

Radical Scavenging Capacity and Antioxidant Activity of the Vitamin E Extracted from Palm Fatty Acid Distillate by Sequential Cooling Hexane

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Abstract

The radical scavenging capacity and antioxidant activity of the vitamin E extracted from palm fatty acid distillate by the sequential cooling hexane were compared to those of the synthetic antioxidants including butylated hydroxyanisole (BHA), 2,6-di-tert-butyl-4-methylphenol (BHT), pyrogallol (PG), *tert*-butylhydroquinone (TBHQ) and commercial α -tocopherol. The extracted vitamin E contained 2,509.57, 9.24, 157.85, 6.70, 1,779.92, 3.97, 2,376.38, 818.80 and 7,662.45 ppm of α -, β -, γ -, δ - tocopherol and α -, β -, γ -, δ - tocotrienol, and total vitamin E, respectively. The maximum 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity of 20 ppm extracted vitamin E was 89%. The antioxidant activity determined by the methods of reducing power and ferric thiocyanate indicated that the 2.5 ppm extracted vitamin E could inhibit the oxidation of linoleic acid emulsion for more than 80% within 1 day. The superoxide scavenging activity of the extracted vitamin E was greater than those of the BHT, commercial α -tocopherol, and PG, respectively. The antioxidant activity of the extracted vitamin E was 91% which was similar to those of the BHT and synthetic α -tocopherol. The total-vitamin E remained 73% after heating at 190°C for 3 h. The tocopherol derivatives were more heat-stable than the tocotrienol derivatives while γ -tocopherol was the most heat-stable derivative. The concentration of total-vitamin E stored at 30°C for 120 days remained about 87% while the concentrations of vitamin E derivatives reduced by 2-20%.

Keywords: vitamin E, extraction, palm fatty acid distillate, scavenging capacity, antioxidant activity

1. Introduction

Several studies have shown that vitamin E exhibits various beneficial properties including antioxidant and anticancer properties (Jung & Min, 1990; Knekt et al., 1992; Maydani, 1995; Evans et al., 2002; Rossi et al., 2007). It performs most actively in scavenging free radicals and possesses the property of antioxidant which is effective in inhibiting free radicals in fat and fatty foods. Vitamin E is also able to reduce blood pressure and blood cholesterol, and prevent skin aging (Qureshi et al., 1988, 1991, 1996; Nesaretnam et al., 2007). Due to its numerous health related properties, vitamin E has been widely used in the manufacture of many health supplements, pharmaceutical products and cosmetics. In addition, vitamin E is widely used in animal feeds due to its antisterility properties (Eitenmiller & Landen, 1999; Gianello et al., 2005; Senevirathne et al., 2006). With the increasing demand for vitamin E, there is a pressing need to develop an efficient method, technique or process to produce sufficient quantities to meet the demand of the market.

Palm fatty acid distillate (PFAD) has been one of the interesting sources for natural vitamin E extraction (Goh et al., 1985; Sanagi et al., 2005). The PFAD is a by-product generated from the combination of high temperature heating approximately about 240-260°C, under vacuum of 2.66-6.66 mbar and direct steam injection of about 2.5%-4.0% by weight of oil at the deacidification-deodorization stage to remove the free fatty acids and odor in palm oil refining process (Leong, 1992). The distillate consists of 81.7% of free fatty acid, 14.4% of triglycerides, 0.8% of

squalene, 0.5% of vitamin E, 2.2% of sterol and others (Gapor et al., 2002). Ng et al. (2004) reported that distillate contains $3,889.2 \pm 165.3$ ppm of vitamin E, comprising 79.5% of tocotrienols and 20.4% of tocopherols. The dominant component of tocopherol and tocotrienol in distillate are α -tocopherol and γ -tocotrienol, respectively, while δ -tocopherol and β -tocotrienol are not found in distillate (Ng et al., 2004). However, Chu et al. (2003) reported that vitamin E in distillate is composed by 21.1, 21.2, 16.7 and 41.0% of α -tocopherol, α -tocotrienol, δ -tocotrienol and γ -tocotrienol, respectively. Purified palm oil contains total vitamin E at approximately 378-890 ppm (Top, 1990). Therefore, the main source of vitamin E is not the vegetable oil itself, but its distillate, a by-product from oil deodorization process (Hunt et al., 2000).

This study was conducted to compare the scavenging and antioxidant activities of vitamin E extracted from the PFAD by using the sequential cooling hexane with those of the synthetic antioxidants including BHA, BHT, PG, TBHQ and synthetic α -tocopherol. Heat and storage stabilities of the vitamin E derivatives were also investigated.

2. Materials and Methods

2.1 Materials

The PFAD samples produced on the same production batch were provided by the Chumporn Palm Oil Industry Public Company Limited, Chumporn province, Thailand. The samples were kept in the closed containers at $30 \pm 5^\circ\text{C}$ and used within 2 months. The BHA, BHT, PG, TBHQ and DPPH were purchased from the Sigma-aldrich. The other chemicals and reagents used for analysis were of analytical reagent grade.

2.2 Extraction of Vitamin E from the PFAD by the Sequential Cooling Hexane

The extraction of vitamin E was operated under the optimal condition by mixing the melted PFAD sample with the hexane at the ratio of 1:4 (w/v) and reducing temperature of the mixture sequentially to a series of pre-determined temperatures including 20°C , 15°C , 10°C , 5°C , 0°C , and -5°C . The solid fractions were removed from the mixture when the temperature of the mixture reaches each of the pre-determined temperatures; and removing the hexane from the mixture for recovering vitamin E from the mixture.

The obtained extracted sample was determined for the composition of vitamin E by analyzing the quantities of its derivatives comprising of total-tocopherol, total-tocotrienol and total-vitamin E using normal-phase HPLC method no. Ce 8-89 (AOCS, 1997).

2.3 Determination of the Physical Chemical Properties of the Extracted Vitamin E

The chemical properties of the extracted vitamin E, including iodine value, saponification value, acid value, peroxide value, volatile matter (hexane), iron, copper, lead, arsenic, palmitic acid, oleic acid, and linoleic acid, were determined in Laboratory Center for Food and Agricultural Products. The property of iodine value was tested with method no. Cd 1b-87 (AOCS, 1997) when the tests for properties of saponification value, acid value, and peroxide value were conducted with method no. 920.16, 969.17, 965.33, respectively (AOAC, 2000). Also, the property of volatile matter (hexane) was tested with in house method based on GC-MS with headspace technique, and the properties of iron, copper, lead, arsenic were measured with method no. 999.10 (AOAC, 2000). In addition, the properties of palmitic acid, oleic acid, and linoleic acid were determined with the method no. 996.06 (AOAC, 2000).

2.4 Determination of Scavenging Activity, Reducing Power and Antioxidant Activity of the Extracted Vitamin E

2.4.1 Scavenging Activity on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical

The extracted vitamin E was determined for the scavenging activity at concentrations from 1.25 to 700 ppm using the DPPH radical as described by Kim (2005) with modification. A volume of 4 ml of vitamin E fraction was dissolved in the mixture of chloroform and methanol in the ratio of 2:1 (v/v) and added to a solution of 1 ml of 0.2 mM DPPH radical in methanol. The mixture was allowed to react for 30 min at room temperature before determining degree of free radical scavenging activity by measuring the absorbance at 517 nm by a spectrophotometer (PerkinElmerTM, UV WINLAB version 2.85.04, UK). The capability of the scavenger on the DPPH radical was calculated using the following equation.

$$\% \text{ DPPH radical scavenging effect} = [(A_0 - A_1 / A_0) \times 100]$$

Where,

A_0 = the absorbance at 517 nm of the control sample, in which the sample treated without adding vitamin E fraction

A_1 = the absorbance at 517 nm of the vitamin E sample

2.4.2 Reducing Power

The reducing powers of the vitamin E fraction at different concentrations (0.00, 1.25, 2.50, 5.00, 10.00 and 20.00 ppm) were determined by a method of Kim (2005) with modification. To 0.1 ml of the sample solution, the 0.25 ml of 0.2 M phosphate buffer at pH 6.6 and 0.25 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] were mixed and incubated at 50°C for 20 min. Then, 0.25 ml of 10% trichloroacetic acid was added to the mixture, before it was centrifuged for 10 min at 3,420 rpm, 25°C. Next, 0.25 ml of the upper layer of the solution was mixed with 0.25 ml of distilled water and 0.05 ml of 1% ferric chloride [$FeCl_3$]. The obtained solution was measured for the absorbance at 700 nm. The high absorbance indicated increasing of the reducing power.

2.4.3 Antioxidant Activity

The antioxidant activity was determined by the ferric thiocyanate (FTC) method. The antioxidant activities of the vitamin E fraction at various concentrations (0.0, 2.5, 5.0, 10.0 and 20.0 ppm) on the inhibition of linoleic acid peroxidation was assayed by a modified method of Kim (2005). The 0.25 ml of the vitamin E fraction at different concentrations was mixed with 2.5 ml of 0.02 M linoleic acid emulsion at pH 7.0 and 2 ml of 0.2 M phosphate buffer at pH 7.0. The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20, and 50 ml of phosphate buffer. Then, the mixture was incubated at 37°C for 6 days. To 0.1 ml of the reaction mixture at 24 h intervals, 4.7 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate, 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl were added. The peroxide value was determined to evaluate the degree of oxidation by measuring the absorbance at 500 nm. The percentage of inhibition of the linoleic acid peroxidation was calculated for each concentration of vitamin E fraction by using the following equation.

$$\% \text{ Inhibition} = 100 - [(A_1/A_0) \times 100]$$

Where,

A_0 = the absorbance at 500 nm of the control sample, in which the sample treated without adding vitamin E fraction

A_1 = the absorbance at 500 nm of the vitamin E sample

2.4.4 Analysis of the Malonaldehyde

The thiobarbituric acid (TBA) test was conducted on the final incubation day (5th day) based on a method described by Kim (2005) to determine the malonaldehyde formation from linoleic acid peroxidation. The sample was prepared as previously described in the FTC method. To 1.0 ml of sample solution, 2 ml of 20% trichloroacetic acid and 2 ml of thiobarbituric acid solution were added. The mixture was then boiled and cooled to about 25°C. After centrifugation of the mixture for 20 min at 4,000 rpm, 25°C, the absorbance of the supernatant was measured at 532 nm.

2.5 Comparison of the Superoxide Radical Scavenging Activity, Antioxidant Activity and Malonaldehyde Inhibitions of the Extracted Vitamin E with the Commercial α -tocopherol and the Synthetic Antioxidants

2.5.1 Superoxide Radical Scavenging Activity

The superoxide radical scavenging activities of the extracted vitamin E, α -tocopherol, BHA, BHT, PG and TBHQ in linoleic acid emulsion were determined based on the method of Kim (2005) with modification. The linoleic acid emulsion was prepared as previously described in the FTC method. The reaction mixture contained 1.2 ml of 0.05 M sodium carbonate buffer adjusted to pH 10.5, 0.1 ml of 3 mM xanthine, 0.1 ml of 3 mM ethylenediaminetetraacetic acid disodium salt [$EDTA_2Na$], 0.1 ml of 0.15% bovine serum albumin, 0.1 ml of 0.75 mM nitroblue tetrazolium chloride (NBT), and 0.1 ml sample solution. After holding at 25°C for 10 min, the reaction process was started by adding 6 mU of xanthine oxidase (XOD) and maintained at 25°C for another 10 min. Next, the reaction was stopped by adding 0.1 ml of 6 mM $CuCl_2$. The absorbance of the obtained mixture was measured at 560 nm. The inhibition rate was calculated by measuring the amount of the formazan formed from NBT in the presence of superoxide using the following equation.

$$\% \text{ Superoxide radical scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

Where,

A_0 = the absorbance at 560 nm of the control sample, in which the sample treated without adding vitamin E fraction;

A_1 = the absorbance at 560 nm of the vitamin E sample.

2.5.2 Antioxidant Activities

The antioxidant activity was measured by the FTC method. The antioxidant activities of different concentrations (0.0, 2.5, 5.0, 10.0 and 20.0 ppm) of the extracted vitamin E were compared to those of the commercial α -tocopherol and the synthetic antioxidants by determining the inhibition of linoleic acid peroxidation (Kim, 2005).

2.5.3 Malonaldehyde Inhibition

The TBA Test was performed to evaluate the malonaldehyde inhibition. The malonaldehyde inhibitions of different concentrations (0.0, 2.5, 5.0, 10.0 and 20.0 ppm) of the extracted vitamin E were compared to those of the commercial α -tocopherol and the synthetic antioxidants by determining the inhibition of linoleic acid peroxidation (Kim, 2005).

2.6 Heat and Storage Stabilities

2.6.1 Heat Stability

A volume of 5 ml of the extracted vitamin E was transferred into a 10 ml vial that was flushed with nitrogen gas before capping. The vial was submerged into the hot silicone oil controlled at $190 \pm 2^\circ\text{C}$ for 0, 1, 2, 3, 4, 5 and 6 h (Nystrom et al., 2007). After heating, the sample was immediately cooled and analyzed for the quantities of vitamin E derivatives, total-tocopherol, total-tocotrienol and total-vitamin E by using the normal-phase HPLC.

2.6.2 Storage Stability

The studied samples were stored in an incubator controlled at $30 \pm 2^\circ\text{C}$ and at $65 \pm 5\%$ of relative humidity for 120 days (U.S. Department of Health and Human Services, 2003). The quantities of the vitamin E derivatives, total-tocopherol, total-tocotrienol and total-vitamin E were determined each 15 days using the normal-phase HPLC.

2.7 Experimental Design and Statistical Analysis

The experiment was carried out in triplicate ($n = 3$) with 2 measurements for each replication and the data was reported as mean \pm standard deviation. The data collected from the experiments was analyzed by using the analysis of variance (ANOVA). The Duncan's New Multiple Range Test (DMRT) was applied to the test significant difference of means at the level of $P \leq 0.05$.

3. Result and Discussion

3.1 Physical Chemical Characterization of the Extracted Vitamin E

The vitamin E extracted from the PFAD by the sequential cooling method was used to determine the scavenging activity, antioxidant activity and stability. The vitamin E mixture was analyzed to determine the components by using the normal-phase HPLC. The study found the mixture contained α -, β -, γ - and δ -tocopherol, α -, β -, γ - and δ -tocotrienol and total-vitamin E of 2,509.57, 9.24, 157.85, 6.70, 1,779.92, 3.97, 2,376.38, 818.80 and 7,662.45 ppm, respectively. The ratio of total-tocopherol and total-tocotrienol were approximately 3:7. The vitamin E sample contained an iodine value of 90 g/100g, saponification value of 189.97 mg KOH/g, acid value of 206.70 mg KOH/g, peroxide value of 0.28 m EqO₂/kg, volatile matter (hexane) >100 ppm, iron of 8.58 mg/kg, copper of 0.56 mg/kg, lead < 0.12 mg/kg and arsenic of 0.088 mg/kg. The sample also contained some other fatty acids such as palmitic acid, oleic acid and linoleic acid at 11.94, 65.64 and 15.70 g/100 g, respectively.

3.2 Bioactive Characterization of the Extracted Vitamin E

3.2.1 Scavenging Activity on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical

Figure 1 shows that the DPPH scavenging effect increased with the increasing concentration of vitamin E in the range of 1.25-20.00 ppm. The concentration of 20.00 ppm was the lowest concentration providing the highest DPPH scavenging effect of $89.00 \pm 0.69\%$, which was enough for performing the activity as effective antioxidant.

The scavenging activities of vitamin E extracted from other sources have been reported. The vitamin E extracted from rice bran by the hydrolysis method using 80% KOH required the concentration of 160 ppm of vitamin E to scavenge the DPPH radical which was equal to the DPPH scavenging effect of $95.17 \pm 0.284\%$ (Kim, 2005). The rice bran vitamin E studied by Kim (2005) contained α -tocopherol, γ -tocopherol, α -tocotrienol and γ -tocotrienol of 68.29, 40.05, 78.71 and 98.24 ppm, respectively. The concentrations of these four derivatives were lower than those obtained in this study. As a result, a higher concentration of vitamin E extracted from rice bran was required for the highest DPPH scavenging effect. The DPPH scavenging effect found in this study was also higher than the one reported by Igbal et al. (2008). In addition, Igbal et al. (2008) reported that vitamin E

extracted from various kinds of wheat bran in Pakistan achieved only 51-79% of the scavenging activity of the DPPH radical. The reason for the low activity might be because vitamin E extracted from wheat bran contained only 22-26 ppm of tocopherols and 59-74 ppm of tocotrienols.

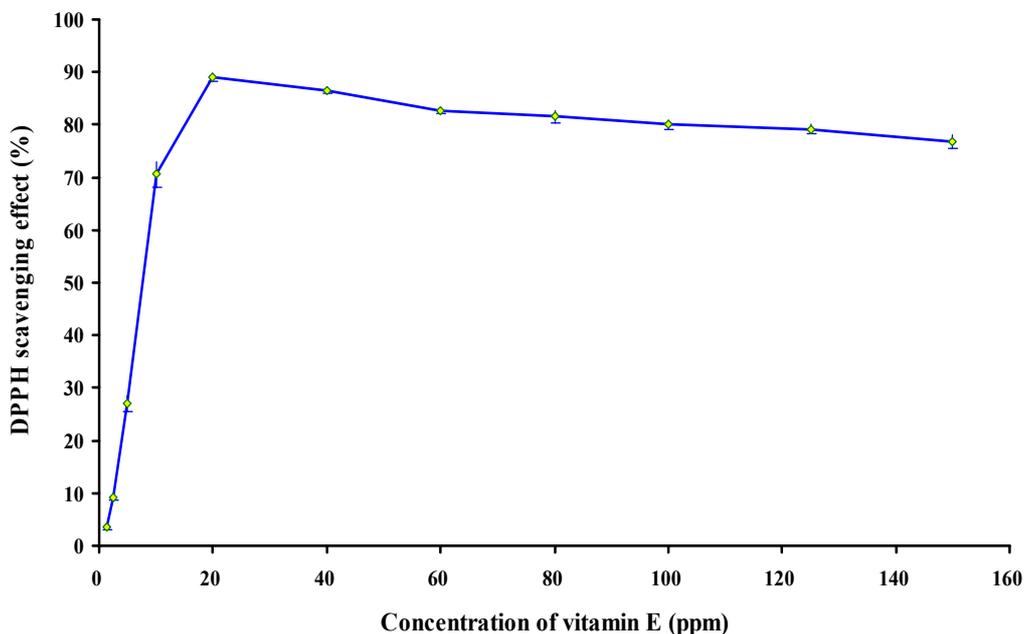


Figure 1. Radical scavenging activity (%) of vitamin E extracted from PFAD on the DPPH free radicals

3.2.2 Reducing Power

The reducing powers were determined by using various concentrations of vitamin E. The reducing powers of vitamin E significantly increased with increasing vitamin E concentrations ($P \leq 0.05$) as shown in Figure 2. Compared to the study of Kim (2005), the vitamin E extracted from rice bran required the concentration of 160 ppm to provide the equal reducing power to the 20 ppm vitamin E obtained from this study.

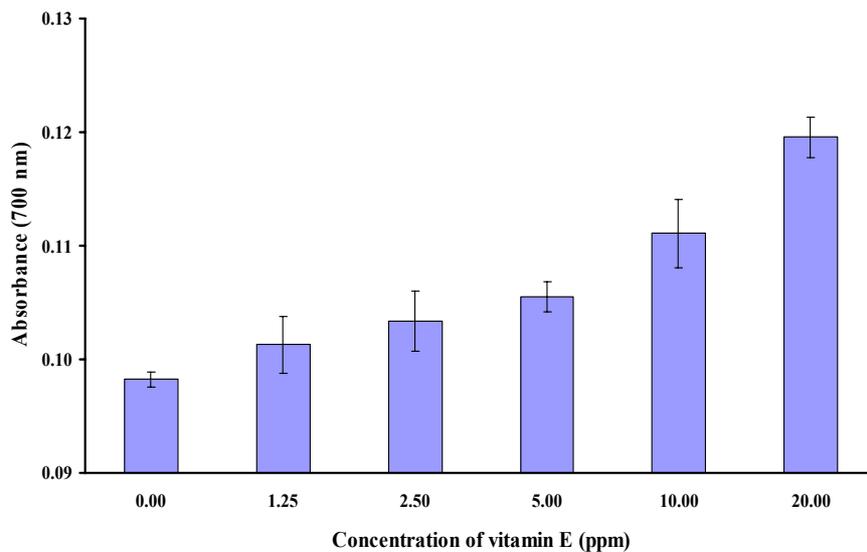


Figure 2. Reducing power (UV absorption at 700 nm) of vitamin E extracted from PFAD

3.2.3 Malonaldehyde Formation from Linoleic Acid Peroxidation

Figure 3 presents the relationship between the absorbance of the vitamin E samples prepared at various concentrations on the linoleic acid peroxidation during storage. The increasing of the absorbance indicated higher malonaldehyde formation, or the lower inhibition of linoleic acid peroxidation. During the first period of autoxidation of linoleic acid, the peroxide level increased sharply during days 1-5 of storage when vitamin E was not added. After day 5, the peroxide decreased as it changed to malonaldehyde, the secondary oxidation product. The higher the absorbance means, the lower the level of the antioxidant activity. However, the emulsion of linoleic acid added with vitamin E provided lower peroxide than the sample without adding vitamin E. The increasing concentrations of vitamin E from 2.50 to 20.00 ppm in the emulsion caused slower rate of oxidation of linoleic acid with no statistical significance ($P > 0.05$).

Figure 4 illustrates the relationship of peroxide inhibition of the vitamin E prepared at various concentrations. The peroxide inhibition increased as a result of increasing vitamin E concentration. The peroxide inhibition sharply increased in 1 day and could inhibit the peroxide formation more than 80%. At day 3, the highest inhibition was achieved, with 96.94 ± 0.65 , 98.33 ± 0.38 , 98.42 ± 0.16 and $98.79 \pm 0.37\%$ at the concentrations of 2.50, 5.00, 10.00 and 20.00 ppm, respectively. The peroxide inhibition during days 1-3 for each vitamin E concentration was different with statistical significance ($P \leq 0.05$) while no significant differences ($P > 0.05$) were observed during days 4-6. Nevertheless, vitamin E concentration of only 2.50 ppm was able to inhibit more than 80% of the peroxide in 1 day.

The correlation between peroxide inhibition and reducing power was found that the reducing power increased when the concentration of vitamin E increased, which was shown by the increasing UV absorption values at 700 nm in Figure 2, which resulted in the increasing percentage of peroxide inhibition. The relative coefficient (R^2) between the reducing power and the peroxide inhibition was 0.8099. This means that the reducing power of the extracted vitamin E was considerably related to peroxide inhibition.

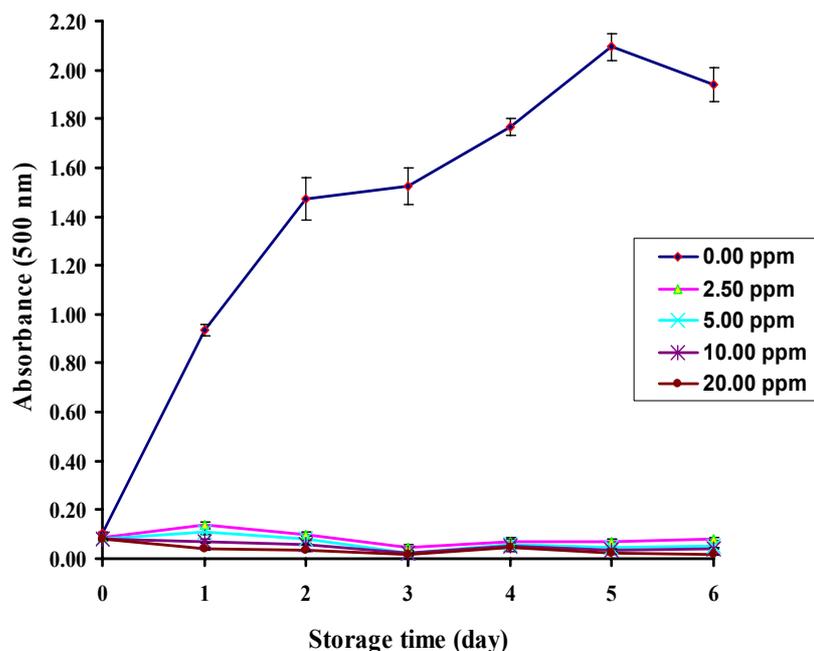


Figure 3. Capability of vitamin E extracted from PFAD to counteract the linoleic acid peroxidation during storage

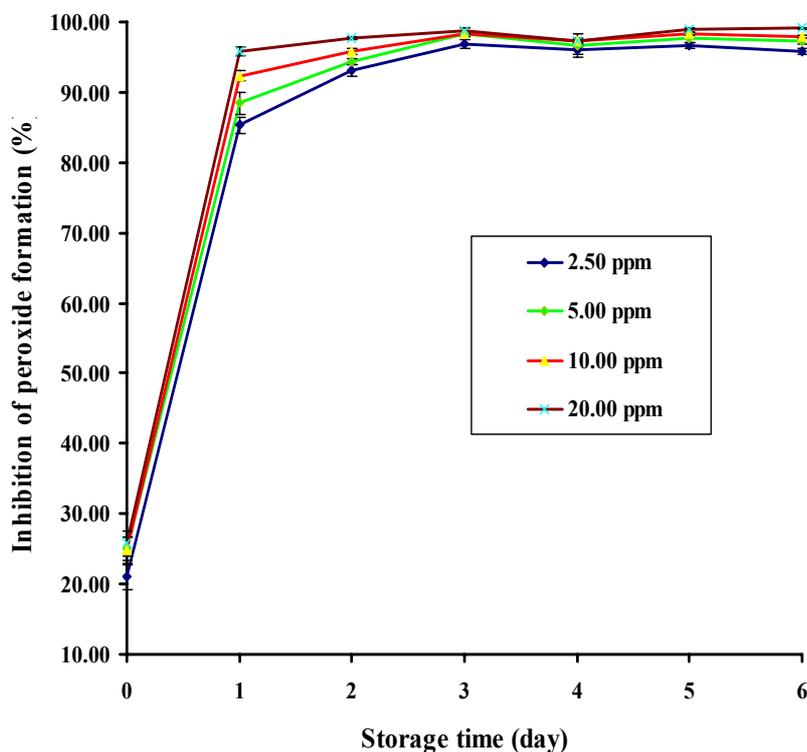


Figure 4. Inhibition of peroxide formation by vitamin E extracted from PFAD at different concentrations under storage conditions

The UV absorption at 532 nm could refer to malondehyde produced in the oxidation of linoleic acid which contained quite high UV absorption at 532 nm. That means a large amount of malondehyde was produced in the oxidation of linoleic acid and contained a decreasing ability as an antioxidant as shown in Figure 5. The experiment found that the increase in concentration of vitamin E indicated the decrease in UV absorption. This means that the increase in ability as an antioxidant demonstrated the decrease in quantity of malondehyde. The vitamin E concentrations of 2.50, 5.00, 10.00 and 20.00 ppm contained a high capability in antioxidation or malondehyde inhibition without statistical significance ($P > 0.05$). It was concluded that vitamin E extracted from PFAD at the concentration of 2.50 ppm can inhibit malondehyde similarly to the higher quantity used. Though the use of higher concentration than 2.50 ppm resulted in the decreasing of malondehyde, but it decreased without statistical significance ($P > 0.05$).

According to Kim (2005), vitamin E extracted from rice bran by the hydrolysis method at the concentrations of 2.50, 10.00, 40.00 and 160.00 ppm provided the UV absorption at 532 nm of 0.225, 0.175, 0.155 and 0.050, respectively. While the vitamin E extracted from PFAD in this study at the concentrations of 2.50, 5.00, 10.00 and 20.00 ppm provided the UV absorption with no statistical significance ($P > 0.05$) as shown in Figure 5. Therefore, it could be concluded that vitamin E extracted from PFAD at the concentration of 2.50 ppm was able to inhibit the malonaldehyde reaction similarly to vitamin E from rice bran at the concentration of 160.00 ppm.

3.2.4 Comparison of the Superoxide Radical Scavenging Activity, Antioxidant Activity and Malonaldehyde Inhibitions of the Extracted Vitamin E with the Commercial α -tocopherol and the Synthetic Antioxidants

3.2.4.1 Superoxide Radical Scavenging Activity

The superoxide radical scavenging property of the extracted vitamin E was compared to those of the commercial α -tocopherol, BHA, BHT, THBQ and PG at the concentration of 20 ppm. Figure 6 presents that the superoxide radical scavenging properties of the test sample varied with a statistical significance ($P \leq 0.05$). In general, the extracted vitamin E possessed high superoxide radical scavenging property and was similar to those of the TBHQ, BHA and BHT. The high superoxide radical scavenging property could be due to the fact that the

vitamin E from PFAD contained as high as 65% tocotrienols, especially γ - and α -tocotrienol. The pronounced inhibiting of superoxide radical of the tocotrienols has been reported (Kim, 2005).

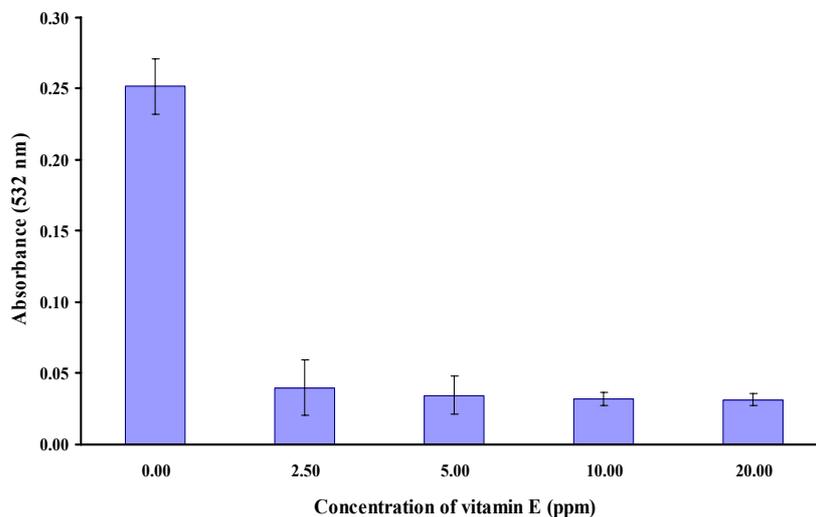


Figure 5. Absorbance of the extracted vitamin E obtained from the thiobarbituric acid method

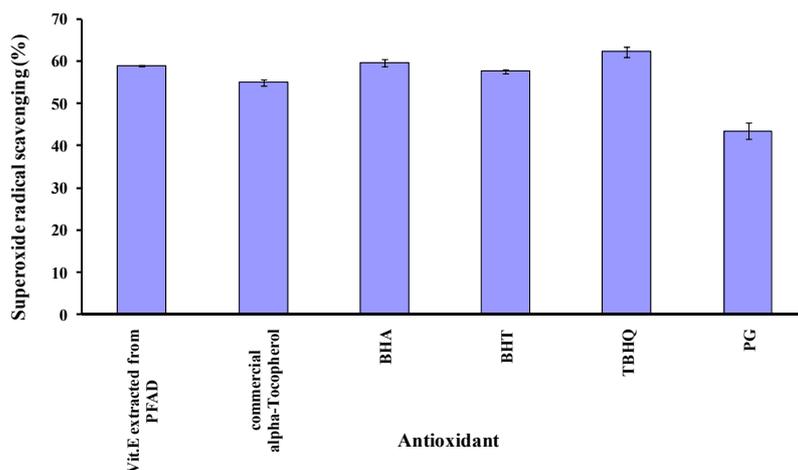


Figure 6. Superoxide radical scavenging activities of the extracted vitamin E from PFAD in comparison to others antioxidants

3.2.4.2 Antioxidant Activity

The result of UV absorption at 500 nm showed the peroxide measured by ferricthiocyanate (Figure 7). If the UV absorption at 500 nm was low, it referred to a high antioxidative property. The experiment found that peroxide, in the first period of autoxidation of linoleic acid emulsion without the addition of vitamin E from PFAD and synthetic antioxidants, sharply increased during days 1-5 of storage. As for the sample of linoleic with vitamin E added from PFAD and synthetic antioxidants, it provided a different peroxide quantity with statistical significance ($P \leq 0.05$) during days 1-2. However, during days 3-6, it provided a different quantity of peroxide with no statistical significance ($P > 0.05$) as linoleic acid system with vitamin E added and synthetic antioxidants provided low peroxide.

At the concentration of 20.00 ppm, the result of antioxidant property revealed that the TBHQ contained the highest antioxidant potential, while BHA, BHT, extracted vitamin E, commercial α -tocopherol and PG contained slightly less activity, respectively (Figure 8). From Figure 9, the antioxidant activity of the 20.00 ppm extracted

vitamin E was higher than those of the commercial α -tocopherol and PG ($P > 0.05$). Low UV absorption at 532 nm referred to a high ability to inhibit malonaldehyde formation. The antioxidants containing high to low malonaldehyde inhibition were the BHA, TBHQ, BHT, the extracted vitamin E, the commercial α -tocopherol, and PG, respectively. The concentration of malonaldehyde in each sample was compared to that obtained from the ferric thiocyanate method to find the relation of the two experiments. Vitamin E from the PFAD contained more intensity in inhibiting linoleic acid oxidation than the commercial α -tocopherol and PG but provided lower ability in inhibiting linoleic acid oxidation than those of the TBHQ, BHA, and BHT. This discrepancy may result from higher tocotrienols in the extracted vitamin E or the synergistic effect of tocopherols and tocotrienols. The tocopherols and tocotrienols were able to interfere in radical chain reaction effectively by providing phenolic hydrogen and lipid peroxy radical. However, the antioxidant activity of tocopherols and tocotrienols may be the difference in food and organism (Kim, 2005).

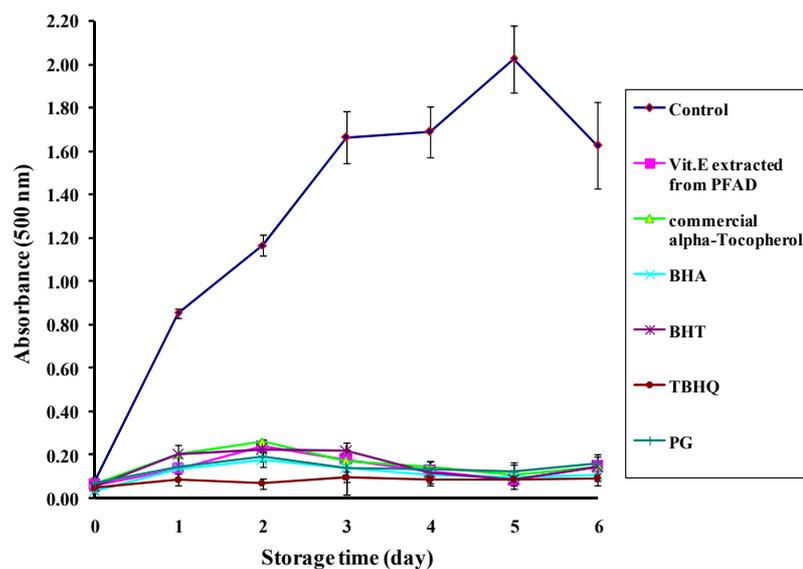


Figure 7. Absorbance of the extracted vitamin E and the other antioxidants on the linoleic acid emulsion peroxidation

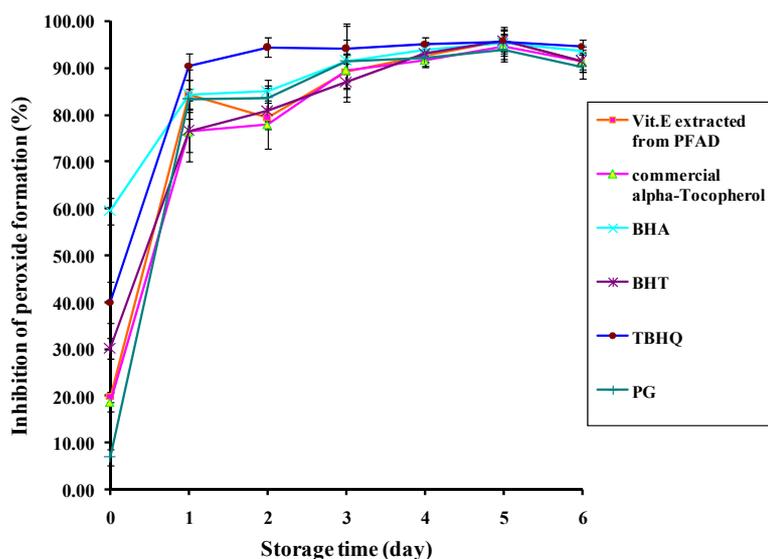


Figure 8. Peroxide inhibitions of the extracted vitamin E and the other antioxidants

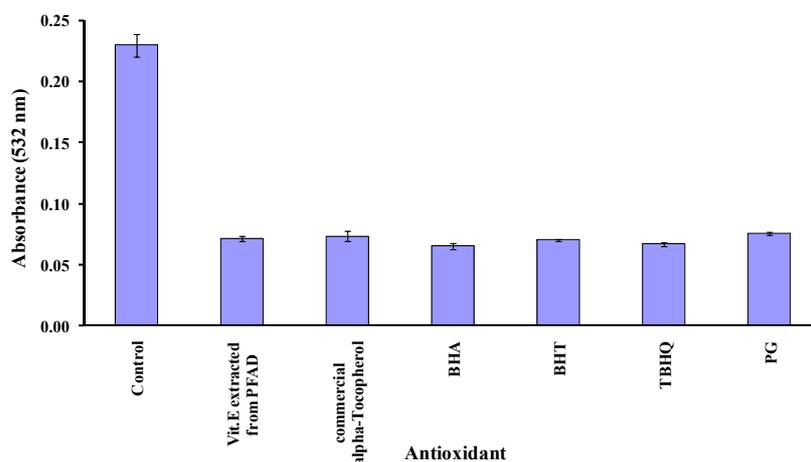


Figure 9. Absorbance of the extracted vitamin E and the other antioxidants obtained from the thiobarbituric acid method

3.2.5 Heat and Storage Stabilities

3.2.5.1 Heat Stability

The heat stabilities at 190°C during 1, 2, 3, 4, 5 and 6 h of vitamin E derivatives, total-tocopherol, total-tocotrienol and total-vitamin E extracted from PFAD were shown in Figure 10. In the consideration of the reducing of the vitamin E derivatives after heating at 190°C for 3 h, the decreasing concentration of α -, β -, γ -, δ -tocopherol and α -, β -, γ -, δ -tocotrienol were 91.59, 73.31, 94.95, 50.08, 64.85, 53.15, 52.88 and 79.35%, respectively. Thus, it could be concluded that most of the vitamin E derivatives remained more than 50% of its initial content. However, the concentrations of vitamin E derivatives, total-tocopherol, total-tocotrienol and total-vitamin E significantly decreased ($P \leq 0.05$) when heating at 190°C for 6 h. The concentrations of α -, β -, γ -, δ -tocopherol, α -, β -, γ -, δ -tocotrienol, total-tocopherol, total-tocotrienol and total-vitamin E decreased by 51.54, 48.19, 91.01, 0.00, 43.04, 27.27, 0.00, 66.13, 51.01, 41.11 and 44.52%, respectively.

Regarding the initial concentrations before heating of the total-tocopherol, total-tocotrienol and total-vitamin E, they were 2,657.24, 5,057.39 and 7,714.63 ppm, respectively. After heating for 6 h, the concentrations changed to 1,355.41 ppm (51.01%), 2,078.85 ppm (41.11%) and 3,434.26 ppm (44.52%), respectively. This result indicated that the concentration percentages of total-tocotrienol were less than those of the total-vitamin E and total-tocopherol, respectively. That means tocotrienols possessed less stability during heating at 190°C for 6 h than the tocopherols, resulting to less quantity and a decreased concentration of total-vitamin E. In addition, the rate of reducing of total-tocopherol (0.47%/h) was less than that of the total-tocotrienol (0.56%/h). This finding agreed with the study of Eitenmiller and Lee (2004) who conducted the research of vitamin E in olein palm oil in simulation frying system at 176 and 185°C for 6 h and discovered that α -tocopherol was more stable than δ -, α - and γ -tocotrienol.

Overall, the extracted vitamin E was stable at 190°C for about 3 h when concentrations of vitamin E derivatives decreased by less than 50% of initial concentrations. At this temperature, it was widely used in frying food processing with addition of vitamin E an antioxidant. However, it was suggested that more vitamin E should be added into frying oil after frying for around 3 h together with considering acid value of the oil, as the index indicating free fatty acid generated in frying oil, because free fatty acid affected the vitamin E stability (Rossi et al., 2007).

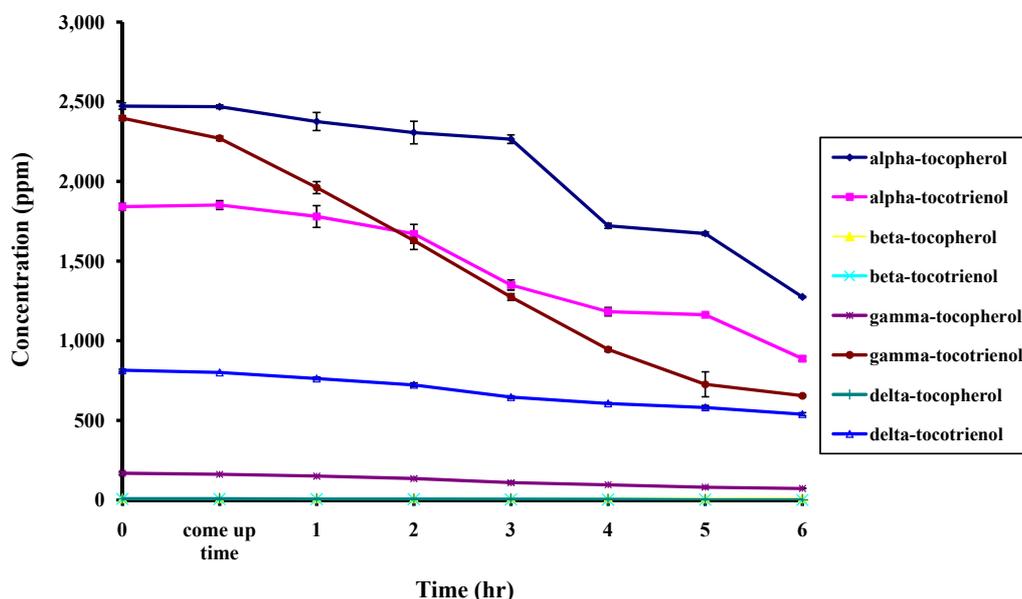


Figure 10. Changes of the concentrations of the vitamin E derivatives during heating at 190°C

3.2.5.2 Storage Stability

The extracted vitamin E was stored at 30°C for 120 days and analyzed for the concentrations of vitamin E derivatives, total-tocopherol, total-tocotrienol and total-vitamin E every 15 days of storage. The results were shown in Figure 11. The concentrations of vitamin E derivatives, total-tocopherol, total-tocotrienol and total-vitamin E significantly decreased ($P \leq 0.05$) during storage. The concentration percentages of α -, β -, γ -, δ -tocopherol, α -, β -, γ -, δ -tocotrienol, total-tocopherol, total-tocotrienol and total-vitamin E decreased to 79.13, 85.30, 95.16, 79.75, 98.16, 91.26, 88.49, 95.94, 80.45, 90.02 and 86.68, respectively. The initial concentrations of total-tocopherol, total-tocotrienol and total-vitamin E were 2,666.53, 4,967.67 and 7,634.20 ppm, respectively. After storing for 120 days, they were 2,142.85 (80.45%), 4,465.53 (90.02%) and 6,608.38 ppm (86.68%), respectively. The result showed that total-tocotrienol contained higher concentration than total-tocopherol, which means that tocotrienols possessed more stability during 120 days of storage than tocopherols.

The decreasing concentrations of the vitamin E derivatives during storage showed that the changes in quantities of vitamin E derivatives decreased with the storage time and the decreasing rate was different depending on the characteristics of those derivatives. Tocopherols showed the higher decreasing rate than the tocotrienols. The decreasing rate of tocopherols and tocotrienols during storage were 0.16 and 0.09% /day, respectively.

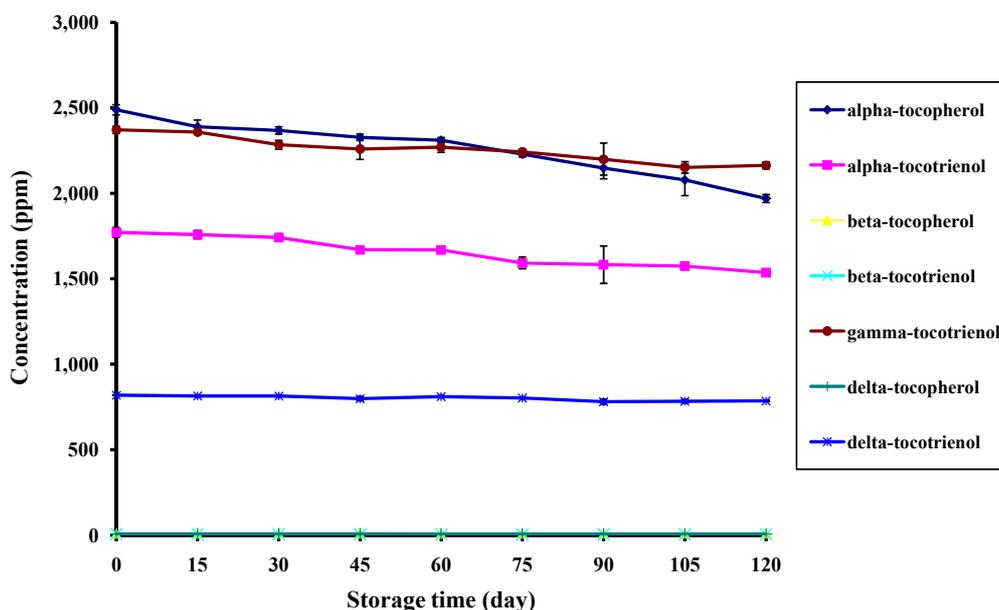


Figure 11. Changes of vitamin E derivatives during storage

4. Conclusion

The study of properties of the vitamin E extracted from PFAD by the sequential cooling hexane confirmed the effective ability of the extracted vitamin E to inhibit the formation of radicals, peroxide generated during lipid oxidation, which could be applied in biological and food system. The concentration of the extracted vitamin E mixture at 20 ppm expressed the maximum DPPH scavenging effect of 89%. According to the methods of reducing power and ferric thiocyanate, the extracted vitamin E mixture at the concentration of 2.5 ppm could inhibit the oxidation of linoleic more than 80% within 1 day. The superoxide scavenging activity of the extracted vitamin E mixture was greater than those of the BHT, synthetic α -tocopherol, and PG, respectively. The total antioxidant activity of the extracted vitamin E was similar to those of the BHT and commercial α -tocopherol. The total-vitamin E remained about 73.35% after heating at 190°C for 3 h and most of the vitamin E derivatives were reduced less than 50%. The tocopherol derivatives were more heat-stable than the tocotrienol derivatives while γ -tocopherol was the most heat-stable derivative. The concentration of total-vitamin E stored at 30°C for 120 days remained about 87%.

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