INTERNATIONAL JOURNAL of OIL PALM

ISSN: 2599-3496 print
ISSN: 2614-2376 online

Volume 2, Number 1, January 2019
Page 46-52
DOI: https://doi.org/10.35876/ijop.v2i1.23

Antioxidant Activity of Bioactive Constituents from Crude Palm Oil and Palm Methyl Ester

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ABSTRACT

Palm oil has many minor components that can act as natural antioxidant. It contains carotenoid and vitamin E. This research was conducted to determine antioxidant activity of non-polar extract from crude palm oil and fatty acid methyl ester. The oil extract obtained from crude palm oil by solvent extraction with hexane (CPO) and transesterification method followed by solvent extraction with hexane (PME). Carotene content from non-polar extracts were analyzed by using UV-visible spectrophotometer, while carotene composition (α- and β-carotene) and vitamin E (tocopherol and tocotrienol) compositions were analyzed by using high performance liquid chromatography. Glycerides and esters content was analyzed by gas chromatography. Antioxidant activity of oil extract was determined by using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay method. Result revealed that PME has higher content carotenoid and vitamin E than CPO. As expected, the concentration of carotenoid and vitamin E in PME increased with transesterification process. Results also showed that all of non-polar extracts exhibited antioxidant activity significantly, as proven by inhibitory concentration 50% (IC50) of PME and CPO is 5.9 μg mL^{-1} and 15.6 μg mL^{-1}. It is suggested that the presence of carotenoid and vitamin E may have a potential effect as natural antioxidant.

Keywords: carotenoid, palm oil, vitamin E

INTRODUCTION

Crude palm oil is a vegetable oil containing minor components such as carotenoids (500-700 μg mL^{-1}) and vitamin E (600-1000 μg m) (Mukherjee & Mitra 2009). CPO has a significant amount of carotene that can be isolated by various methods. Some researchs has developed carotene isolation process from palm oil such as solvent extraction, transesterification (Bharin *et al*. 1998), saponification (Panjaitan *et al*. 2008), adsorption, membrane (Chang *et al*. 2002) and solvolytic micellization (Chang *et al*. 2002). Transesterification is general term used to describe transformed ester form into other through interchange of the alkoxy group. This reaction will produce esters (PME), glycerol and carotene (Khalid & Khalid 2011).

Carotene is tetraterpene that characterized by a conjugated system of double bonds. The extreme hydrophobic character of carotenes has function as

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antioxidant and play important roles in protection of body tissues from damage cause by free radicals (Boon *et al*. 2010). Carotene and vitamin E has potential as a supplement, as well as a source of antioxidants in pharmaceutical preparations such as creams, ointments and gels (Bayerl 2008). Yeh and Hu (2003), mentioned carotene effective as lung cancer drug, degenerative eye disease and cataract (Leung *et al*. 2005) and decrease blood glucose level (Hamid & Moustafa 2014).

Antioxidants have used to preserve fats and oils without degradation. These substances inhibit oxidative damage in oil content (Izbaim *et al*. 2009). Several methods had developed to measure the free radical scavenging activity (Rubalya & Neelamegam 2012).

DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical scavenging assay method is common applied in determining antioxidant activity of natural product. This assay method also used to study the scavenging activity of antioxidant in oils. Until now, IOPRI not yet determine the ability of free radical reduction activity of carotene from CPO and PME. These researches describe about free radical scavenging activity of carotene extract from CPO and PME.

MATERIALS AND METHODS

Reagents and Standards

All the solvents used for sample preparation and extraction were of analytical grade obtained from Merck (Darmstadt, Germany). All solvents used for HPLC analysis and UV-visible analysis were obtained from Merck (Darmstadt, Germany). β-carotene standards, tocopherol and tocotrienol standards were purchased from Sigma Chemical Co. (Sigma-Aldrich Company, St. Louis, MO, USA).

Instrumentation

Total of carotenoid contents was analysed by using spectrophotometer UV-visible (1700, Shimadzu). α- and β-carotene composition and vitamin E (tocopherol and tocotrienol) analysed by using HPLC (Perkin Elmer), equipped with a YMC (Tokyo, Japan) C30 column (250x4.6 mm I.D., 5 µm particle size) and Agilent Technologies (Santa clara, USA) C18 Column (4.6x150 mm, 2.7 µm particle size), respectively. Glyceride and ester contents were analyzed by using gas chromatography (GC-14B, Shimadzu), equipped with DB-5 HT capillary column (0.53 mmx5 m).

Extraction Process of Carotenoids

Extraction from CPO

The carotene extraction process of CPO using solvent extraction according to Ahmad *et al*. (2009) with slightly modification. CPO and hexane were mixed at a ratio of 1:5 (CPO:hexane) in vortex for 10 minutes. The product was extracted carotene from CPO and used in the next analysis.

Extraction from PME

Production of PME by using transesterification process according to Panjaitan *et al*. (2008) CPO and methanol were mixed at a ratio 1:9 (CPO:methanol) with KOH as a base catalyst for 60 minutes at 60 °C. The glycerol produced was removed while the methyl ester obtained washed with water and hexane. The rich carotene in hexane layer collected and used in the next analysis.

Analysis of Non-Polar Extract

HPLC Analysis of Carotenoids

Identification of α- and β-carotene composition from CPO and PME analysed by using HPLC according to Strati *et al*. (2012) with slightly modification.

HPLC Analaysis of Total Vitamin E (Tocopherol and Tocotrienol)

Identification of total tocopherol and tocotrienol from CPO and PME were analyzed by using HPLC according to Ahmadi *et al*. (2012) with slightly modification.

Spectrophotometer UV-Visible Analysis of Total Carotenoids

Extracted carotene from CPO and PME were analyzed by using spectrophotometer UV-visible at 446 nm according to MPOB test method P2.6:2004 (MPOB 2004).

Gas Chromatography of Glyceride and Ester Contents

Analysis of glycerides and ester content were prepared according to MPOB test method C2.11 by using gas chromatography (MPOB 2004).

Analysis of DPPH Radical Scavenging Activity

Antioxidant activity of carotenes from CPO and PME were analyzed by scavenging activity of stable DPPH. This method was according to Rubalya and Neelamegan (2012) with slightly modification. The carotene was examined with six different concentration (1.5-9.0 µg mL^{-1}) with hexane and chloroform as solvent at ratio 2:3. Amount 0.5 mL mixed solution from each concentration were placed into tube with adding 4 mL 0.5 mM of DPPH ethanolic solution. The mixture measured by using spectrophotometer UV-visible at 515 nm. Antioxidant activity calculated by plotting percentage inhibition against different concentrations. Inhibitor concentration (IC_{50}) is an antioxidant concentration that inhibits the DPPH reaction by 50% under experimental conditions.

RESULTS AND DISCUSSION

HPLC Analysis of Carotenoids in CPO and PME

The HPLC chromatogram of α- and β-carotene composition using HPLC presented in Figure 1 and Figure 2. Based on those results, both contain high β-carotene about 66.6% and 64.6% for CPO and PME respectively, whereas α-carotene about 34.3% and 35.4% for CPO and PME respectively. It shows that the CPO and PME contain the high β-carotene. These results are consistent with the study by Panpipat and Chaijan (2011), who have reported that β-carotene and α-carotene is the major component contained in palm about 80-90% of the total carotenoids. According to Sthal and Sies (2003), β-carotene is a pro-vitamin A,

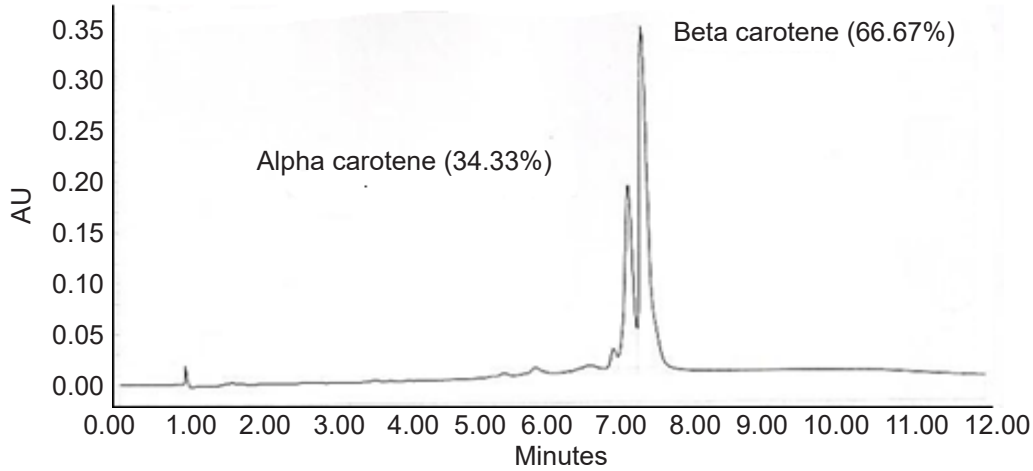

Figure 1 Carotenes composition from CPO.

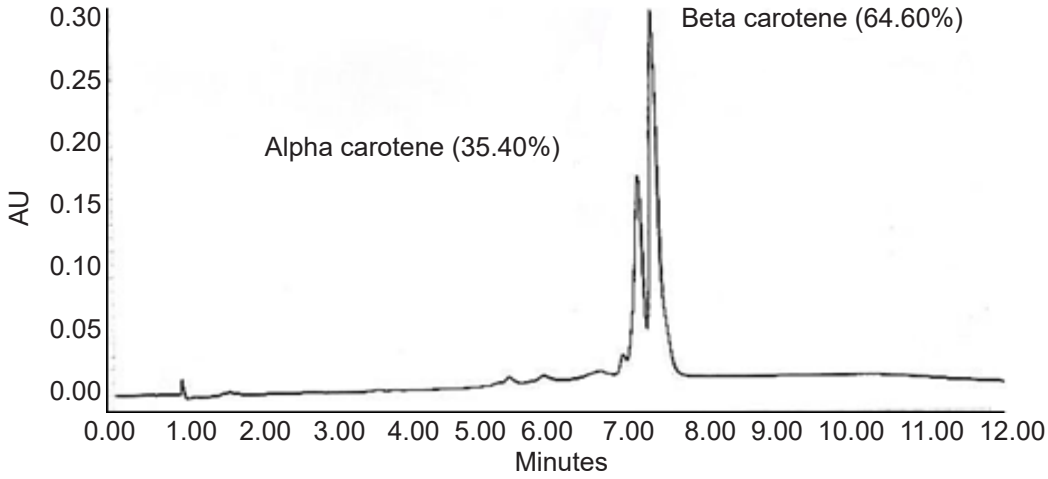

Figure 2 Carotenes composition from PME.

which can be utilized as a source of vitamin A. Thus, high levels of β-carotene on CPO and PME chance as the largest producer of vitamin A.

Spectrofotometer UV-Visible Analysis of Total Carotenes

The results of the carotene concentration analysis using UV-visible spectrophotometer presented in Table 1. The result shows that carotene extract from PME achieved 599 μg mL^{-1}, it is higher than CPO which achieved only 510 μg mL^{-1}. Carotene in CPO still highly bound to the triglyceride so that carotene extract lower than PME that do not contain triglyceride. It was proven in PME only containing monoglycerides, diglycerides and triglycerides under 0.5%. Based on the study by Hasibuan *et al*. (2012), the transesterification method has been successfully transformed glyceride into ester so that carotene more soluble in the solvent.

Gas Chromatography Analysis of Glycerides and Esters

The results of the glycerides and esters content analysis of CPO and PME presented in Figure 3 and Figure 4. Results shows that CPO contains high levels of triglycerides about 76.84%, whereas PME does not contain triglycerides. The high triglyceride concentration can affect the purity of carotene extracted according to Panjaitan *et al*. (2008), triglyceride very strong binds to carotene, so thatmake difficult to obtain pure carotene with high triglyceride concentrations. Meanwhile, the PME does not contain triglycerides but contain high levels of esters about 93.80%. This happens because the transesterification process has turned into a glyceride ester compound (Khalid & Khalid 2011).

Table 1 Characteristic of carotene extract from CPO and PME

Parameters	Crude Palm Oil	Palm Methyl Ester
Triglyceride (%)	76.84	nd
Diglyceride (%)	14.37	nd
Monoglyceride (%)	nd	0.17
Ester (%)	11.41	93.80
Carotene (μg mL^{-1})	510	599

nd: not detected

HPLC Analysis of Total Vitamin E (Tocopherol and Tocotrienol)

The results of total vitamin E (tocopherol and tocotrienol) contents from CPO and PME presented in Table 2. Results shows that CPO has no content tocopherol and tocotrienol about 14.16%, whereas

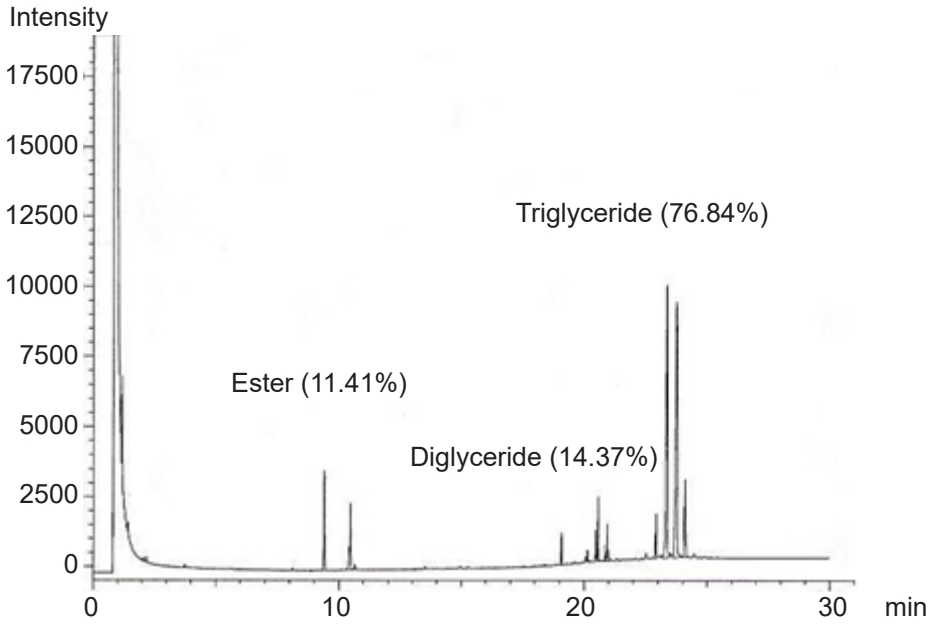

Figure 3 Chromatogram glycerides and esters content of CPO.

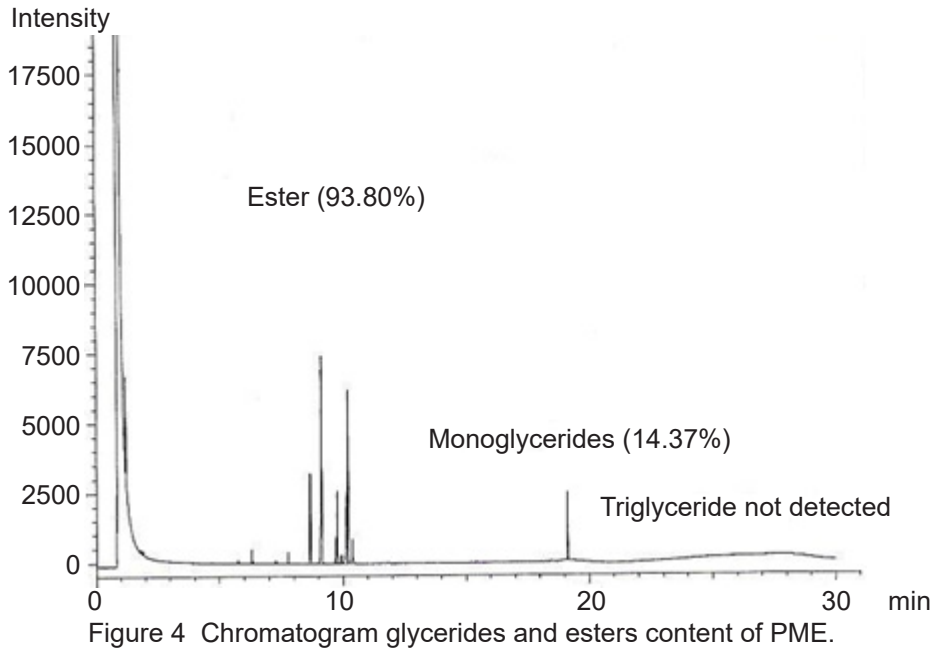

Figure 4 Chromatogram glycerides and esters content of PME.

Table 2 Total vitamin E (Tocopherol and Tocotrienol) Composition

Extract Sources	Tocopherol (%)	Tocotrienol (%)
Crude Palm Oil	nd	14.16
Palm Methyl Ester	0.12	83.26

nd: not detected

PME has tocopherol and tocotrienol about 0.12% and 83.26%, respectively. Extract from CPO obtained little amount of tocotrienol, while tocopherol was not detected. This can occur due to the involvement of the high triglyceride contents, possibly that can interfere extraction process using

non polar solvent. Extract from PME contains high tocopherol and tocotrienol, which shown in the process of more than 90% triglycerides has transformed into ester (Hasibuan 2012).

DPPH Radical Scavenging Activity

Table 3 shows free radical activity of carotene extract from CPO and PME. The results indicate that antioxidant activity of carotene extract from PME stronger than CPO. Figure 5 shows that antioxidant activity of crude palm oil at 9 µg mL^{-1} was 30.1% lower than PME was 69.3%. The IC50 results showed PME stronger than crude palm oil approximately 5.9 µg mL^{-1} and 15.6 µg mL^{-1}, respectively. According Sinaga *et al*. (2012), high IC_{50} values was below 50 µg mL^{-1}. Some studies reported that high levels of α-carotene and β-Carotene could increase antioxidant activity of a product (Panpipat & Chaijan 2011). Based on the HPLC results, β-carotene concentrations from palm oil was higher than PME. However, the antioxidant activity of PME stronger than crude palm oil. This expected, due to changes trans-β-carotene to cis-β-carotene isomers during transesterification process. Levin and Mokady (1994), reported that 9-cis-β-carotene has a higher antioxidant potency than that of the all-trans isomer.

Table 3 DPPH antioxidant activity of carotene extract from CPO and PME

Concentrations (µg mL^{-1})	Antioxidant Activity (%)	
	CPO	PME
1.5	7.5	15.9
3	12.3	26.4
4.5	17.9	36.5
6	22.6	54.8
7.5	24.8	63.9
9	30.1	69.3

Carotene concentrate from PME has antioxidant activity stronger than CPO. Possibility carotene does not act as free radical scavenger directly due to high triglyceride content. However, both sources of carotene concentrate has high antioxidant activity and further we conclude that carotene from palm oil is a potential candidate for natural antioxidant.

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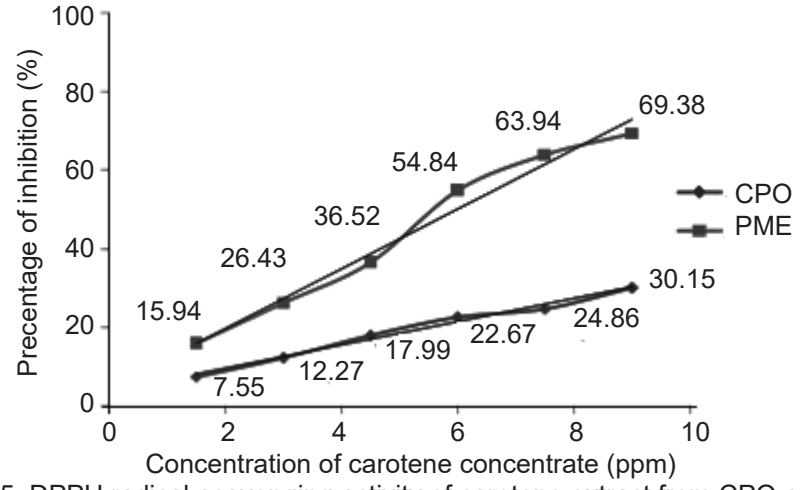

Figure 5 DPPH radical-scavenging activity of carotene extract from CPO and PME.

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