w/w)  | 95.61 ± 0.69  | 11.77 ± 0.02  |
| Vitamin E content (%-w/w) | 0.57 ± 0.01  | 0.48 ± 0.01  |

3.2. Effect of extraction process

The Mg-PFAD salt produced from neutralization was further extracted using hexane as an organic solvent. In this process, the percentage of vitamin E and FFA content in the extract in the process stages will be reviewed. Figure 2 shows the vitamin E and FFA extracted with three-stages process. The amount of extracted vitamin E and FFA increased with the addition of the extraction stage. Hexane solvent has the ability to extract vitamin E and remaining FFA in Mg-PFAD with three extraction steps up to 84.4% and 94.2%, respectively. Due to vitamin E and fatty acids are nonpolar compounds and hexane has low polarity [8], these compounds will be extracted with the solvent easily. Vitamin E is relatively more polar than FFA, which tends to make FFA extracted more than vitamin E [10]. However, the condition of Mg-PFAD which was homogeneously dispersed in hexane form favorable interaction between solvent and material and most of vitamin E extracted from Mg-PFAD salt. Neutralization followed by extraction process could concentrate vitamin E about 0.62%(w/w).
3.3. Effect of batch adsorption process

The extract produced from extraction process was further concentrated using batch adsorption with silica as adsorbent. Silica was polar adsorbent with large surface areas [10]. Table 2 shows uptake percentages (adsorption) and recovery percentages (desorption) of vitamin E and FFA at equilibrium. Since vitamin E is relatively more polar than hexane and FFA, it gets adsorbed to a polar adsorbent. Although FFA can be adsorbed into surface of silica, the resulting uptake of vitamin E was higher than FFA. Adsorption was an important step for purify vitamin E due to could adsorbed more vitamin E than FFA in the extract.

Table 2. Percentage uptake and recovery of vitamin E and FFA from batch adsorption process.

| Component | %uptake (mg component adsorbed/mg total component) | %recovery (mg component desorbed/ mg component adsorbed) |
|-----------|--------------------------------------------------|----------------------------------------------------------|
| Vitamin E | 98,0 ± 1,11                                       | 96,9 ± 0,14                                              |
| FFA       | 51,2 ± 0,90                                       | 13,7 ± 0,06                                              |

Same as adsorption process, recovery percentage of vitamin E was higher than FFA. FFA recovery percentage was lower than the uptake percentage indicating that lots of remaining FFA was still in surface of the adsorbent. High polarity isopropanol desorbed vitamin E molecules better from the adsorbent. Thus, Isopropanol solvent is more selective to release vitamin E than FFA from the adsorbent. This seems beneficial considering that FFA is another adsorbate that can be adsorbed besides vitamin E and its presence can affect the level of purity of vitamin E.

3.4. Final product overview

Vitamin E was mainly found in isopropanol solvent with high degree of recovery (>95%). This indicates that batch adsorption was successful in concentrating vitamin E in the samples. To produce the final concentrate, batch adsorption process followed by evaporation. Table 3 shows characteristic of pre-concentrate (concentrate from neutralization followed by extraction) and final product concentrate from a combination of neutralization, extraction, and adsorption methods.
Table 3. Characteristic of pre-concentrate and final product concentrate.

| Characteristic            | Pre-concentrate | Final product concentrate |
|---------------------------|-----------------|---------------------------|
| Visual concentrate        |                 |                           |
| Concentrate yield (mg/g PFAD) | 395.9          | 51.7                      |
| Vitamin E content (ppm)   | 1350 (0.62%-w/w) | 12282 (5.6%-w/w)         |
| FFA content (mg FFA/mg concentrate) | 0.20           | 0.09                      |
| IC50 (ppm)                | 247.9 (weak activity) | 23.3 (strong activity)   |

Removal of FFA in the neutralization process followed by extraction and batch adsorption greatly concentrated vitamin E in the sample compared with concentrate without adsorption process. There was a visual change from a solid yellow suspension to a thick brown concentrate. The concentration of final concentrate was obtained with vitamin E purity of 5.6% (12282 ppm) and the amount increased 9.2 times compared to pre-concentrate (0.62%). Moreover, there was a decrease in the FFA concentration 2.3 times compared to pre-concentrate. In terms of the antioxidant activity, IC50 value is a measure of the effectiveness of a substance inhibiting the oxidation. The lowest value of IC50 means the highest antioxidant activity due to lots of antioxidant molecules in the sample [11]. The IC50 value of <50 ppm indicates the sample has a strong antioxidant activity, whereas >150 ppm indicates has a weak antioxidant activity [12]. Thus, pre-concentrate has a weak antioxidant activity and final concentrate has a strong antioxidant activity due to the IC50 value of 23.3 ppm.

4. Conclusion
Neutralization process followed by extraction and batch adsorption process successfully concentrated vitamin E from PFAD. There was a change in antioxidant activity from weak (concentrate from neutralization followed by extraction) to strong activity (neutralization-extraction-batch adsorption method). The methods described in this study used low temperature (60-65°C) and in mild alkali conditions (neutralization process) which minimize degradation of vitamin E during separation. The methods were relatively simple and not energy-intensive compared to the existing methods such as molecular distillation. This combination method could be easily adapted to the existing process in industry for vitamin E purification with some modifications.

Acknowledgments
This research was funded by Badan Pengelola Dana Perkebunan Kelapa Sawit (BPDPKS) Indonesia. Another support by PT Tunas Baru Lampung Tbk for providing PFAD.

References
[1] Top A G M 2010 Production and utilization of palm fatty acid distillate (PFAD) Journal of Lipid Technology 22 (1) 11-13
[2] Damrongwattanakool N and Raviyan P 2018 Enrichment of vitamin E in palm fatty acid distillate using sequential-cooling urea-fatty acid complexation Songklanakarin J. Sci. Technol. 40 (5) 1175-1180
[3] Maarasyid C, Muhamad I I, and Suproyanto E 2014 Potential Source and Extraction of Vitamin E from Palm-Based Oil: A Review Jurnal Teknologi 69 (4) 43-50

[4] Posada L R, Shi J, Kakuda Y, and Xue S J 2007 Extraction of tocotrienols from palm fatty acid distillate using molecular distillation Journal of Separation and Purification Technology 57 218-227

[5] Top A G M, Leong L W, Ong A S H, Kawada T, Watanabe H, and Tsuchiya N 1993 Production of high concentration tocopherols and tocotrienols from palm oil by-product United States Patent 5 190 618.

[6] Chu B S, Baharin B S, Che Man Y B, and Quek S Y 2003 Separation of tocopherols and tocotrienols from palm fatty acid distillate using hydrolysis-neutralization-adsorption chromatography method Journal of Food Lipids 10 (2) 141-152

[7] Chu B S, Baharin B S, Che Man Y B, and Quek S Y 2004 Separation of vitamin E from palm fatty acid distillate using silica: I. Equilibrium of batch adsorption Journal of Food Engineering 62 97-103

[8] Chu B S, Baharin B S, Che Man Y B, and Quek S Y 2004 Separation of vitamin E from palm fatty acid distillate using silica: III. Batch desorption study Journal of Food Engineering 64 1-7

[9] Wong M, Trimms R, and Goh E 1988 Colorimetric determination of total tocopherols in palm oil, olein, and stearin Journal of the American Oil Chemists’ Society 65 258-261

[10] Chu B S, Baharin B S, Che Man Y B, and Quek S Y 2005 Comparison of selected adsorbents for adsorption and desorption of vitamin E from palm fatty acid distillate Journal of Food Lipids 12 23-33

[11] Jadid N, Hidayati D, Hartanti S R, Arraniry B A, Rachman R Y, and Wikanta W 2017 Antioxidant activities of different solvent extract of Piper retrofractum Vahl. using DPPH assay AIP Conference Proceedings 1854 020019

[12] Tristantini D, Ismawati A, Pradana B T, and Jonathan J G 2016 Pengujian aktivitas antioksidan menggunakan metode DPPH pada daun tanjung (Mimusops elengi L) Prosiding Seminar Nasional Teknik Kimia “Kejuangan” ISSN 1693-4393