

Antioxidant Activity of Vitamin E Concentrate from Magnesium Salts of Palm Fatty Acid Distillate (Mg-PFAD)

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Abstract

Vitamin E concentrate was produced through saponification of palm fatty acid distillates (PFAD) and magnesium oxide to form Mg-PFAD, followed by three-stages vitamin E extraction with isopropanol, hexane, or ethanol. The vitamin E-rich extracts were evaporated to remove solvent and produced vitamin E concentrate. The objectives of this research were to investigate the effect of organic solvent's types and solvent to Mg-PFAD mass ratios on vitamin E concentration, solvent selectivity, and antioxidant activity of the vitamin E concentrate. Vitamin E concentrates obtained after isopropanol extraction had vitamin E concentration of 784 ppm with vitamin E recovery of 16 mg tocopherol/100 mg tocopherol in Mg-PFAD, while vitamin E concentrates obtained after hexane extraction had vitamin E concentration of 574 ppm with vitamin E recovery of 35 mg tocopherol/100 mg tocopherol in Mg-PFAD. Isopropanol extraction produced vitamin E concentrate with the highest selectivity for vitamin E and the highest antioxidant activity of 79% IC. It was found that vitamin E concentration was not proportional to the antioxidant activity of the vitamin E concentrate.

Keywords: Direct solvent extraction, palm fatty acid distillate, saponification, vitamin E, unsaponifiable matter

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INTRODUCTION

National Institute of Health (2020) stated that human need to consume vitamin E for as much as 15 mg per day. As one of the countries with the largest population, Indonesia requires reliable resources to

supply and fulfill domestic demands for vitamin E. Vitamin E is contained in various plants and animals, but most of the commercial sources are naturally not available in Indonesia, such as almond, hazelnut, sunflower seed, wheat seed, cod fish, salmon, kiwi,

etc. Therefore, Indonesia imported 1900 tons of vitamin E in 2018 to fulfill its need (International Trade Center, 2018).

Vitamin E was found to be contained in palm fatty acid distillate (PFAD), a byproduct of crude palm oil deodorization process (Quek *et al.*, 2007, Top *et al.* 2010, Maarasyid *et al.*, 2014). PFAD is available in a great number in Indonesia as the largest producer of palm oil in the world. PFAD production is approx. 4% of the crude palm oil (CPO) (Kuvaini, 2016), or approx. 2 million tons from 51.8 million tons of CPO in 2019 (Indonesian Palm Oil Association, 2020). PFAD contains 0.1-0.4% vitamin E, consist of 70% tocotrienol and 30% tocopherol. High tocotrienol content in PFAD may also be beneficial, not only for the possibility of a more efficient isolation process of Vitamin E (Ngoc Doan, 2021), but also due to its antioxidant activity that is three times higher than tocopherol (Panfili *et al.*, 2013).

This research investigated antioxidant activity of vitamin E concentrate, which was extracted from PFAD via saponification with metal oxide prior to organic solvent extraction. Briefly, PFAD was saponified with metal oxide, e.g. magnesium (MgO), before the extraction stages. The result of PFAD saponification was magnesium salts of PFAD (Mg-PFAD) which was insoluble in most organic solvents and solidify at room temperature. The solubility characteristic of Mg-PFAD enables separation of vitamin E by solvent extraction, resulting in vitamin E rich extract. The objectives of this research were to investigate the effect of organic solvent's types and solvent to Mg-PFAD mass ratios on vitamin E concentration, solvent selectivity, and antioxidant activity of the vitamin E concentrate.

MATERIAL AND METHOD

Saponification of PFAD

The saponification of PFAD was conducted following the method from Listianingrum, *et al.* (2018) by modification. Briefly, about 1.5 kg PFAD was melted in the 15 L jacketted reactor at 60°C. MgO powder was added to the reactor with a molar ratio of MgO to PFAD of 1.1 mol/mol after the PFAD was melted. The mixture was mixed by using blade stirrer at the bottom part of the reactor. After the mixture became homogeneous, 10 mL of demineralized water was added as the catalyst of saponification reaction. The end of reaction was indicated by the formation of the solid magnesium salts of PFAD (Mg-PFAD) which occurred within 5 to 10 minutes.

Vitamin E Extraction from Mg-PFAD

The extraction was conducted by contacting about 100 g of Mg-PFAD salts and the extracting solvent (hexane, isopropanol, or ethanol) at 60°C for 45 minutes. The extraction temperature was adjusted by using a heating mantle. The variation of solvent to Mg-PFAD mass ratios (3 or 4 kg/kg) was chosen based on the result from the preliminary experiment (unpublished result). After the extraction was

completed, liquid extract was separated from the solid by decantation through filter paper. Depend on the state of Mg-PFAD and solvent mixture, the filtration between each stages was completed between 30 to 60 minutes. The solid was re-extracted with fresh solvent at the 2nd stage extraction, and the procedure was repeated for the 3rd stage extraction. Due to the long duration of filtration step, we only investigated the 2 and 3-stages of extraction. The liquid extract obtained from each stage of extraction was evaporated to remove the solvent. The solvent-free extract, which was to be stated as vitamin E extract concentrates, was weighed and analyzed for total tocopherol to express the vitamin E concentration, acid value to express the FFA concentration, and inhibitory concentration to express antioxidant activity.

Total Tocopherol Analysis

Total tocopherol content expressed the vitamin E concentration in the extract concentrates which was analyzed following the procedure from Wong *et al.* (1988). Briefly, standard p.a. tocopherol was diluted in toluene to a certain concentration to make a series of standard solution. Next, about 200 ± 10 mg of the solution was added by 5 mL of toluene, 3.5 mL of 2,2-bipyridine, 0.5 mL of FeCl₃·6H₂O solution, and ethanol p.a. in a 10 mL volumetric flask. The absorbance of each solution was measured using a spectrophotometer at a wavelength of 212 nm. The procedure was repeated to measure the samples of vitamin E extract concentrates in order to obtain the total tocopherol by plotting the absorbance values in the standard tocopherol calibration curve. Vitamin E concentration in extract concentrate (expressed in mg tocopherol/kg concentrate) was calculated using Equation 1. Recovery of vitamin E in extract concentrate from Mg-PFAD (expressed in mg of tocopherol/100 mg of tocopherol in Mg-PFAD) was calculated using Equation 2.

$$C_{\text{vitamin E}} = \frac{m_{c,t}}{m_c} \quad (1)$$

$$\text{Recovery of Vitamin E} = \frac{m_{c,t}}{m_{100 \text{ g Mg-PFAD},t}} \quad (2)$$

Acid Value Analysis

Acid value expressed the FFA content in the extract concentrates which was conducted following the method from Bockisch (1998). Briefly, about 0.1 g of sample weighed. The KOH-solvent solution was prepared in a burette to a certain volume. A neutral solution consisting of 25 ml of chloroform, 25 ml of solvent, and 3 drops of phenolphthalein was mixed in an Erlenmeyer flask. The neutral solution was neutralized with KOH-solvent by dropping it into the flask until it became slightly pink. Next, the 0.1 g sample was added to the flask then the neutralization continued. The volume of KOH-solvent used for titration was recorded to calculate the acid value using Equation 3.

$$\text{Acid Value} = \frac{56.1 \times V_{KOH} \times N_{KOH}}{m_s} \quad (3)$$

FFA concentrations in the sample and the amount of FFA in the concentrate were calculated using the Equation 4 and 5 using the acid value obtained from the previous step.

$$\%FFA \text{ in Sample} = \frac{Mr_{PFAD} \times \text{Acid Value}}{(1000 \times Mr_{KOH})} \quad (4)$$

$$m_{s,f} = \%FFA \text{ in Sample} \times m_s \quad (5)$$

The percentage of extracted FFA which expressed the amount of FFA contained in the concentrate of the amount of FFA in the 100 grams of Mg-PFAD can be calculated using Equation 6.

$$\%FFA \text{ Extracted} = \frac{m_{c,f}}{m_{100 \text{ g Mg-PFAD},f}} \quad (6)$$

The amount of tocopherol and FFA data obtained from each sample was used to calculate solvent selectivity using Equation 7.

$$\text{Solvent Selectivity} = \frac{m_{c,t} (mg)}{m_{c,f} (g)} \quad (7)$$

Antioxidant activity analysis of the vitamin E extract concentrate was conducted with the DPPH method from Tristantini, et.al. (2016). Briefly, 1 mL of vitamin E extract concentrate as the sample was added to 2 mL of 0.1 mM DPPH. Then, the mixture was shaken and incubated in a dark place at room temperature for 30 minutes. A blank solution was made with the same treatment with substituting the sample with ethanol p.a. The absorbance value of each solution was measured using a spectrophotometer at a wavelength of 516 nm. The obtained datas were used to calculate antioxidant activity with Equation 8.

$$\%IC = \frac{A_0 - A_s}{A_s} \quad (8)$$

RESULTS AND DISCUSSION

Effect of solvent type on yield and physical characteristic of vitamin E concentrates

Vitamin E extraction from Mg-PFAD soap using different solvent produced different characteristics, not only during processing but also as concentrate as the final product. The process characteristics describe the mixture behavior of the Mg-PFAD and solvent during extraction and extract concentration process. Concentrate characteristics consist of the concentrate visual attributes, vitamin E concentration and recovery, and also the concentrate yield from Mg-PFAD. Table 1 shows the comparison of vitamin E concentrate produced from three-stages

extraction using various organic solvents and solvent to Mg-PFAD mass ratio.

The recovery of vitamin E in the concentrates were slightly higher to the previous studies by Lestari, et. al. (2018). In previous studies using ethanol as extraction solvent, recovery of vitamin E were approx. 9-14.5% and yield of concentrates were approx. 27 g concentrate/kg PFAD. Therefore, this study can considerably be used as a reference for applications on a larger scale. The difference that occurred during the process was presumed to be due to the solvent-feed mixture. The resulting mixture of Mg-PFAD with isopropanol or ethanol was a rough suspension, in contrast to the result of Mg-PFAD and hexane mixture which was a thick homogeneous slurry-like solution. The mixture in the form of a rough suspension caused difficulty when stirring with an overhead stirrer, while a homogeneous solution can flow so that it was easily stirred. Mg-PFAD solid in the coarse suspension of ethanol variation had a harder, more compact texture and clump in the middle of the reactor, whereas the solid was soft and easier to stir in isopropanol variation.

Table 1. Comparison of vitamin E concentrate produced from extraction using various organic solvents and solvent to Mg-PFAD ratio.

Solvent Types Solvent to Mg- PFAD ratio (kg/kg)	Experiments' Variation					
	Hexane		Isopropanol		Ethanol	
	3	4	3	4	3	4
Results Comparison						
Behavior during Process	Homogeneous dispersion.		Soft solid suspension.		Hard solid suspension.	
Process Superiority	Solvent and feed contact was very good. Stirring was occurred very well.		Solvent and feed contact was adequate. Stirring was adequately occurred.		Solvent and feed contact was the worst. Stirring was hardly to be occurred.	
Separation Process Difficulty	Extract and raffinate separation was done for about 18 hours.		difficulties were not found.		Any considerable difficulties were not found.	
Visual of Concentrates	Yellow to orange solid.		Orange viscous liquid.		Yellowish solid suspension.	

Based on the behavior of the mixture of the three solvents, the best quality of contact with the solvent and the feed was given by hexane, isopropanol, and ethanol, respectively. However, the ease of separation after extraction was more likely to be obtained in ethanol and isopropanol variations. Behavior during the extraction process affected the downstream process of the whole process of extracting vitamin E from Mg-PFAD, namely the separation of vitamin E extracts from raffinate (Mg-PFAD). Extracts resulting from variations of ethanol and isopropanol were very easily separated from raffinate,

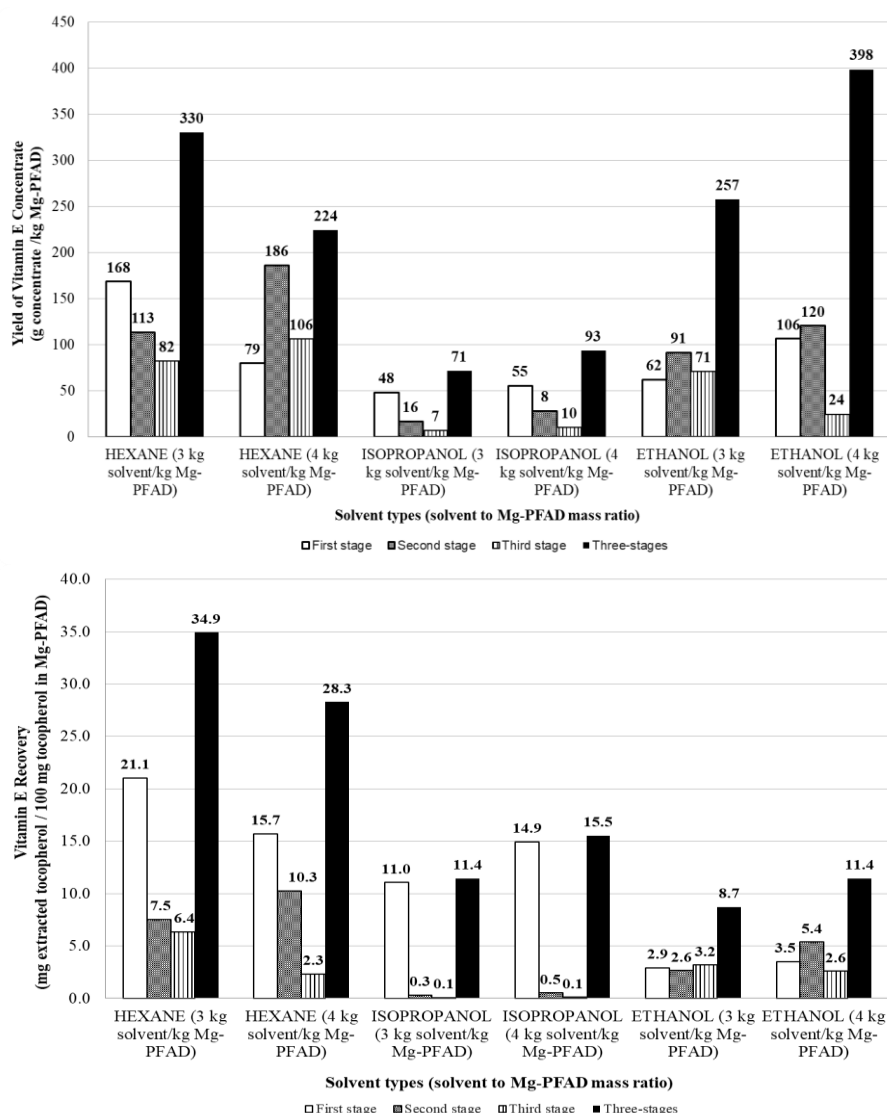


Figure 1. Effects of Organic Solvent, Solvent to Mg-PFAD Mass Ratio (kg/kg), and the Extraction Stage on the (a) Yield of Vitamin E Concentrate (g concentrate/kg Mg-PFAD), and (b) Recovery of Vitamin E

only requiring a simple decantation and filtration process using filter paper. Meanwhile, extracts from hexane variations cannot be decanted and must be filtered for about 18 hours.

The concentrates produced as the final extraction product of each variation differed in terms of its appearance and color, which was influenced by high palmitic acid and oleic acid content in PFAD. Extraction using ethanol produced concentrates which were similar to those produced in previous studies, which were liquid and solid phases. The liquid phase was yellow, had a high concentration of tocopherol, and a low FFA concentration, while the solid phase was white, had a low concentration of tocopherol, and a high FFA concentration. In contrast to extraction using ethanol, extraction using isopropanol produced homogeneous liquid phase concentrates, orange in color, and high viscosity. Meanwhile, the concentrations of hexane variations were found in a

yellowish-orange solid phase. The concentrates had color and turbidity that vary slightly at each stage, which may be indicated different composition of the extracted components.

Effects of Types of Organic Solvents, the Increase in Solvent to Feed Ratio, and the Number of Extraction Stages on the Solvent Selectivity

Solvent selectivity was calculated from the extracted tocopherol (vitamin E) to FFA mass ratio of the concentrate. The most suitable solvent should give the highest extracted tocopherol to FFA mass ratio to produce concentrate with high purity of vitamin E. Figure 2 shows the effects of organic solvent, solvent to Mg-PFAD mass ratio (kg/kg), and extraction stage on the solvent selectivity for tocopherol. In general, isopropanol showed the highest selectivity for tocopherol, followed by hexane, and ethanol.

Separate concentrates produced from the 1st, 2nd, and the 3rd stage of extraction using isopropanol and hexane showed gradual reduction of solvent selectivity to tocopherol. Ethanol selectivity for tocopherol fluctuated but the change seemed to be insignificant. An increase of solvent to Mg-PFAD mass ratio had an insignificant impact on isopropanol and ethanol solvents. On the other hand, the increase of solvent to Mg-PFAD mass ratio in hexane extraction decreased solvent selectivity, which may be caused by the high amount of extracted FFA.

According to Hu, et.al. (1996), oil extraction using alcohol as the solvent will produce a higher content of non-glyceride components, such as phosphatides, and unsaponifiable components due to the polarity of alcohol which is relatively high. Although the current research showed that the selectivity of isopropanol was higher than hexane, the same result was not obtained in ethanol variation.

The selectivity of ethanol was obtained in a smaller value than hexane. This can be caused by the solubility of oleic acid, one of the dominant free fatty acids in PFAD, which is higher in ethanol compared to isopropanol and ethanol. In addition, this can also be due to the physical nature of the ethanol concentrate which contained a high fat but low tocopherol solid phase that could physically be separated. The higher polarity of ethanol caused its affinity for fatty acids to be no higher or equal to hexane, which led to fatty acids separation as the concentrates were exposed to a lower temperature environment that occurred after the extraction process.

Antioxidant Activity of Vitamin E Concentrates

The antioxidant activity was carried out by measuring %IC (inhibitory concentration) at a 500 ppm of product concentrate, or as much as 500 mg concentrate in 1 L solution. Figure 3a shows antioxidant activity of concentrate expressed in %IC compared to vitamin E concentrations in the

concentrates (Figure 3b). According to Irshad, et. al., (2012), antioxidant activity of concentrate was proportional to the amount of antioxidant substances in the concentrate. However, this research showed that sample with the highest %IC was obtained in the concentrate produced from the 3rd stage extraction by using 3 kg isopropanol/kg Mg-PFAD with %IC of 79.2%, which had the lowest concentration of vitamin E of 29 ppm. This indicated that antioxidant activity of concentrate was not only dictated by vitamin E concentration but also other compounds, such as phytosterols and squalene. Phytosterols and squalene content in PFAD were 0.68% and 0.76%, respectively based on result from Pittoyo (1991), where the composition of these two compounds were greater than vitamin E in PFAD which was only 0.034%. In addition, phytosterols and squalene has a tendency to be more non-polar compared to vitamin E, so the increase in concentrations in concentrate will be proportional to the increase in free fatty acid concentrations which will increase the antioxidant activity of the concentrate.

In addition to this, approx. 70% of vitamin E in PFAD was in the form of tocotrienol. Although tocotrienol and tocopherol had similar respon during total tocopherol analysis, the antioxidant activity of tocotrienol was theroretically higher than tocopherol as proven in several studies by Serbinova & Packer (1994), Komiyama, et. al. (1989), Sundram, et. al. (1989). Based on comparative studies conducted by Serbinova, et. al. (1991), it was estimated that unsaturated hydrocarbon side chains of tocotrienols had better mobility and were less restricted in their interactions with radical lipids in membranes than tocopherols. As a result, the potential antioxidant activity of tocotrienol was thought to be higher than tocopherol. In addition, similar polarity properties can cause the distribution of tocotrienols and tocopherols in concentrates to be non-uniform.

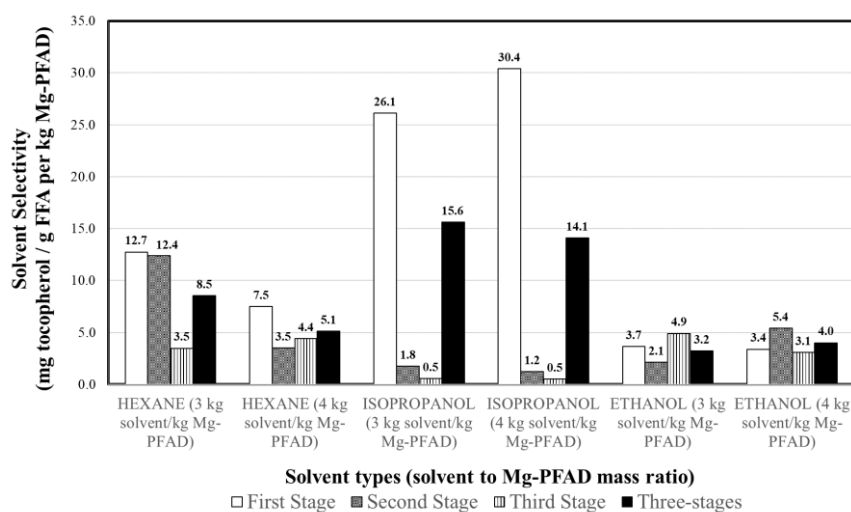
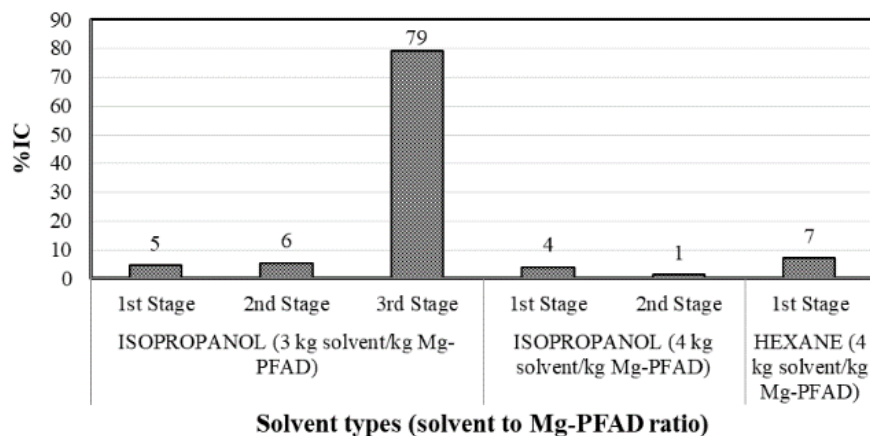
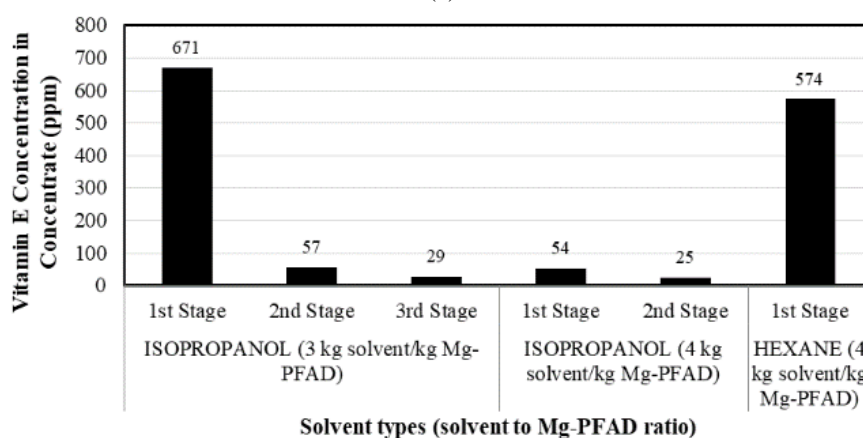


Figure 2. Effects of Organic Solvent, Solvent to Mg-PFAD Mass Ratio (kg/kg), and Extraction Stage on the Solvent Selectivity (mg tocopherol/g FFA in concentrate per kg Mg-PFAD)



(a)



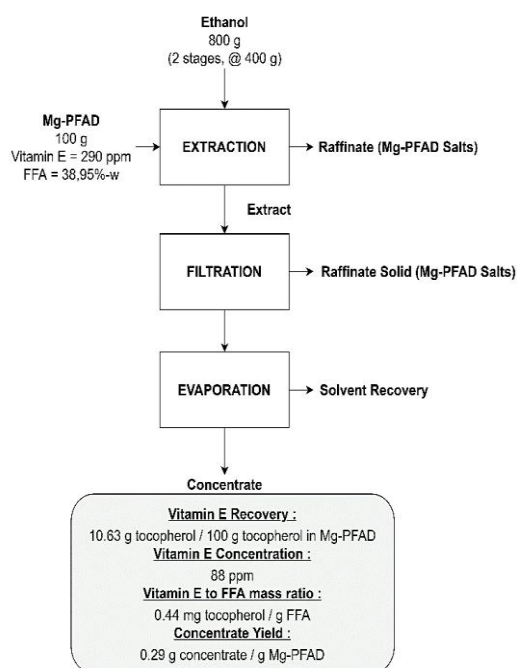
(b)

Figure 3. Antioxidant Activity (a) and Vitamin E Concentration (b) on Extract Concentrates After Three Stages Extraction Using Various Solvent

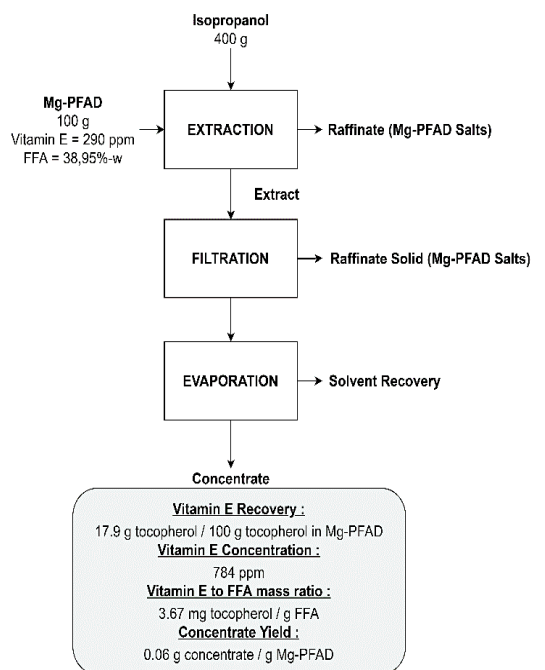
Recommendations for Vitamin E Extraction

Figure 4 shows several recommendations for larger scale of vitamin E extractions. Process parameters to be considered included high recovery, high purity of vitamin E, and ease of handling during processing. Apart from the aspect of product characteristics, the sustainability aspect of the process was also considered, especially which aimed for the minimal use of extraction solvent.

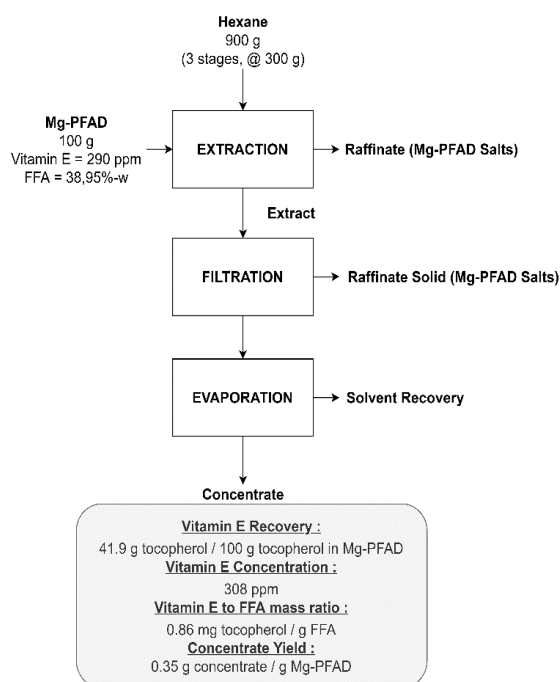
Among the studied variations, the best extraction method to produce vitamin E concentrate with the largest vitamin E recovery was the 3 stages extraction by using hexane with solvent to Mg-PFAD mass ratio of 3 kg solvent/kg Mg-PFAD. On the other hand, if the desired concentrate was the one with the lowest free fatty acid content and highest vitamin E content, then one stage extraction by using isopropanol with solvent to Mg-PFAD mass ratio of 4 kg solvent/kg Mg-PFAD may be chosen. Addition of the number of stages on the vitamin E recovery in isopropanol extraction had no significant effect on vitamin E recovery, so one stage was thought to be adequate.



(a)



(b)



(c)

Figure 4. Vitamin E Extraction from Mg-PFAD Block Flow Diagram with (a) Ethanol, (b) Isopropanol, and (c) Hexane Solvent

In addition, if the desired concentrate result was a vitamin E-rich concentrate with a physical characteristic that was most easily handled after the process, extraction with ethanol solvent can be chosen. Extraction with ethanol solvent using 4 kg solvent/kg Mg-PFAD in two stages may be most preferred

because it showed to produce relatively high concentrate yield. Technology development for scaling up vitamin E extraction from Mg-PFAD is necessary to commercialize vitamin E concentrate products. Therefore, the concentrate results of this study should be linked to market needs and compared with commercially available vitamin E products. The daily requirement of vitamin E per person is 15 mg per day, whereas according to Willy (2019), vitamin E supplement tablets generally contain 200 to 400 IU or around 134-2268 mg vitamin E per tablet. Vitamin E is also generally applied to beauty products, for example, anti-aging products that have vitamin E levels of 0.5 to 1%-w/w based on Keen & Hassan (2016). Based on this study, samples with the highest concentrations of vitamin E obtained were 784 mg vitamin E per kg concentrate which could be said to be too low for applications as supplement tablets and beauty products. Therefore, the extraction method and the results of this study can be utilized as an initial stage of the isolation of vitamin E which is then followed by other separation methods, such as adsorption (Quek et al, 2007, Sari et al, 2021) to increase the concentration of vitamin E which needs further investigation.

CONCLUSION

Based on this study, we concluded that the recovery of vitamin E increased with the decreased in solvent polarity. The highest recovery of vitamin E was obtained respectively in hexane, isopropanol, and ethanol variations in the amount of 15.7 - 34.9; 11.0 - 15.5; and 2.9 - 11.4 mg of tocopherol per 100 mg of tocopherol in Mg-PFAD. The highest concentration of vitamin E was inversely proportional to the amount of extracted FFA as shown by the comparable value of vitamin E concentrations in the concentrate with the selectivity of the solvent for vitamin E compared to FFA. The highest concentrations of vitamin E were obtained from variations of isopropanol (467-784 ppm) followed by hexane (307-574 ppm), and ethanol (92-136 ppm). The highest solvent selectivity was obtained from isopropanol, followed by hexane and ethanol, respectively 0.51 - 0.78; 0.31 - 0.57; 0.08 - 0.14 mg tocopherol per g FFA. The highest antioxidant activity (%IC) was obtained from variations of 3rd stage extraction by using 3 kg isopropanol/kg Mg-PFAD with %IC of 79.2%.

For further research, saponifying Mg-PFAD as an extraction feed that still contains relatively high amount of FFA can be done to maximize the saponification of FFA in order to prevent a low purity vitamin E concentrate result. Separation of solid phase obtained in the extracts resulting from ethanol solvent variation can be separated in order to produce vitamin E concentrates with higher purity. Extraction sequences by combining extraction solvents at different stages should be carried out to increase the recovery and concentrations of vitamin E.

NOTATION

A_o	= Blank absorbance
A_s	= Sample absorbance
$C_{\text{vitamin E}}$	= Vitamin E concentration (ppm)
IC	= Inhibitory concentration
$m_{100 \text{ g Mg-PFAD},f}$	= FFA mass in 100 g Mg-PFAD (g)
$m_{100 \text{ g Mg-PFAD},t}$	= Tocopherol mass in 100 g Mg-PFAD (mg)
m_c	= Concentrate mass
$m_{c,t}$	= Tocopherol mass in concentrate
$m_{c,f}$	= FFA mass in concentrate
Mr_{KOH}	= Molar mass of KOH (g/mol)
Mr_{PFAD}	= Molar mass of PFAD (g/mol)
m_s	= Sample mass
$m_{s,f}$	= FFA mass in sample
N_{KOH}	= Normality of KOH solution (N)
V_{KOH}	= Volume of KOH solution (mL)

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