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Bioactive compounds from palm fatty acid distillate and crude palm oil

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Abstract. Crude palm oil (CPO) and palm fatty acid distillate (PFAD) are rich sources of bioactive compounds. PFAD is a by-product of palm oil refinery that produce palm frying oil. Physical refining of palm oil by deodorization produces palm fatty acid distillate. CPO and PFAD contain some bioactive compounds such as vitamin E (tocopherol and tocotrienols), phytosterol, and squalene. Bioactive compounds of CPO and PFAD are vitamin E, phytosterols, and squalene. Vitamin E of CPO and PFAD mainly comprised of tocotrienols and the remaining is tocopherol. Phytosterols of CPO and PFAD contained beta sitosterol, stigmasterol, and campesterol. Tocotrienols and phytosterols of CPO and PFAD, each can be separated to produce tocotrienol rich fraction and phytosterol rich fraction. Tocotrienol rich fraction from PFAD has both antioxidant and cholesterol lowering properties. Bioactive compounds of PFAD silmultaneously have been proven to improve lipid profile, and have hepatoprotector effect, imunomodulator, antioxidant properties, and lactogenic effect in animal test experiment. It is possible to develop separation of bioactive compounds of CPO and PFAD integratively with the other process that utilizes fatty acid.

1. Introduction

Physical refining of crude palm oil (CPO) to produce frying oil passes through several steps including degumming, bleaching, and deodorization. Palm fatty acid distillate (PFAD) is a by-product of deodorization in palm oil refinery. The major components of PFAD are free fatty acid, lipid oxidation products, and other compounds such as tocopherols, tocotrienols, phytosterols, and squalene [1]. PFAD utilization currently is limited to oleochemical industries, while the use of its bioactive compounds is almost neglected. The production of PFAD is 4% based on CPO [2]. It is about 3.66 ton of PFAD is produced from every 100 ton of CPO [3]. PFAD is commonly used as fatty acid source for non-food industries [4] such as soap, feed, and oleochemical industries [5].

According to Liu *et al*. [6], as much as 5-57% of tocotrienols and tocopherols lost from CPO during deodorization. Most of tocotrienols and tocopherols are accumulated in PFAD with the concentration of 0.7-1.0%. Goh and Gee [7] indicated that hydrocarbon in PFAD is squalene as major component and n-alcane ($C_{12}H_{26}$-$C_{36}H_{74}$) as minor component. PFAD contains high amount of squalene that reaches 1.03%, and this quantity is higher than that found in other vegetable oil [8].

PFAD also contains free fatty acids and glycerides 96.1%, and minor bioactive compounds such as tocopherols and tocotrienols (0.48%), phytosterols (0.37%), squalene (0.76%), and other hydrocarbons (0.71%) [1]. Meanwhile the composition in CPO is vitamin E 222 ppm, phytosterols 17,322 ppm, squalene 535 ppm, and β carotene 643 ppm [9]. PFAD has been used integratively as a source of bioactive compounds and free fatty acids for oleochemical industries [10].

Bioactive components of PFAD and CPO are accumulated in unsaponifiable fraction (USF) [9, 11]. USF can be separated from saponifiable fraction by simple saponification. Two fractions are formed during saponification, unsaponifiable fraction that contains bioactive compounds and saponification fraction that rich in free fatty acids or soap [9, 10].

USF from PFAD contains tocopherols 4.05% and tocotrienols 8.04% [12, 13] and phytosterols [14]. Purification of USF produces vitamin E rich fraction with vitamin E concentration as high as 33.88% [15], and phytosterols rich fraction with phytosterols concentration of 17.33% [14]. Our previous study [10] showed that USF from PFAD had vitamin E of 0.80%, phytosterols of 9.16%, squalene of 1.14%, meanwhile polycosanol and co-enzyme Q10 were not detected. USF from CPO contained vitamin E 1.94%, α tocotrienol 0.86%, δ tocotrienol 0.14%, γ tocotrienol 0.94%, total tocotrienol 1.94%, total phytosterols 65.80%, β sitosterol 41.94%, campesterol 4.47%, stigmasterol 19.39%, β carotene 0.054%, and squalen 31.69%.

Vitamin E of PFAD dan CPO is different from other sources that rich in tocotrienols. The composition of vitamin E in PFAD is α tocopherols (20%), α tocotrienols (22%), γ tocotrienols (46%), and δ tocotrienols (12%). Beside rice bran oil, palm oil has high tocotrienols content [16, 17]. Meanwhile the composition in CPO is α tocopherols (0%), α tocotrienols (65%), γ tocotrienols (26%), and δ tocotrienols (9%) [9]. Tocotrienol is an important compound for pharmaceutical product, foods, and food supplements. Tocotrienols have hypocholesterolaemic, antioxidant, antithrombotic, anti atherogenic, anti inflammation, and immunomodulator properties. Tocotrienols are also known to have the ability to reduce blood LDL cholesterol level [18]. Tocotrienols are able to inhibit HMG Co-A reductase, an enzyme that is responsible to convert HMG into mevalonate in cholesterol biosynthesis. This conversion is a rate-limiting step in the cholesterol biosynthesis, and the primary statin target [19].

Phytosterols of PFAD comprises of campesterol (13%), β sitosterol (60%), stigmasterol (24%), and cholesterol (3%) [20]. In CPO, phytosterols consists of campesterol (0.5%), β sitosterol (98.8%), and stigmasterol (0.7%) [9]. Phytosterols lower blood cholesterol level by inhibiting cholesterol absorption. The required dose of phytosterols to have beneficial effect on health is 2-3 g/day [21]. Phytosterols also have the ability to increase breast milk production, as well as anti inflammation, anti cancer, immunomodulatory [22], antithrombotic, and hypocholesterolaemic [23, 24, 25]. So far, the mechanism of cholesterol lowering properties of phytosterol is still unclear, but Bonsdorff-Nikander [26] proposed some theories that phytosterols compete with cholesterol in micelle formation, phytosterols cocrystallize with cholesterols, and phytosterols inhibit ACAT (Acyl CoA:Cholesterol Acyl Transferase) activity in cholesterol synthesis.

Squalene as one of bioactive compounds in PFAD and CPO, has anti cancer and cholesterol lowering properties [20]. Squalene has the ability to increase fecal excretion of bile acid that leads to reduce blood cholesterol level [27, 28, 29]. Other study showed that squalene increase cholesterol level, due to incresed rate of cholesterol synthesis [19]. It is hypothesized that bioactive compounds of USF synergistically affect lipid profile improvement.

2. Bioactive Compounds Of PFAD, CPO, and USF

Bioactive compounds found in PFAD and CPO are vitamin E, phytosterols, and squalene. Loganathan et al. [20] revealed that palm oil contained vitamin E 600-1.000 ppm, phytosterols 300-620 ppm, carotenoid 500-700 ppm, squalene 250-540 ppm, phospholipids 20-100 ppm, co-enzyme Q10 10-80 ppm, and poliphenol 40-70 ppm. Meanwhile, our previous study [30] showed that PFAD contained bioactive compound that comprised of α-tocopherol 8.5-134.62 ppm, α-tocotrienol 11.97-51.63 ppm, δ-tocotrienol 4.56-63.65 ppm, γ-tocotrienol 32.18-117.98 ppm, total vitamin E 64.70-280.63 ppm, β-

sitosterol 381.55-3,575.55 ppm, stigmasterol 10.99-1548.32 ppm, campesterol 18.96-1,720.30 ppm, total phytosterols 407.00-6011.72 ppm, squalene 205.73-1,273.64, but coenzyme Q10 and polycosanol were not detected.

Distillate of deodorizer from various vegetable oils had different composition of bioactive compounds. Ceriani and Mirelles [31] reported that soybean oil distillate deodorizer contained tocopherols, and β sitosterol; wheat oil only had tocopherols; meanwhile canola oil contained β sitosterol and tocopherols. Benites et al. [32] also showed that soybean oil distillate of deodorizer contained tocopherols. Furthermore, Khatoon et al. [11] showed that phytosterols were also found in soybean oil distillate deodorizer. Bondioli et al. [33] revealed that olive oil distillate deodorizer had squalene. Distillate deodorizer of dried grain contained phytosterols, steryl ferulic, tocopherols, tocotrienols, and carotenoid.

Generally, phytosterols are the most prominent bioactive compound found in PFAD from several palm oil refineries in Java, Indonesia (Table 1). Phytosterols also the major component bioactive compounds in soybean oil distillate of deodorizer [11] and canola oil, meanwhile tocopherols are dominant in wheat oil [32], and squalene in olive oil [33].

Table 1. Bioactive compounds of PFADs from several palm oil refineries.

Palm Oil Refinery	Vitamin E (ppm)	Phytosterol (ppm)	Squalene (ppm)	Polycosanol (ppm)	Co-enzyme Q10 (ppm)
1	195.60	7476.56	2373.27	nd	nd
2	64.70	407.00	462.87	nd	nd
3	280.63	6011.72	2767.08	nd	nd
4	200.76	2310.52	1380.16	nd	nd
5	172.47	1956.15	2222.41	nd	nd
6	208.82	3915.22	nd	nd	nd

nd = not detectable
Reference: Estiasih et al. (2013) [30]

PFAD, CPO, and their corresponding USF have different concentration and bioactive compound composition. Generally, USF has higher bioactive compounds than PFAD (Table 2). According to Khatoon et al. [11], bioactive compounds of PFAD are accumulated in unsaponifiable fraction. Vitamin E increase 245% in USF as compared to PFAD, phytosterols decrease by 73%, and squalene increase by 13611%. The increase of vitamin E and squalene was related to elimination of free fatty acid and triglycerides, as the major component of PFAD and CPO respectively, during saponification. But, factors that affect the decrease of phytosterols was still unknown. Our previous studies [9, 10] showed that all bioactive compounds increased in USF after PFAD and CPO saponification, including phytosterols with the concentration of 9.18%.

Our previous studies [9, 10] that investigated the optimization of saponification showed that USF from PFAD contained vitamin E of 0.80%, phytosterols of 9.18%, and squalene of 1.14%. USF from optimum conditions for maximum yield comprised of 0.47% vitamin E, 7.77% phytosterols, and 16.26% squalene. The bioactive compounds in USF are expected to synergetically reduce blood cholesterol level in hypercholesterolaemia condition.

The concentration of vitamin E in USF from PFAD was high (800 mg/100 g). Consumption 1 g USF per day will provide 8 mg that meets the daily requirement of vitamin E or 57% of daily reference value [10]. Other sources of vitamin E are rice bran (90.9 mg/100 g), wheat sprout (153.7 mg/100 g), coconut (1.1 mg/100 g), soybean (7.8 mg/100 g), and olive (11.9 mg/100 g) [34].

Phytosterols was the highest concentrated bioactive compounds in USF from PFAD (9,18%). The concentration was high as compared to other sources such as crude corn fiber oil (8.79%), crude corn kernel oil (7.94%), refined corn kernel oil (1.11%), and commercial corn oil (0.74%) [35]. The study of Jiang and Wang [36] showed that a by product of cereal processing (rice bran, wheat bran, wheat sprout, oat bran, and oat hull) contained phytosterols of 9.35 mg/g oil.

Table 2. Bioactive compounds of unsaponifiable fraction of PFAD from the optimum conditions of saponification.

Bioactive Compounds	Concentration	
	ppm	% w/w
Vitamin E	7,968.04	0.80
• α tocopherol	644.11	0.06
• α tocotrienol	1,860.54	0.19
• δ tocotrienol	4,853.78	0.49
• γ tocotrienol	609.61	0.06
• Total tocotrienol	7,323.93	0.73
Phytosterol	91,846.30	9.18
• Beta sitosterol	81,932.59	8.19
• Campesterol	61.87	0.01
• Stigmasterol	9,851.83	0.99
Polycosanol	nd	nd
Squalene	11,436.66	1.14
Co-enzyme Q10	nd	nd

nd = not detected
Reference = Estiasih et al. (2012) [9]

Sabir et al. [37] found the variability of phytosterols in several vegetable oils such as mustard (64 mg/g), corn (23 mg/g), soybean (9 mg/g), rapeseed (5 mg/g), and coconut (0,8 mg/g). Generally, crude vegetable oil contains 1-5 g/kg phytosterols, but rice bran oil contains phytosterols 30 g/kg [38]. In palm oil, phytosterol is a minor component that comprises of campesterol, stigmasterol, and β-sitosterol [17].

Squalene was the bioactive compound in USF with concentration of 1.14% or in 1 g of USF would provide 114 mgsqualene. It is estimated that average daily consumption of squalene in food stuff is 28 mg/day [39]. Olive oil contains 200 to 700 mg squalene/100 g [40].

3. Bioactivity of USF

Hypercholesterolaemia condition was achieved by administration the rats with cholesterol. In USF, the bioactive compounds that were supposed to act as cholesterol lowering agents were phytosterols and squalene, although vitamin E also had a role to reduce cholesterol level. Phytosterols have similar action to cholesterol by micelle formation before being carried by enterocytes. The absorption of phytosterols is inhibited by efflux transporter activity, and this transporter contained a pair of ATP binding protein known as ABCG5 and ABCG8. ABCG5 and ABCG8 transport phytosterols and unesterified cholesterol from enterocytes to digestive lumen [41].

Squalene is the major bioactive compound in USF. Squalene from food is absorbed and converted into cholesterol, and the increase in cholesterol synthesis is not related to the increase of blood cholesterol level but increase of cholesterol excretion in the feces [42]. Squalene was also reported to increase pravastin absorption and a combination of pravastin and squalene was effective to reduce cholesterol [43].

Squalene has a role to increase cholesterol excretion into feses [42]. Several studies showed different effect of squalene on lipid profile. Shin et al. [29] showed the hypolipidaemic effects of amaranth squalen (AS) that were evident in both serum and liver. AS markedly increased faecal excretions of cholesterol and bile acid, and slightly inhibited 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. The preliminary study suggested that the cholesterol-lowering effect of AS may be mediated by the increased faecal elimination of steroids through interference with cholesterol absorption, and that different sources of squalene (plant versus animal) may affect cholesterol metabolism differently. Cholesterol-lowering effect of amaranth is associated, at least in

part, with its squalene content. The effect of squalene may be attributed to enhanced excretion of faecal steroids through interference of cholesterol absorption.

Previously, the study of Qureshi et al. [28] showed that all amaranth varieties contain tocotrienols and squalene compounds which are known to affect cholesterol biosynthesis. Serum total cholesterol and LDL-cholesterol were lowered by 10-30% and 7-70%, respectively, in birds fed amaranth-containing diets. HDL-cholesterol was not affected by amaranth supplementation. Activities of liver cholesterol 7 alpha-hydroxylase (the enzyme responsible for cholesterol breakdown into bile acids) were 10-18% higher than those of controls for birds fed most forms of amaranth and its oil, whereas activities of liver 3-hydroxy-3-methylglutaryl coenzyme A reductase (the rate-limiting enzyme for cholesterol biosynthesis) were lowered by about 9% of popped, milled amaranth and its oil. The lack of marked inhibition of this enzyme suggests the presence of some other potent cholesterol inhibitor(s) apart from tocotrienols and squalene in amaranth. Also, the study of de Castro *et al*. [30] showed that amaranth oil, and its squalene, increased the excretion of bile acids but did not have a hypocholesterolemic effect in hamsters fed on a diet containing high amounts of saturated fat and cholesterol.

Another bioactive compound in USF is vitamin E. Tocotrienols down-regulate the liver pathway of HMGCo-A reductase. The advantage of tocotrienols over statin is that tocotrienol does not block coenzyme Q10 pathway as statin does. Therefore, tocotrienols lower cholesterol production without side effects. Gamma and delta tocotrienols subtly affect the genes that code HMG reductase. Delta tocotrienol also uniquely down-regulate SREBP (sterol regulatory element binding protein), a nuclear factor that naturally regulate other genes linked to low density lipoprotein (LDL). This effect may also down-regulate excessive triglyceride synthesis. Other than delta tocotrienols, none of the other seven vitamin Es substantially regulates SREBP [44].

4. Conclusions

CPO and PFAD are the valuable sources of bioactive compounds. Unsaponifiable fraction (USF) of PFAD and CPO contain vitamin E, phytosterols, and squalene, and USF is a potential candiadte for food supplement and fortificant. All of the bioactive compounds in USF are expected to act synergystically. For fortification, USF may be prepared by microemulsification and microencapsulation, as well as by direct fortification into several food products.

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