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Effect of Solvent Ratio and Extraction Temperature on the Purity of Vitamin E from Palm Fatty Acid Distillate Extract

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Abstract. The presence of vitamin E in palm fatty acid distillate (PFAD) mixes with free fatty acids (FFA) so separation process is required. Vitamin E can be separated through a process technology that removes fat components and non-saponifiable components. The combination of separation process technology used are homogenization, neutralization and extraction. Extraction was carried out using multiple solvents including n-hexane, ethanol, and water to bind each component based on its polarity index. This study aims to determine the significant effect of solvent composition and extraction temperature on the yield and purity of vitamin E. The purity of vitamin E is strongly influenced by the variables, the lower the ethanol ratio and the higher the extraction temperature, the higher the yield and purity of vitamin E obtained. The highest yield of vitamin E to PFAD was 13.07%-w/w in the solvent composition n-hexane:ethanol:water 45:10:45 at 65◦C. A high purity vitamin E concentrate was obtained with the lowest acid number of 15.50 mg KOH/g sample and very strong antioxidant activity (IC_{50} value 17.07 ppm) on the composition of n-hexane:ethanol:water 40:20:40 at 60◦C.

Keywords: *free fatty acid; PFAD; polarity index; solvent extraction; vitamin E.*

1 Introduction

Vitamin E was found in crude palm oil with good antioxidant activity by tocopherol and tocotrienol compounds as antioxidants which inhibit the formation of free radicals and prevent various diseases such as cancer and premature aging of the skin. The amount of vitamin E increases five times as CPO is processed and refined into cooking oil which produces palm fatty acid distillate (PFAD) by-product. Vitamin E concentrations in PFAD obtained in Top [1] are 4000-6000 ppm. In addition, the price of PFAD as a by-product is quite low and its availability in Indonesia is quite large (reaching 2 million tons from 51.8 million tons CPO production) according to Indonesian Palm Oil Association [2].

This shows the potential of PFAD as a raw material for obtaining vitamin E. PFAD has a composition of bioactive components which are naturally mixed with free fatty acids. PFAD consists of 81.70-94.70% free fatty acids (FFA) and 1.40-14,40% glycerides mentioned in previous studies by Top [1]. To obtain vitamin E from PFAD, a separation process from free fatty acids is required so that the purity increases. Various separation process technologies in previous studies have been carried out to separate vitamin E including saponification, adsorption, distillation, esterification, enzymatic processes, and solvent extraction.

This research will examine the process of extracting vitamin E including the condition of the solvent composition used based on the polarity index of the solvent and the extraction temperature. PFAD as a raw material will be homogenized through dispersion with water to increase the contact area. Furthermore, it will be neutralized using an oxide, namely MgO to remove the fat component so that the Mg-PFAD salt is formed. The Mg-PFAD salt was then extracted using n-hexane, ethanol and water at various extraction temperatures. The results of the extraction will then be filtered to separate the solid salt and filtrate. Then the filtrate was evaporated to obtain vitamin E concentrate. The best extraction conditions were obtained with the characteristics of high yield of vitamin E, low acid number value, high total tocopherol content and concentrate with high antioxidant activity. The purpose of this study was to determine the significance of the influence of the solvent composition and extraction temperature variables on the yield and purity of vitamin E.

2 Material and Method

2.1 Material

The main raw material PFAD has slip melting point of 45-50◦C and acid value of 195.75 mg KOH/ g sample. The magnesium oxide and solvent used were technical grades, the material used for analysis were analytical grades.

2.2 Homogenization of PFAD

Homogenization of PFAD was carried out using dispersion of PFAD with water at a mole ratio between PFAD and water of 1:40 based on US Patent 4,235,794. PFAD is preliminary heated in reactor at temperature 60◦C. After completely melted, water is added to the reactor. The homogenization process was carried out in the reactor using a homogenizer at a speed of 6000 rpm for 4 minutes with a reactor agitator speed of 10-20 Hz.

2.3 Neutralization of PFAD

After homogenization, magnesium oxide (MgO) is added with a mole ratio PFAD:MgO of 1:1.1. The neutralization was a modified fusion method Patent US 2,890,232. Neutralization process carried out for 7 minutes with agitator stirring at 10-20 Hz until formed Mg-PFAD salt.

2.4 Extraction

The extraction carried out using hexane, ethanol and water as an organic solvent. Mg-PFAD salt produced from neutralization added to the reactor. The mass ratio Mg-PFAD:solvent of 1:4. The extraction process is carried out in one steps for 60 minutes with the solvent ratio and temperature variated. The variation used are shown in Table 1. After the extraction process complete, the mixture are then filtered. The solvent mixture evaporated to separate the solvent and vitamin E concentrate.

Table 1 Experimental design and research variations.

Run	Solvent Percentage (%)			Temperature(◦C)
	N-hexane	Etanol	Water	
1	20	60	20	30
2	20	60	20	60
3	40	20	40	30
4	40	20	40	60
5	33.33	33.33	33.33	45
6	45	10	45	25
7	45	10	45	65
8	15	70	15	25
9	15	70	15	65
10	33.33	33.33	33.33	45

2.5 Analysis

2.5.1 Determination of Acid Number Value

Total of free fatty acid in the sample was analyzed by titration method. A sample of 0.1 g was mixed with the solution. The solution containing ethanol and chloroform (25 ml for each solution), and phenolphthalein pH (three drops) then neutralized until the color change to pink. The sample mixture titrated with

0.1 N KOH. The titration volume of KOH used for calculate the acid value using Equation 1.

$$Acid\ Value\ = \frac{56.1\ \times Volume\ KOH\ \times KOH\ normality}{Sample\ mass} \tag{1}$$

2.5.2 Determination of total tocopherol

To determine the total tocopherol value in sample the colorimetric method by Wong et al. [3] are used. The sample solution was made up to 10 ml, that contains 0.2 g samples that was mixed in 5 ml of toluene, 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) with ethanol. The sample solution was then shaked for one minute, then analyzed in spectrophotometer to read the absorbance at 520 nm. The concentration range for calibration curve of α-tocopherol in toluene used was 0-1045 ppm.

2.5.3 Analysis of antioxidant activity

The antioxidant activity was evaluated based on IC50 value. To determine the value, analysis proceed by the decolorization of methanol solution. 2 ml of DPPH (0.1 mM in methanol) was mixed with 8 ml of sample. The solution was then shaked for one minute and incubated at room temperature for 30 minutes in the dark to see the color difference. The same thing was done with other samples with different concentration. Put the mixture in spectrophotometer and the absorbance was read at 517 nm against a blank. To obtain the IC50 value from regression, the inhibition concentration percentage (%IC) obtained by Equation 2.

$$\%IC = \frac{Blanko\ absorbance - Sample\ Absorbance}{Sample\ Absorbance} \tag{2}$$

3 Result and Discussion

The yield of Mg-PFAD salt resulting from the neutralization process is 1.66 g Mg-PFAD/g PFAD. Characteristic analysis of PFAD and Mg-PFAD are shown in Table 2. Total tocopherol in PFAD showed vitamin E content in the range of 0.48-0.6%-w/w according to the literature Top [1]. The value of the Mg-PFAD acid salt number resulting from neutralization decreased by 89.74% and the FFA value decreased by 88.81% from this preliminary process. The percentage of vitamin E content did not change indicating that vitamin E was

not degraded during neutralization. In the Mg-PFAD salt produced from this neutralization process there is still 11.20% of unsaponified free fatty acids. The free fatty acid content is lower than the research conducted by Lestari et al. [4] and Sari et al. [5] namely 21.52% and 12.34%. This is because in this study dispersing PFAD in water increased the contact area so that the mixing was more homogeneous and more materials reacted to become salt during the neutralization process.

Table 2 Characteristic analysis of PFAD and neutralized Mg-PFAD salt.

Parameters	PFAD	Mg-PFAD
Acid value (mg KOH/g sample)	195.75	20.07
FFA (%-w/w)	89.20	9.98
Vitamin E (%-w/w)	0.60	0.60

In the extraction process, product yield, FFA levels and tocopherol levels in the resulting extract will be reviewed. The yield is the ratio of the cumulative amount of the compound produced to the total mass of PFAD used. Yield of product mass to PFAD, FFA content and tocopherol content is shown in Table 3. High product yields were obtained in the combination of using ethanol solvents with a low ratio compared to water and n-hexane solvents.

Table 3 Characterization of extracted vitamin E concentrate.

Solvent Percentage (%)			T (◦C)	Yield (%-w/w)	FFA Level (%)	Tocopherol Level (%)
Hexane	Ethanol	Water				
20	60	20	30	1.69	0.54	0.78
20	60	20	60	4.80	0.96	2.21
40	20	40	30	1.41	0.16	3.55
40	20	40	60	5.52	0.04	16.98
33,33	33,33	33,33	45	6.70	0.39	10.41
45	10	45	25	7.33	1.70	6.46
45	10	45	65	13.07	0.72	18.69
15	70	15	25	2.13	0.92	0.55
15	70	15	65	1.55	0.58	0.29
33,33	33,33	33,33	45	2.78	0.23	4.64

The highest yield gain of vitamin E to PFAD was obtained when the extraction conditions were at a temperature of 65◦C and the percentage of n-hexane:ethanol:water was 45:10:45 resulting in a yield of 13.07%. The highest product mass gain was obtained at low ethanol solvent composition. This shows that the recovery of vitamin E concentrate decreased upon extraction with a large amount of ethanol (with a high polarity index polarity index 5.10) solvent composition. Tocopherol in vitamin E is non-polar so it will dissolve in solvents with a low polarity index such as n-hexane (polarity index 0.10). Based on the research conducted, decreasing the polarity of the mixed solvent will increase the recovery of vitamin E. This is in line with research conducted by Lestari *et al.* [4] which showed an increase in the acquisition of vitamin E in n-hexane solvents compared to ethanol and isopropanol solvents which have a high polarity index.

Increasing the extraction temperature causes an increase in the gain of vitamin E concentrate. This is because an increase in temperature will increase the diffusion coefficient and solubility of compounds with high antioxidants such as tocopherol in the solvent. In addition, an increase in temperature promotes the release of bound compounds by breaking down the cellular constituents of cells according to Wang *et al.* [6]. However, too high a temperature can degrade vitamin E. According to Chu *et al.* [7], vitamin E will be damaged in the temperature range of 180◦C-260◦C.

To determine the success of the extraction process, the purity of the resulting product needs to be observed. In this case, the levels of free fatty acids in the vitamin E concentrate are reviewed. The lower the FFA level in vitamin E, the higher the purity will be. The lowest FFA content in vitamin E concentrate was obtained at a temperature of 60◦C and the percentage of n-hexane:ethanol:water was 40:20:40 which was 0.04%. Under these conditions the tocopherol content was obtained at 16.98% with a concentration of 19.31 mg tocopherol/g sample. This shows an increase compared to research by Sari *et al*. [5], with the yield of vitamin E for 3-stage extraction and adsorption stages of 0.55 mg tocopherol/g sample. The products obtained from this study were vitamin E concentrate in the form of a viscous liquid, neutralized Mg-PFAD salt and extracted Mg-PFAD salt. Product visualization from this research can be seen in Figure 1.

(a) (b) (c)

Figure 1 Products gained through the process: (a) neutralized Mg-PFAD salt, (b) vitamin E concentrate, (c) extracted Mg-PFAD salt

The resulting vitamin E concentrate is a viscous liquid with a brownish yellow color. The appearance and color are affected by the high content of palmitic acid and oleic acid in PFAD. The neutralized Mg-PFAD salt and the extracted Mg-PFAD salt are produced in a yellowish white form in the form of hard solids such as granules, the total ethanol and hexane used in the extraction process are affecting the hardness of Mg-PFAD salt. The more ethanol used in the solvent ratio, the harder the extracted Mg-PFAD salt formed, this is similar to Lestari et al. [8] research on products extracted using ethanol due to the material not being mixed homogeneously with the solvent in the extraction process

3.1 Effect of Temperature and Solvent Ratio on Acid Number Value

The acid number is expressed as the number of milligrams of KOH needed to neutralize the free fatty acids present in one gram of oil or fat. The higher the FFA contained in the oil/fat, the greater the acid number obtained. The value of this acid number can be converted into the concentration of free fatty acids. Figure 2 shows the contour plot of the effect of temperature and extraction ratio on the acid number of the product obtained.

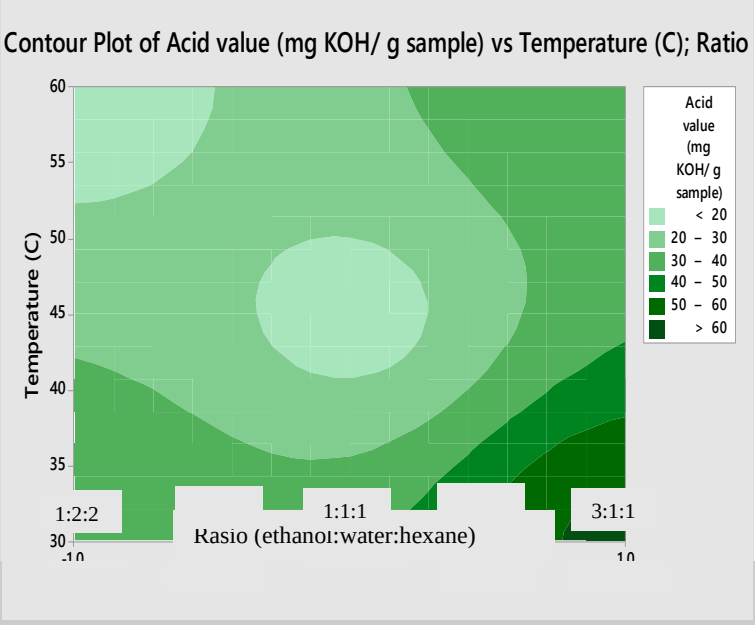

Figure 2 Contour plot solvent ratio and temperature to acid number value responses

Figure 2 shows a contour plot that provides a visual interpretation of the interaction between the two variables tested and acid number value responses. Different contour shapes represent the different interactions between variable. When there is complete interaction between the independent variables, the ellipse contours are formed. Results of this study shows that a low acid number value obtained in a condition at high temperatures (45°C and 60°C) where the ratio of ethanol was low with an acid number value of <20 mg KOH/g sample. This is related to the solubility of free fatty acids in the solvent used. In a study conducted by Choo *et al.* [9] showed that the solubility of palmitic and oleic fatty acids (the main component of PFAD) was greater in the ethanol fraction when the extraction was carried out using a mixed solvent of ethanol and n-hexane. The solubility of palmitic acid in ethanol was 34.58% and oleic acid was 33.75%. In another study, ethanol was used as a mixed solvent and could dissolve oleic acid well. The solubility of oleic acid in ethanol shows a greater number in the dissolved phase of 48.9% Estiasih *et al*. [10]. The effect of temperature and solvent ratio on the extraction process showed a significant effect on the acid number, and the interaction between the two variation factors show a significant effect (P value less than α=0.05). The lowest acid number is obtained at a temperature of 60◦C and the ratio of ethanol:water:n-hexane is 1:2:2 with an acid number value of 15.5 mg KOH/g sample. This value is lower than the previous study conducted by Sari

et al. [5] with a value for the concentrated acid number resulting from batch adsorption purification of 18.96 mg KOH/g sample.

3.2 Effect of Temperature and Solvent Ratio on Total Tocopherol

Optimal extraction conditions will help separate the tocopherol and tocotrienol components from free fatty acids and other compounds so that it is expected to increase the recovery of vitamin E. Figure 3 shows the contour plots of the effect of temperature and extraction ratio on the recovery of total tocopherol in the product.

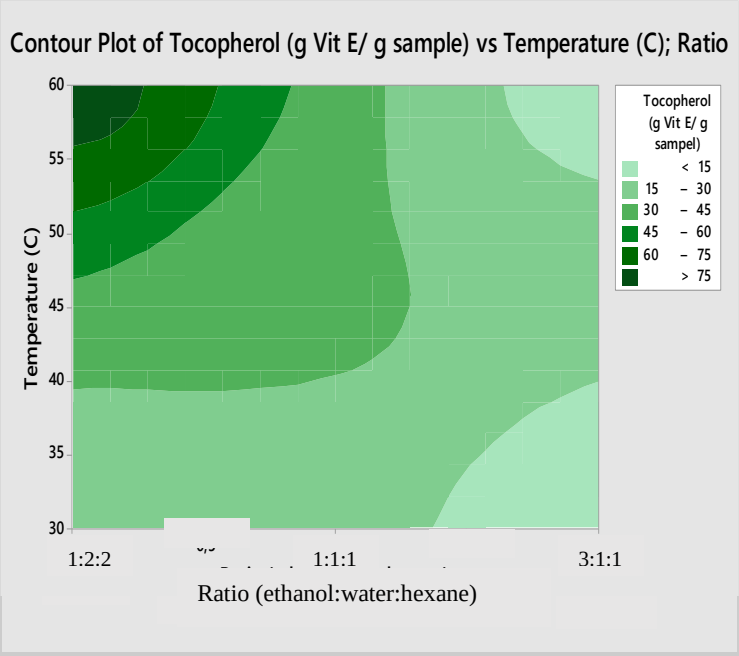

Figure 3 Contour plot solvent ratio and temperature to total tocopherol responses

The higher the temperature of the variation used and the lower the ratio of ethanol to n-hexane and water, the higher the total tocopherol in the product. From the contour plot, at 60°C and the ratio of ethanol: water: n-hexane was 1: 2: 2, a total mass of tocopherol was obtained > 75 g of tocopherol/g sample. The use of n-hexane solvent which is higher in the solvent ratio results in a high total tocopherol recovery, this is in line with the study of Sari *et al.* [5] where the concentration of total tocopherol in the concentrate extracted from n-hexane solvent is higher than that of ethanol. High total tocopherol in the product

indicates that the FFA content in the concentrate decreases or optimal separation of FFA by solvent occurs. The effect of temperature and solvent ratio and the interaction between the two variation factors on the extraction process showed a significant effect on the total tocopherol in the product.

3.3 Effect of Temperature and Solvent Ratio on IC_{50} Value

The percentage of antioxidant activity was obtained from the analysis results and resulted in the IC_{50} value from the regression results. The IC_{50} value is the effective concentration of the extract required to reduce 50% of the total DPPH. The lower the IC50 value, the better the antioxidant properties because it only requires a low concentration to absorb 50% of the free radicals present. According to Tristantini *et al.* [11], the strength of antioxidant activity is based on the IC_{50} value. IC_{50} value < 50 indicates very strong antioxidant activity, IC_{50} value of 50-100 indicates strong antioxidant activity and IC_{50} value> 100 indicates moderate to weak antioxidant activity.

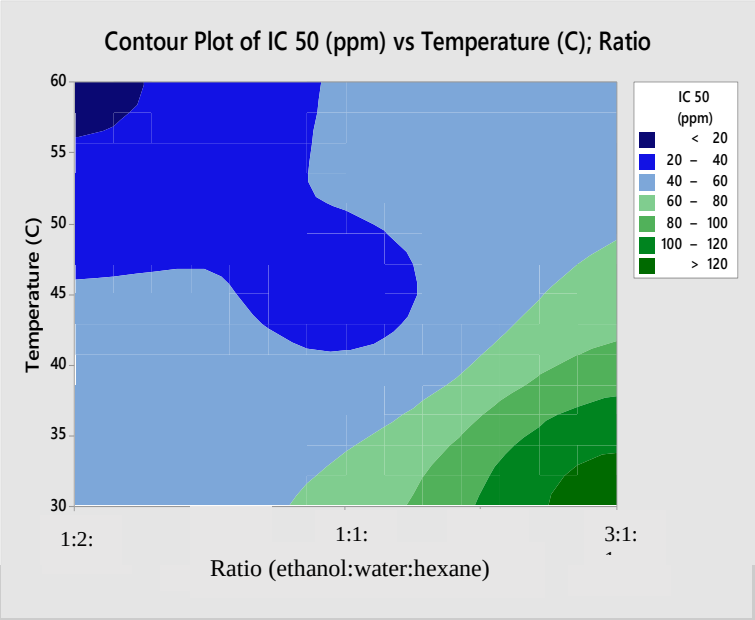

Figure 4 Contour plot solvent ratio and temperature to IC_{50} value responses

Figure 4 shows the contour plot of the effect of temperature and extraction ratio on the IC_{50} value. Very strong antioxidant activity (low IC_{50} value) was shown at a low ethanol ratio. The higher the temperature of the variation used and the lower the ratio of ethanol to n-hexane and water, the lower the IC_{50} value. In this study, the lowest final concentration of vitamin E was obtained with IC_{50} values ranging from 17.07 ppm with a single stage extraction process. This indicates that the

final concentrate of vitamin E in this study has stronger antioxidant activity than previous studies by Sari *et al*. [5] regarding the purification of vitamin E from PFAD using a batch neutralization, extraction, and adsorption process to obtain the final concentrate of vitamin E with an IC_{50} value of 23.3. The raw material, namely PFAD, has weak antioxidant properties with IC_{50} value obtained 489.77 ppm. Decreasing the IC_{50} value of the extracted product indicates that a series of processes from neutralization to extraction can increase antioxidant activity. From PFAD raw materials to concentrate products, there was an increase in antioxidant activity of 90%. The increase in antioxidant activity was due to the greater concentration of vitamin E contained in the sample along with the decrease in impurities or FFA in the material due to conditions of temperature variations and the ratio of solvents used. Low impurities such as FFA in concentrates can also increase antioxidant properties. The effect of temperature and solvent ratio on the extraction process showed a significant effect on the IC_{50} valuet, but he interaction between the two variation factors did not show a significant effect (P value greater than α=0.05)..

4 Conclusion

Solvent extraction carried out succeeded in increasing the purity of the vitamin E concentrate by reducing the total free fatty acids in the product. From PFAD as a raw materials to concentrate products, there was an increase in antioxidant activity of 90%. The use of several solvents can bind each component based on its polarity index. The purity of vitamin E is strongly influenced by the variables, the lower the ethanol ratio and the higher the extraction temperature, the higher the yield and purity of vitamin E obtained. The highest yield of vitamin E to PFAD was 13.07%-w/w in the solvent composition n-hexane:ethanol:water 45:10:45 at 65◦C. High purity vitamin E concentrate was obtained with the lowest acid number of 15.50 mg KOH/g sample and very strong antioxidant activity (IC_{50} value 17.07 ppm) on the composition n-hexane:ethanol:water 40:20:40 at 60◦C To determine the best process conditions, optimization will be studied using a response surface methodology analyzer in the the next paper.

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