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ANTIOXIDANT ACTIVITY OF UNSAPONIFIABLE FRACTION OF PALM FATTY ACID DISTILLATE ON PEROXIDATION RATS

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ABSTRACT

Unsaponifiable Fraction (USF) from palm fatty acid distillate (PFAD) contained multicomponents of bioactive compounds, including vitamin E, phytosterols, and squalene. Vitamin E of USF from PFAD was dominated by tocotrienols that exhibited better antioxidant activity than tocopherols. In this study, USF from PFAD was examined its ability to protect from lipid peroxidation induced by thermally oxidized frying oil. Six groups of rats were treated by USF at dose of 0, 100, 200, 500, and 1000 mg/kg bw/day. The normal rats was used as a control group. Antioxidant activity of USF was measured by blood malondialdehyde (MDA) level and superoxide dismutase activity. The results showed that USF from PFAD was effective antioxidant for protection from peroxidation, indicated by decreasing blood MDA level and increasing liver SOD activity. The effective dose of USF was 500 mg/kg bw/day. At higher dose (1000 mg/kg bw/day) USF caused higher blood MDA level and lower SOD acitivty than dose of 500 mg/kg bw/day.

Keywords: antioxidant activity, malondialdehyde, peroxidation, superoxide dismutase, thermally oxidized frying oil

INTRODUCTION

Lipid peroxidation is a the result of oxidative stress that leads to several diseases such as cancer (Gonenc *et al.*, 2006). Lipid peroxidation produces free radicals (Mohan dan Priya, 2009) and in oxidative stress condition these compounds were produced over the capacity of antioxidant defense system. Free radicals can attack unsaturated free fatty acid in cell membrane that cause cell damage because lipid bilayer is very important in cell functions such as receptor and enzyme acitivites (Evans, 2000).

Human body has antioxidant defense system that comprises of antioxidant vitamin such as vitamin E and C, and also antioxidant enzymes such as superoxide dismutase, gluthation peroxidase, and catalase (Hamden *et al.*, 2009). The imbalance between pro-oxidant/antioxidant in the body, will result to excesive free radicals and antioxidant enzyme inactivation. MDA (malonaldialdehyde) is one of oxidative stress markers (Gonenc *et al.*, 2006).

Lipid peroxidation prevention by lipohyllic antioxidants such as vitamin E can suppress oxidation in human body. Tocopherols and tocotrienols are vitamin

E that posses antioxidant activity (Loganathan *et al.*, 2009) and have a role in preventing oxidative stress (Meydani, 2000; Niki and Noguchi, 2004; Choi and Lee, 2009; Mitei *et al.*, 2009; Yoshida and Niki, 2009; Ikeda *et al.*, 2010). In the body, tocotrienols are more effective antioxidant than tocopherol (Schaffer *et al.*, 2005) because tocotrienols are more efficiently absorbed and tranported into cells (Yamada *et al.*, 2002). Also, tocotrienols are easiser to incorporate into lipid bilayer in cell membrane (Packer, 2001). Furthermore, Fairus *et al.* (2006) explained that tocotrienols are more efficient in preventing and reducing lipid peroxidation due to higher intramembrene mobility and easier to react with free radicals.

Unfortunately, the sources of tocotrienols are still limited. Palm fatty acid distillate (PFAD) is a by-product of palm oil refining in deodorization step. This by-product contains glyceride 96.1% and other minor bioactive components such as tocopherol (0,48%), phytosterols (0.37%. squalene (0.76%), and other hydrocarbons (0.71%) (Gapoor *et al.*, 2002). These bioactive compounds were found in unsapobiable fraction (Khatoon *et al.*, 2010). Our previous research showed that this fraction contained tocopherols 4.05% and tocotrienols 8.04% (Ahmadi dan Estiasih, 2009, 2010). Palm oil is the source of tocotrienols beside rice bran oil (Ng *et al.*, 2004; Puah *et al.*, 2007).

Our previous research (Estiasih et al., 2013) showed that tocotrienol rich fraction (TRF) from PFAD was effective antioxidant to protect liver from peroxidation, indicated by decreasing liver MDA level, increasing liver SOD activity, and increasing liver catalase activity, as well as impairment in hepatocytes after TRF force feeding.

This research aimed to assess the ability of unsaponifibale fraction (USF) from PFAD that contained vitamin E, mainly tocotrineols, as antioxidant that tested in vivo by using thermally oxidized frying oil induced peroxidation rats.

MATERIALS AND METHODS

Materials

PFAD was kindly obtained from a palm oil refinery at Surabaya, Indonesia. Chemical reagents used were vitamin E standard (α tocopherol, α tocotrienol, β tocotrienol, δ tocotrienol), (Santa Cruz Biotech USA), HPLC grade solvents (Merck) and other chemical reagents for analysis (Merck), Wistar male rats with body weight of 150-200 g and age of 8-12 weeks, commercial diet, thermally oxidized frying oil.

Preparation of USF

The preparation of USF was conducted by saponification method (Estiasih *et al.*, 2012). Samples of PFAD and USF were analyzed for vitamin E and chemical properties including peroxide value (Hills and Thiel, 1946) and anisidine

value (ISO, 2006), as well as free fatty acid content (AOCS, 1990), and total oxidation value.

Vitamin E analysis

This procedure was according to Ball (1988). About 1 mg of unsaponifiable fraction of PFAD was added by ethanol 1 mL and then filtered. 20 µL of sample solution was injected into high performance liquid chromatography (Shimadzu LC20AT) with C18 PPODS column 250×4.6 mm and uv vis detector (Shimadzu SPD20A) at wavelength of 295 nm. Mobile phase was methanol: water (95:5 v: v) with flow rate of 1 mL/min. Identification and quantification was conducted by using α tocopherol, α tocotrienol, β tocotrienol, δ tocotrienol and γ tocotrienol standards (Santa Cruz Biotech, USA) that injected into HPLC separately [24].

Bioassay of Antioxidant Acivity of USF

The protocol of bioassay had been approved for ethical clearence No. 190-KEP-UB from Animal Care and Use Committee, Brawijaya University. As many as 36 Wistar male rats were used in this study. Each rat was caged individually and adapted to laboratory enviroment for 7 days. During adaptation, rats were fed by commercial diet that comprised of protein 5.56%, fat 1.13%, carbohydrate 18.05% and calorie 104.62 kkal/100g. Rats were divided into 6 groups and each group comprised of 6 rats. One group was normal rats without USF treatment. Other five groups were peroxidation rats treated by USF 0, 100, 200, 500, and 1000 mg/kg bw/day. Peroxidation was induced by force feeding of thermally oxidized frying oil daily in the dose 2 ml/rat/day for ... days. Thermally oxidized frying oil was obtained by repeatedly heating frying oil untill peroxide value reached more than 100 meq/kg. After 4 week treatment, bood samples were taken from heart for analysis of MDA and SOD.

Serum Blood SOD Analysis (Ukeda *et al.*, 2001)

The principle of SOD measurement is the reaction by xanthine and xanthine oxidation with EDTA as anion donor to produce superoxide radicals. Superoxide radicals by NBT (*Nitro Blue Tetrazolium)* is reduced to formazone with purple in color. One unit of SOD activity is indicated by the amount of enzyme that inhibits NBT reduction by reacting with superoxide radical to produce O_2 and H_2O_2. Measurement is conducted by colorimetric method by using spectrophotometer. The concentration of SOD was calculated based on standard curve.

Blood Serum MDA Analysis (Pasha and Sadasivadu, 1984)

The principle of MDA measurement is by measuring malondialdehyde as secondary product of lipid that react with TBA in acidic condition (pH 2-3) at 97-100°C, to form pink color. Absorbance was measured at 532 nm. The blood was taken from heart and then centrifuged at 4000 rpm at 10 min. 250 µL blood was

added by 625 µL TCA 10%, and then was added by HCl 1 < 100 µL and homogenized by vortex. Aquabidest 0.5 mL was added and then homogenized again by vortex. Na-thiol 1% 100 µL was added and homogenized again. The mixture was heated at 100°C in water bath shaker for 30 min, and then was centrifuged for 3000 rpm for 15 min. Supernatant was taken and added by 3 mL aquabidest. The absorbance was measured at 532 nm.

RESULT AND DISCUSSION

Chemical Characteristics of PFAD and USF

The chemical characteristics of PFAD and its USF are shown in Table 1. Data in PFAD contained free fatty acid of 88% that fulfilled the PORIM (Palm Oil Research Institute of Malaysia) standard of PFAD. PORIM standard requires free fatty acid concentration of minimum 70% (Affandi, 1994). Saponification decreased free fatty acid concentration (Table 1), because this compound saponified into soap. The maesured free fatty acid in unsaponifiable fraction indicated undesirable free fatty acid residue.

Table 1. Chemical characteristics of palm fatty acid distillate (PFAD) and unsaponifiable fraction (USF)

Characteristis	PFAD	USF
Free fatty acid (%)	87.83	1.37
Peroxide value (meq/kg)	1.53	12.18
Anisidine value	6.92	10.35
Total oxidation value	9.98	34.71

The oxidation level of PFAD and USF was indicated by primary oxidation product (peroxide value) and secondary oxidation product (anisidine value). Total oxidation value revealed the past oxidation and recent oxidation. The oxidation level of PFAD is very low compared to USF. Oxidation level increased in USF due to accumulation of these products in USF after saponification. Unsaponifiable fraction is not water soluble and non polar. The oxidation products of PFAD reacted with oxygen to form peroxide and further decomposed into smaller compounds. These oxidation products are supposed to be non polar and non volatile that accumulated in USF. Mostly, fatty acid of PFAD is long fatty acid therefore its oxidation product tended to be water insoluble..

Data in Table 2 shows the profile of vitamin E ini PFAD and USF. Usually, palm oil contained vitamin E of 600-1000 ppm. PFAD has vitamin E of 0.1956% or 1956 ppm (Table 2) that higher than palm oil. During deodorization, vitamin E is vaporized and accumulated in deodorizer distillate. Vitamin E The concentration of vitamin E increased 10 fold in USF compared to PFAD. The concentration of vitamin E in USF is high compared to other sources such. JECFA has defined an

Acceptable Daily Intake (ADI) of 0.15-2 mg/kg bw/day calculated as alpha-tocopherol (EFSA, 2008) or 10,5-140 mg for individu wirth body weight of 70 kg.. Consumption of USF 1 g/day will provide vitamin E 19,6 mg that meets the daily requirement of this vitamin.

Table 2. Vitamin E composition of PFAD and USF

Bioactive Compounds	PFAD		USF	
	ppm	%	ppm	%
Vitamin E	**195.60** /	**0.1956**	**19,600.00**	**1.96**
• α tocopherol	37.81	0.0038	3,145.80	0.31
• α tocotrienol	35.81	0.0036	6,546.40	0.65
• δ tocotrienol	4.54	0.0005	4,429.60	0.44
• γ tocotrienol	117.44	0.0117	5,478.20	0.55
• Total tocotrienols	157.79	0.0158	16,454.20	1.65

The advantage of palm oil as a source of vitamin E is rich in tocotrienols. Other sources of vitamin E, except rice bran (Theriault et al., 1999, Ng et al., 2004), is dominated by tocopherols such as soybean oil distillate deodorizer (Wan et al., 2008), coconut, cacao, soybean, barley, germinated wheat, sunflower oil, groundnut oil, walnut, sesame oil, and oilve oil (Heinonen and Piironen, 1991). Tocotrienols have higher antioxidant activity than tocopherols that able to prevent microsomal and liver mitochondria peroxidation as well as dioleoylphosphatidylcholine liposome oxidation (Serbinova and Packer, 1990; Packer *et al.*, 2001).

Vitamin E in palm oil comprises of several isomers i.e. α tocopherol, α tocotrienol, γ tocoferol, γ tocotrienol, and δ tocotrienol) (Table 3). Palm oil has vitamin E of 600-1000 ppm with the composition α tocopherol 20%, α tocotrienol 22%, γ tocotrienol 46%, and δ tocotrienol 12%. Palm oil is unique due to high tocotrienol concentration (Puah *et al.*, 2007). Data in Table 3 showed that tocotrienols are the main vitamin E in PFAD.

Table 3. Vitamin E profile (% relative) of PFAD and USF

Vitamin E (%)	PFAD	USF
α tocotrienol	18.31	33.40
δ tocotrienol	2.32	22.60
γ tocotrienol	60.04	27.95
α tocopherol	19.33	16.05
Total tocotrienols	80.67	83.95

Among tocotrienols, generally PFAD is dominated by γ tocotrienol. This research is in accordance to that reported by Puah *et al.* (2007) that γ tocotrienol is

the main vita,in E in palm oil. Commonly, the concentration of vitamin E in PFAD is $\gamma > \delta > \alpha$ tocotrienol.

Tocotrienols are found in high concentration in palm oil and rice bran (Theriault *et al.*, 1999). Other sources, such as coconut oil, cocoa butter, soybean, barley, germinated wheat, sunflower oil, groundnut, walnut, sesame, and olive, only contain tocopherols (Heinonen and Piironen 1991).

Compared to other vitamin E containing plant sources, PFAD and USF contain high amount of vitamin E that is 0.20% (200 mg/100 g) in PFAD and FTT 1.96% (1,960 mg/100 g) (Table 2). Vitamine E concentration increased up to 9.8 times in USF compared to PFAD. This enhancement was due to saponification that removed free fatty acid and increased unsaponifiable fraction

***In Vivo* Antioxidant Acivity of Unsaponifiable Fraction**

MDA Level

Malonaldialdehyde (MDA) is a product of tertiary lipid oxidation that produced by human body especially in oxidative stress condition. MDA is an indicator of peroxidation in the body that the higher MDA level indicated the higher oxidative stress and the lower body antioxidant status. This indicator is measured from blood serum and is a biomarker of oxidative stress because it is a final product of lipid peroxidation. Rush *et al.* (2005) stated that free radicals in the from of reactive oxygen species (ROS) is constantly produced in low level in normal condition because it functions in intercell signalling. ROS is eliminated as long as cel has endogen antioxidant defense system to counteract the oxidants.

Free radicals or ROS, such as superoxide anion, hydrogen peroxide, and radical hydroxy could damage lipid, protein, and nucleic acid. The main target of ROS is polyunsaturated fatty acids in lipid membrane Polyunsaturated fatty acids are degradated by free radicals and form MDA. MDA level in blood serum is one of cell damage marker caused by free radicals. In the case ROS can not neutraize by antioxidants, MDA level will increase significantly that leads to oxidative stress (Jyothi *et al.*, 2008). According to Najeeb *et al.* (2012), lipid peroxidation is a chain reaction that continuously produces free radicals from polyunsaturated fatty acid oxidation in cell membrane. This oxidation causes oxidative damage of the cells. MDA is a final product of this lipid peroxidation and a marker for oxidation.

Vitamin E, mainly tocotrienols, containing USF feeding is able to reduce peroxidation level in vivo. It wast indicated by MDA level of blood serum of rats treated by USF was lower than that was not fed by USF (Figure 1). Perricon (2008) that tocotrienol is an effective antioxidant indicating by acting faster 40-60 times than α tocoferol in preventing peroxdation due to free radical action. Tocotrienols have better antioxidant activity and is able to reduce MDA level better than tocopherols.

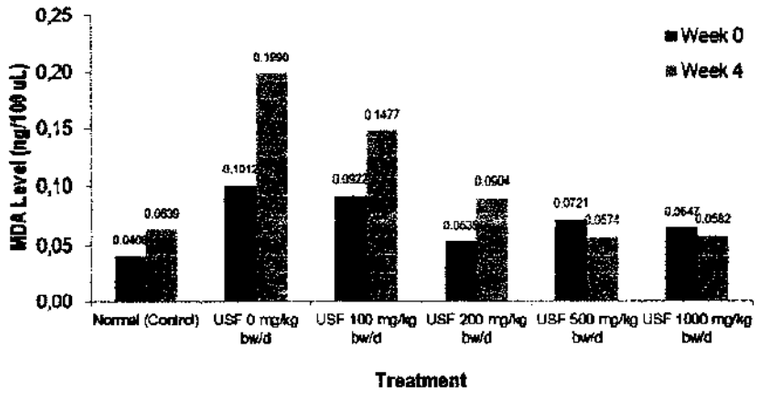

Figure 1. Blood serum MDA level of groups of rats treated by various doses of USF

USF contained vitamin E of 1.96% that comprised of 1.58% tocotrienols and 0.32% tocopherol. Biological activity of tocopherols are lower than tocotrienols (pustaka tambah lagi).

Blood serum MDA level of rats decreased in accordance to the increase of ESF level feeding. USF is able to act as antioxidant to scavenge free raducals in stress oxidative condition. Murray *et al.* (2000) explained that vitamin E is lipophillic compound that able to penetrate into cell membrane to prevent lipid peroxidation and act as chain beraking antioxidant. The highest decrease in MDA level appeared in USF dose of 500 mg/kg bw/d. At dose of USF 100 mgkg bw, MDA level was the highest after 4 week feeding, but still lower than control. Increasing USF doses resulted in decreasing MDA level.

In USF, vitamin E concentration is 1.96% with tocotrienol content of 1.58%. USF feeding of 100, 200, 500, and 1000 mg/kg bw/d provided vitamin E intake of 1.96; 3.92; 9.80; and 19,60 mg/kg bw/d, respectively. At these doses, tocotrienols intake was 1.58; 3.15; 7.89; and 15.77 mg/kg bw/d, respectively.

After 4 week feeding, groups of rats fed by USF doses of 500 and 1000 mg/kg bw/d revealed lower MDA level than normal group. It means that USF acted as antioxidant and was able to decrease oxidation caused by thermally oxidized frying oil. This oil had peroxide value of 112 and 116 meq/kg.

Week 0 in Figure 1 indicated the MDA level of blood serum of rats before treating by USF. The MDA level at week 4 increased at USF doses of 0, 100, and 200 mg/kg bw/d compared to MDA level at week 0, but MDA level was lower at doses of 500 and 1000 mg/kg bw/d at week 4 compared to week 0. It means that at USF doses of 100 and 200 mg/kg bw/d, USF was not effective to prevent peroxidation. At higher doses, USF effectively prevents oxidation caused by thermally oxdized frying oil feeding.

USF contained tocotrienols as the major vitamin E, and tocotrienols had the ability to scavenge peroxyl radicals 1.5 times greater in liposome than α

tocopherol (Packer *et al*, 2001). In liver microsomal, tocotrienols are 40 times more effective to protect Fe(II)NADPH that affected lipid peroxidation . The difference in their activities are due to the difference of phytil structure of both types of vitamin E. Cromanoxyl α tocotrienol (α tocotrienoxyl), a radical of tocotrienol, is recycled faster in membrane and lipoprotein than α tocopheroxyl radical, a radical of tocopherol (Serbinova *et al.*, 1991).

High level of MDA as a final product of lipid peroxidation, indicates high free radical formation that leads to reduction of body primary antioxidant such as superoxide dismutase (SOD). High MDA level was related to thermally oxidized frying oil feeding.

Favier (1995) revealed that MDA is final produc of lipid peroxidation. The high level of MDA is affected by lipid peroxide level as indicator of free radicals. Antioxidant acts to reduce peroxide radicals thus prevent oxidative stress. The study of Daga *et al.* (2003) showed that vitamin E feeding at 400 IU or 268.46 mg for 12 weeks, effectively reduced MDA plasma level by inhibiting lipid peroxidation due to free radicals. The average of MDA plasma reduction was 42.8%.

Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) is an enzymatic antioxidant defense system in cells. This enzyme has an important role to protect cells from negative effects of superoxide anion that produced from various biological (Najeeb *et al.*, 2012).

ROS such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), peroxynitryl ($ONOO^-$), are known to cause oxidative damage and contribute to several chronic diseases such as cancer, heart disease, and brain damage (Poljsak and Milisav, 2013). Protection and elimination of ROS could be done by enzymatic or non enzymatic reactions. To scavenge free radicals, cells have a biological defense system by enzymatic antioxidants. One of these enzymes is superoxide dismutase (SOD) that convert ROS into oxygen and hydrogen peroxide. Furthermore, hydrogen peroxide is converted by catalase into water (Fang *et al.*, 2002). According to Devaki *et al.* (2011), ROS formation occurs initially in stress oxidative condition and the amount of ROS formation depends on oxidative stress intensity. In extreme condition, antioxidant defense system is interfered that causes by disturbance of enzyme production or enzyme inactivation.

Group of normal rats was fed by standard diet and was not treated either by thermally oxidized frying oil or USF. This group revealed non stress oxidative condition indicated by high SOD activity (Figure 2). Conversely, group of rats treated by thermally oxidized frying oil without USF feeding showed the lowest SOD activity after 4 week treatment. USF contained vitamin E that acts as primary antioxidant by scavenging free radicals.

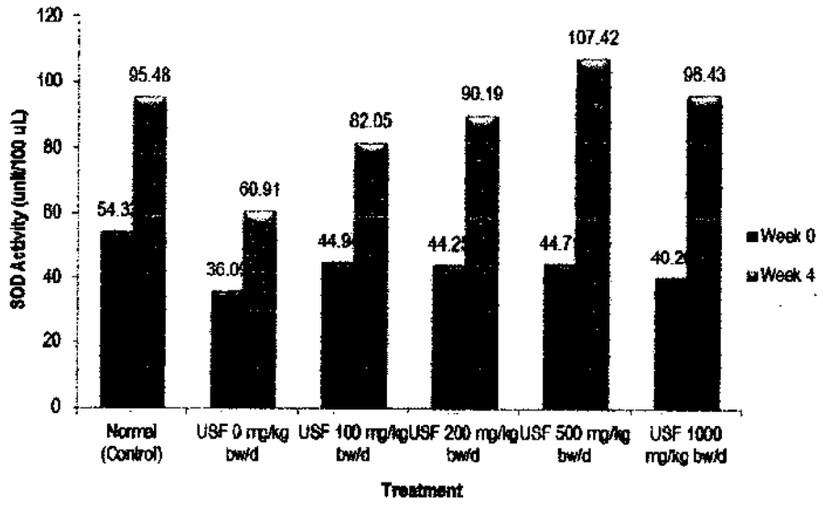

Figure 2. Blood serum SOD activity of groups of rats fed by USF at various doses

Thermally oxidized frying oil that was obtained by repeatedly heating frying oil, contained free radicals that measured by peroxide value more than 100 meq/kg. This condition caused SOD as primary antioxidant in the body to neutralize free radicals. Therefore, group of rats treated by thermally oxidized frying oil without USF feeding, showed the lowest SOD activity after 4 week treatment. Yamaguchi *et al.* (1994) and Favier (1995) explained that SOD is a primary enzymatic in antioxidant defense system that catalyzes dismutation of superoxide radicals into hydrogen peroxide and protects the body from destructive superoxide radicals.

Groups of rats without USF feeding revealed oxidative stress condition indicated by decline in SOD activity as main antioxidant in the body. Accumulation of oxidation products such as MDA in mitochondria is accompanied by oxidative destruction that leads to reduction in intracellular enzyme activities such as SOD, glutathion peroxidase (GPx) and catalase (CAT). Denev *et al.* (2012) explained that in normal condition, there is a balance between antioxidant and prooxidant. But, due to several factors such as stress, radiation, nutrition, air pollution, and smoking, there is the disturbance of this balance that leads to oxidative stress condition. The later condition requires sufficient antioxidants from diet.

SOD converts superoxide anion to hydrogen peroxyde (H_2O_2) and oxygen (O_2), as well as protects from ROS formation intra or outside cells (Koyama *et al.*, 2013). In high free radical concentration, there is an imbalance between antioxidants and oxidants. Zaidi and Banu (2004) stated that in oxidative stress, there is a reduction in SOD, glutathione-S-transferase (GST), catalase, and glutathione peroxidase (GPx) activites in the brain. Furthermore, Yang *et al.* (2008) explained that insufficiency of enzymatic antioxidants to detoxify ROS led

to antioxidant-oxidant imbalance. Previously, Palmieri and Bledorio (2007), indicated that the increase of oxidative stress is an indicator of the inability of antioxidant defense system to inactivate execive ROS production.

The relationship betweed USF dose feeding and SOD activity after 4 week treatment is shown in Figure 2. SOD activity increased in accordance to increasing doses of USF to 500 mg/kg bw/d. Further increase in USF dose (1000 mg/kg bw/d) did not reveal higher SOD activity. The highest SOD activity was found in the group of normal rats and the lowest was occured in the group of peroxidation rats without USF feeding. Induction of peroxidation by thermally oxidized frying oil force feeding 2 ml/rat/d did not show similar initial antioxidant status (week 0). Perhaps, this was due to the effects of several factors such as different adaptability of each individual rats, different stress condition, and different initial antioxidant status. USF feeding into groups of peroxidation rats showed improvement in antioxidant status after 4 week treatment.

The highest antioxidant activity was found in the group of rats terated by USF 500 mg/kg bw/d that indicated this dose is effective to improve antioxidant status. USF from PFAD contained vitamin E with tocopherol 0.32% and tocotrienols 1.58%. This composition of vitamin E is effective to protect from stress oxidation. Packer *et al* (2001) showed that vitamin E in cell membrane sel effectively inhibits lipid peroxidation. Vitamin E (tocopherols and tocotrienols) scavenges free radicals in propagation cahin of peroxyl radicals. The study of Zaidi and Banu (2004) indicated that vitamin E is more effective than vitamin A and C to increase glutathion level and SOD, GST, and CAT activities.

Vitamin E in USF contains higher amount of tocotrienols than tocopherol. Tocotrienols have better ability as antioxidant than tocopherols due to unsaturated chain of phytil side chain in tocotrienols. According to Inokuchi *et al.* (2003) unsaturated side cahin of tocotrienols affect their ability to penetrate into lipid bilayer in cell membrane. Moreover, tocotrienols are regererated faster than tocopherols due to their existence near membrane surface. Also, tocotrienols penetrate into cell membrane easier and distribute more uniformly than tocopherols. This makes tocotrienols are easier to react with free radicals and have better *in vivo* antioxidant activity (Packer, 2001).

Group of rats terated by the highest dose of USF (1000 mg/kg bw/d) showed lower SOD activity at week 4 than that treated by USF 500 mg/kg bw/d (Figure 2). This showed that effective dose was obtained at dose of 500 mg/kg bw/d. Higher dose of USF did not reveal better SOD activity. This phenomemon related to the prooxidant activity of vitamin E at high concentration.

CONCLUSION

USF can be obtaind by saponification of PFAD and vitamin E concentration of USF is higher than PFAD. Vitamin E of PFAD and USF is dominated by tocotrienols. USF has in vivo antioxidant activity that indicated by reducing blood

serum MDA level and increasing SOD activity in peroxidation condition induced by thermally oxidized frying oil. The occurance of tocotrienols contributes to high antioxidant activity of USF. Increasing USF feeding doses increased antioxidant status of peroxidation rats. The effective dose of USF was found at 500 mg/kg bw/d and higher dose of USF did not reveal better antioxidant status.

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YAMAGUCHI UNIVERSITY

September02, 2014

Subject: Acceptance for Presentation

Dear Teti Estiasih
Faculty of Agricultural Technology,
Brawijaya University, Indonesia

We are pleased to inform you that your research abstract entitled "ANTIOXIDANT ACTIVITY OF UNSAPONIFIABLE FRACTION OF PALM FATTY ACID DISTILLATE ON PEROXIDATION RATS" has been accepted for presentation at the 10th Young Scientist Seminar on November 16 – 17, 2014 at Seminar Park, Yamaguchi, JAPAN. Type the presentation is "Oral Presentation".

Please note that the desirable duration of oral presentation is 10 minutes; question and discussion 5 minutes.

Thank you for your interest and cooperation.
We look forward to seeing you at the seminar.

Yours sincerely,

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