IDENTIFICATION OF CRUDE PALM OILS BY THE ANALYSES OF FATTY ACIDS, VOLATILE ORGANIC COMPOUNDS, AND CAROTENOIDS

Somluk Sanorkham

A Thesis Submitted in Partial Fulfillment of the Requirements
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Miss Somluk Sanorkham

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การบ่งชี้เอกลักษณ์ของน้ำมันปาล์มดิบด้วยการวิเคราะห์กรดไขมัน กลุ่มสารประกอบอินทรีย์ระเหย และแคโรทีนอยด์

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การวิเคราะห์เชิงลึกของน้ำมันปาล์มดิบมีความสำคัญต่อการแยกความแตกต่างของน้ำมัน ปาล์มดิบจากแหล่งทางภูมิศาสตร์ที่แตกต่างกัน เพื่อวัตถุประสงค์ในการควบคุมการบริหารเพื่อป้องกันและแก้ไขปัญหาการลักลอบนำเข้าน้ำมันปาล์มดิบจากต่างประเทศมายังประเทศไทย ในงานวิจัยนี้ได้ศึกษาน้ำมันปาล์มดิบจำนวน 11 ตัวอย่างจาก 5 จังหวัดในประเทศไทย ด้วยการศึกษาองค์ประกอบของกรดไขมัน สารประกอบอินทรีย์ระเหยง่าย และแคโรทีนอยด์ด้วยเทคนิค GC-FID, GC-MS และ HPLC-DAD ตามลำดับ จากการศึกษาพบว่า ตัวอย่างของน้ำมันปาล์มดิบในงานวิจัยนี้มีโครมาโตแกรมของกรดไขมันที่เหมือนกัน ซึ่งองค์ประกอบหลักของกรดไขมันคือกรดปาล์มมิติก กรดโอเลอิค กรดลิโนเลอิคและกรดสเตียริค รวมถึงองค์ประกอบของกรดไขมันส่วนน้อยนั้นพบในปริมาณที่ใกล้เคียงกันมากในทุกตัวอย่างน้ำมันปาล์มดิบ ในการศึกษาองค์ประกอบของสารประกอบอินทรีย์ระเหยง่ายพบว่า สารประกอบอินทรีย์ระเหยง่ายของน้ำมันปาล์มดิบที่ต่างกันมีโครมาโตแกรมที่เหมือนกัน ในการศึกษาแคโรทีนอยด์ในน้ำมันปาล์มดิบ พบว่า โครมาโตแกรมของแคโรทีนอยด์มีความคล้ายคลึงกันมากในทุกตัวอย่างน้ำมันปาล์มดิบ โดยมีอัตราส่วนของแอลฟ่าต่อเบต้าแคโรทีนอยู่ในช่วง 0.70-0.91 และมีค่าความคลาดเคลื่อน ±0.02 ซึ่งมีความเสถียรที่สูงและมีศักยภาพในการนำไปใช้บ่งชี้ชนิดของน้ำมันปาล์มดิบ อย่างไรก็ดีอัตราส่วนนี้ของน้ำมันปาล์มดิบที่มาจากแหล่งต่างกันมีค่าใกล้เคียงกัน ดังนั้นจึงต้องมีการศึกษาตัวอย่างน้ำมันปาล์มดิบที่ระบุสายพันธุ์ แหล่งที่มาและฤดูกาลเพิ่มเติม เพื่อยืนยันในการบ่งชี้ความแตกต่างของน้ำมันปาล์มดิบจากแหล่งที่ต่างกัน

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Somluk Sanorkham : Identification of Crude Palm Oils by the Analyses of Fatty Acids, Volatile Organic Compounds, and Carotenoids. Advisor: Assoc. Prof. SIRIPORN JONGPATIWUT, Ph.D.

An in-depth analysis of crude palm oil is important to differentiate crude palm oil (CPO) from different geographic provenance for administrative control purposes to prevent and solve the problem of smuggling CPO from abroad to Thailand. In this study, eleven CPO samples from five provinces in Thailand were studied for their composition. The compositions of fatty acids, volatile organic compounds (VOCs), and carotenoids were characterized by GC-FID, GC-MS, and HPLC-DAD techniques, respectively. The chromatograms of fatty acids distribution were similar for all samples. The main components of fatty acids are palmitic acid, oleic acid, linoleic acid, and stearic acid. In the study of volatile organic compounds, it was found that volatile organic compounds chromatograms of different CPO were similar. Moreover, the chromatograms of the carotenoids were similar in all CPO samples. The alpha to beta-carotenes ratios were in the range of 0.70 to 0.91 with ±0.02 of the tolerance. This ratio can be considered as a characteristic of a CPO. However, the ratios of CPOs from some sources were similar. Hence, more CPO samples with specified species, locations, and seasons must be further investigated to develop the identification of CPO.

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CHAPTER 1
INTRODUCTION

Palm oil is the most consumed vegetable oil in the world which provides the ingredients for edible products, personal care products, and biodiesel. In Thailand, when the market price fell down, the Thai government subsidizes the crude palm oil (CPO) price for domestic usage which making CPO price in Thailand higher than in neighboring countries. For this incentive, the domestic selling price may cause the problem of smuggling CPO imports from abroad.

In recent years, identification of CPO by their geographical provenance is challenging and interesting because CPO has several factors affecting its composition (i.e. genetic variety, growing conditions, seasonal variation, fertilization regime, oil processing techniques and etc.) (Tres and van Ruth, 2011). The fatty acid composition and carotenoid contents in CPO have different amounts for each region (Clegg, 1973). The characterization of CPO by geographical origin on continental scales (South-East Asia vs. South America vs. Africa) using fatty acid composition (Obisesan *et al.*, 2017; Pérez-Castaño *et al.*, 2015; Ruiz-Samblás *et al.*, 2013; Tres *et al.*, 2013) and regional scales (Central Malaysia vs. Northern Malaysia vs. East coast Malaysia vs. Southern Malaysia vs East Malaysia) using stable isotope profile (Muhammad *et al.*, 2018a) were successfully used fingerprinting techniques with chemometrics such as principal component analysis (PCA) and partial least square discrimination analysis (PLS-DA). Besides, volatile organic compounds (VOCs) was unique for CPO in Peninsular and Sabah of Malaysia using Gas Chromatography - Ion Mobility Spectrometry (GC-IMS) combined with chemometrics (Goggin *et al.*, 2020). In addition, the major compounds of fatty acid methyl esters (FAMEs) of CPO were similar in different countries (Cameroon, Ghana, and Nigeria) but the minor compounds of FAMEs of CPO were different (G. P. P. Kamatou and A. M. Viljoen, 2017).

As discovered by most studies, the aim of this research is to investigate fatty acids, VOCs, and carotenoids in CPOs from different parts in Thailand, i.e. Surat Thani, Krabi, Chumphon, Prachuap Khiri Khan and Kanchanaburi by a gas

chromatograph coupled with a flame ionization detector (GC-FID), a gas chromatograph coupled with mass spectrometry (GC-MS), and a high-performance liquid chromatograph coupled with a diode array detector (HPLC-DAD), respectively.

CHAPTER 2
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Crude Palm Oil

Crude Palm Oil (CPO) is extracted from the mesocarp and kernel of the oil palm fruits which called crude palm oil (CPO) and palm kernel oil (PKO), respectively. Moreover, this oil can be extracted by using different methods of mechanical process (traditional methods, small-scale mechanical units, medium-scale mills and large industrial mills). The quality of CPO depends on the postharvest care especially in the management of the fresh fruit bunches (FFBs) and the process that are implemented.

In general, CPO is being semi-solid at room temperature and the color of CPO is dark orange to red because of high content of carotenoids. Otherwise, PKO has different color from CPO because the content of carotenoids is lower than CPO. The common properties of CPO and PKO is shown **Table 2.1** and **Table 2.2** (Mba *et al.*, 2015).

Generally, The Palm tree is the *Elaeis* genus, this genus has two main species as follows:

1) *Elaeis guineensis (E. guineensis)* originates from West Africa.
2) *Elaeis oleifera (E. oleifera)* originates from South America.

The same somatic chromosome number of 32 is in both *E. guineensis* and *E. oleifera* and easily hybridized to improve their species to meet economic needs and their properties are between their parents.

Today, *E. guineensis* oil palm is economical important species to produce palm oil which can divided into following 3 main parts of fruit forms:

1) Dura such as Deli Dura, Dumpy Dura and African Dura etc.
2) Pisifera such as AVROS (Zaire), La Me (Cote d'Ivoire) and Ekona (Cameroon)
3) Tenera is the result of a cross between the Dura and Pisifera species and use Tenera for trading.

In facts, every country has developed their palm species which have different name. However, popular species of oil palm is Tenera crossbreed.

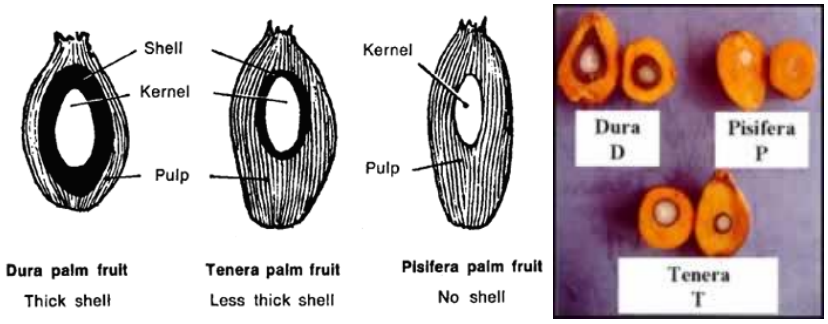


Figure 2.1 Different palm fruit forms (Zou *et al.*, 2012).

Table 2.1 Properties of crude palm oil (MPOB, 2011)

Characteristics	**Range**
Apparent density (g/mL at 50 °C)	0.8889-0.8896
Refractive index (n_D 50 °C)	1.4521-1.4541
Saponification value (mg KOH/g oil)	194-205
Unsaponifiable matter (% by weight)	0.19-0.44
Fatty acid composition (wt% as methyl esters)	
C12:0	0.0-0.5
C14:0	0.9-1.5
C16:0	39.2-45.8
C16:1	0.0-0.4
C18:0	3.7-5.4
C18:1	37.4-44.1
C18:2	8.7-12.5
C18:3	0.0-0.6
C20:0	0.0-0.5
Iodine value (Wijs)	50.4-53.7
Slip melting point (°C)	33.8-39.2
Total carotenoids as β-carotene (mg/kg)	474-689

Table 2.2 Properties of palm kernel oil (MPOB, 2011)

Characteristics	Range
Refractive index (n_D 50 °C)	1.4500-1.4518
Saponification value (mg KOH/g oil)	243-249
Unsaponifiable matter (% by weight)	0.1-0.8
Fatty acid composition (wt%) as methyl esters	
C6:0	0.1-0.5
C8:0	3.4-5.9
C10:0	3.3-4.4
C12:0	46.3-51.1
C14:0	14.3-16.8
C16:0	6.5-8.9
C18:0	1.6-2.6
C18:1	13.2-16.4
C18:2	2.2-3.4
others	Trace -0.9
Iodine value (Wijs)	16.2-19.2
Slip melting point (°C)	25.9-28.5

2.2 Crude Palm Oil Chemical Composition

Palm oil is one of natural fats and oils. The major component is the mixture of triglycerides or triacylglycerols which mainly consist of glycerides and three fatty acids. The minor components contains approximately 1 % which consist of carotenoids, vitamin E (tocopherols and tocotrienols), free fatty acids (FFA), moistures, and other trace components (May, 1994; YCW, 2014).

2.2.1 Fatty Acid of Crude Palm Oil

Fatty acids are long chains aliphatic hydrocarbon containing a carboxylic acid group. The carbon counting of fatty acids begins with the carbon of the carboxylic acid group. The length of fatty acid hydrocarbon chains has mainly even numbers of carbons from C4 to C28 and there are 3 parts to describe different types of fatty acid as follows:

(a) The length of the hydrocarbon tails divided to the number of carbons atom.

- Short-chain fatty acids (SCFA) are fatty acids carbon fewer than six atoms, i.e., butyric acid.
- Medium-chain fatty acids (MCFA) are fatty acids with carbons between 6 to 12 atoms.
- Long-chain fatty acids (LCFA) are fatty acids with carbons between 13 to 21 atoms.
- Very long chain fatty acids (VLCFA) are fatty acids carbons longer than 22 atoms.

(b) The degree of unsaturation or double bond is one parameter affecting the characteristic of fats and oils which can describe the degree of unsaturation as follows:

- Saturated fatty acid is fatty acid without double bond in their structure and most saturated fatty acids in nature have an even number of carbon atoms in unbranched structures.
- Unsaturated fatty acid is fatty acid with one or more double bond in the hydrocarbon chain length which can be further categorized as monosaturated fatty acids containing one double bond while polyunsaturated fatty acids containing two or more double bonds in its structure. Besides, the present of double bond in fatty acid molecule introduce a complication because a carbon atom can easily rotate around its single bond to its neighbor. However, the position of double bond is fixed between the two carbons that they link which have two possible configurations depending on the relative positions of hydrocarbon chain of the fatty acid that are cis and trans isomers. The most naturally occurring unsaturated fatty acid exists is cis isomers which have a lower melting point comparing to trans isomers. The molecular structure of cis and trans are shown in **Figure 2.2**.

(c) The position of the double bonds is defining the type of fatty acid and its functionality. The omega carbon or n is carbon number at the methyl end which indicates the different fatty acid. For example, C18:3 linolenic fatty acids in **Table 2.3**. alpha-linolenic acid (18:3 n3) which has double bonds positions at 9, 12, 15 in fatty acid chain and omega carbon at position 3. Whereas gamma-linolenic acid which has double bonds position at 6, 9, 15 in fatty acid chain and omega carbon at position 6. Since fatty acids occurring in nature fats are different lead to different triglycerides are found. Therefore, triglycerides are named by fatty acid that they contain. For example, triolein is the triglyceride of oleic acid, tripalmitic is triglyceride of palmitic. On the other hands, mono palmitin-distearin is one molecule of palmitic acid and two of stearic acid. In the nature, the various fatty acid are found, but only important commercial fatty acid are palmitic acid, oleic acid, stearic acid, linoleic acid, linolenic acid, lauric acid and myristic acid (Tinker, 2016; YCW, 2014).

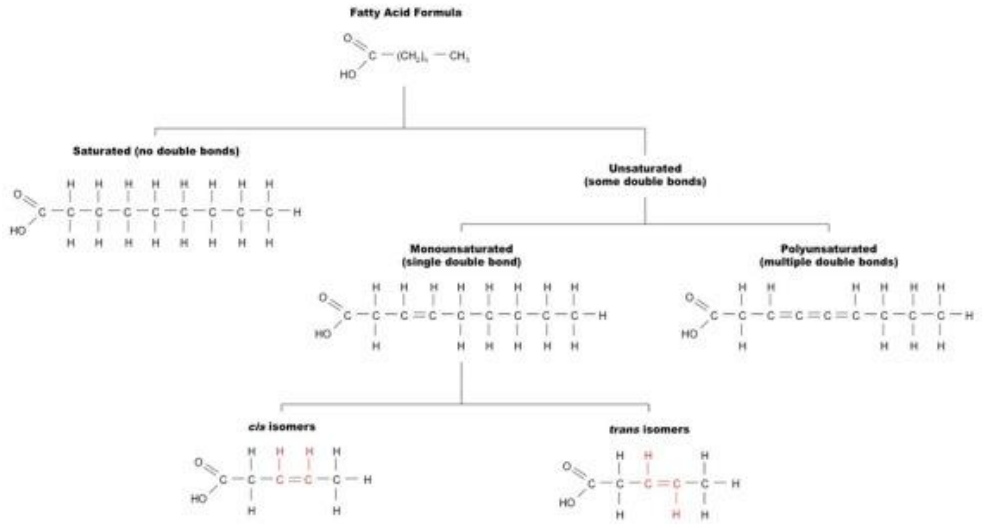
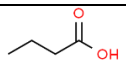
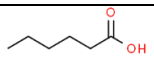
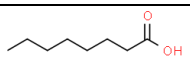
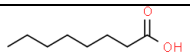
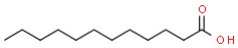
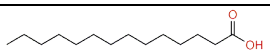
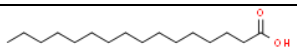
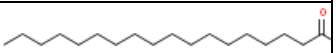
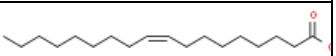
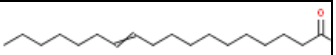
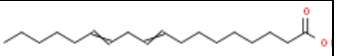
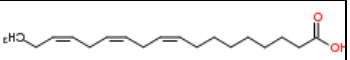
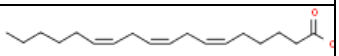


Figure 2.2 Types of fatty acids (YCW.,2014).

Table 2.3 Chemical names and descriptions of some common fatty acid (YCW.,2014)

Common Name	Carbon Atoms	Double Bonds	Scientific Name	Structure
Butyric acid	4	0	Butanoic acid	
Caproic acid	6	0	Hexanoic acid	
Caprylic acid	8	0	Octanoic acid	
Capric acid	10	0	Decanoic acid	
Lauric acid	12	0	Dodecanoic acid	
Myristic acid	14	0	Tetradecanoic acid	
Palmitic acid	16	0	Hexadecenoic acid	
Palmitoleic acid	16	1	9-hexadecenoic acid	
Stearic acid	18	0	Octadecanoic acid	
Oleic acid	18	1	9-octadecenoic acid	
Vaccenic acid	18	1	11-octadecenoic acid	
Linoleic acid	18	2	9,12-octadecadienoic acid	
Alpha-linolenic acid (ALA)	18	3	9,12,15-octadecatrienoic acid	
Gamma-linolenic acid (GLA)	18	6	6,9,12-octadecatrienoic acid	

Clegg (1973) reported fatty acid composition of palm oil in different species and country, the results show that palmitic acid and oleic acid content in *E. guineensis* oil from Malaysia, Indonesia and Zaire is the major content along with fatty acid contents varied as shown in **Table 2.4**. Comparing fatty acid contents of palm oil from Zaire between Clegg (1973) and Loncin and Jacobsberg (1963), it was found that fatty acid contents were in the same range. It can conclude that fatty acid contents of palm oil are varied between country while the same country have the same contents of fatty acid in palm oil. Furthermore, Clegg (1973) also reported fatty acid composition in different species of palm oil, it indicates that fatty acid composition of *E.oleifera* oil are rather different from *E.guineensis* oil because unsaturated fatty acid percentage of *E.oleifera* oil are higher than *E.guineensis* oil and *E. guineensis x E. Oleifera* oil have the contents between them as shown in **Table 2.5**.

Table 2.4 Fatty acid composition of palm oil in different countries (Clegg, 1973)

Fatty acid composition of palm oil (percentage of total acids)				
Fatty acid	**Commercially product (all source)**	**Zaire**	**Indonesia**	**Malaysia**
		Range	**Range**	**Range**
Lauric	trace	0	0-Trace	0-Trace
Myristic	0.5-0.9	1.2-2.4	0.4-0.8	0.5-0.8
Palmitic	32-51	41-43	46-50	46-51
Stearic	2-8	4.4-6.3	2-4	1.5-3.5
Oleic	38-52	38-40.2	38-42	40-42
Linoleic	5-11	9.9-11.2	6-8	6-8
Iodine value	44-54	-	49-53	50-54

Table 2.5 Fatty acid composition of palm oil in different species (Hardon, 1969)

Fatty acid	Percentage of total fatty acid			
	E. Oleifera	E. Oleifera x E. guineensis		
	Congo	Congo	Malaya	Colombia
Lauric	0.05	0.01	0.1	0.07
Myristic	0.3	0.47	0.9	0.77
Palmitic	25.0	31.7	32.5	27.3
Stearic	1.2	4.1	3.4	6.1
Arachidic	0.1	0.11	0.1	0
Palmitoleic	1.4	0.05	0.2	0.45
Oleic	68.6	49.5	48.0	58.5
Linoleic	2.1	13.4	13.8	11.35
Linolenic	0.9	0.5	0.4	1.3
Iodine value	81.3	70.7	62.0	69.8

Some studies indicate that the fatty acid composition (FA) were varied in several factors in the geographical origin. Tres *et al.* (2013) studied fatty acid composition using GC-FID in continental scale which were Southeast Asia, West Africa and South America. The results show that C12:0 (lauric acid), C16:0 (palmitic acid) or C20:1 n-9 (gondoic acid) considerably varied among the three continents. Some saturated fatty acid such as C12:0 (lauric acid), C14:0 (myristic acid) and C16:0 (palmitic acid) of CPO in Southeast Asia was higher than the other two continents. Monounsaturated fatty acid from the n-9 series such as C18:1 n-9 (oleic acid) and C20:1 n-9 (gondoic acid) in South America were higher than the two continents. CPO from South America has higher contents of oleic acid and lower contents of palmitic acid than two continents which respect to Clegg (1973) and Sambanthamurthi *et al.* (2000). They combined data with chemometric analysis such as Principal Component Analysis (PCA) as shown in **Figure *2.3*** First two factors of the PCA scores plot on the fatty acid fingerprint (auto-scale data) of crude palm oils of three different continents (Tres et al., 2013).. The data of PCA consisted of 94 rows which from samples and 21 variables which from fatty acid. The results showed that the CPO

sample from Southeast Asian probably form a narrower cluster, while CPO sample from African and South American showed more variability that agreed with Clegg (1973) reporting narrow range of fatty acid contents in palm oils from different location in Malaysia

The fatty acid composition were different between continents might because of the differences in the botanical identity (i.e., varieties, breeds), fruit ripeness at harvesting among others, climate and soil conditions (Tres *et al.*, 2013). Also, genetic variation, climate and other geographical are the factors that could be related to this variation and drastically affected by fertilization regime, seasonal variation, and oil processing techniques.

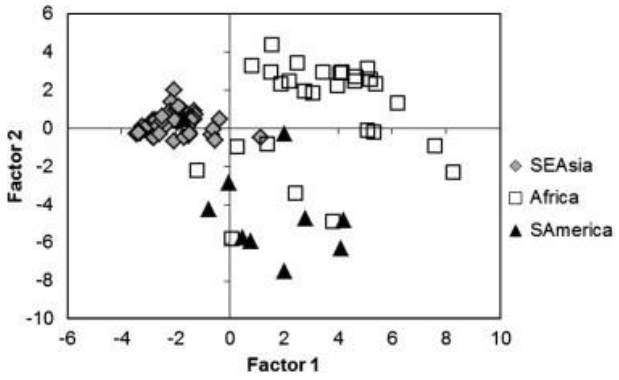


Figure 2.3 First two factors of the PCA scores plot on the fatty acid fingerprint (auto-scale data) of crude palm oils of three different continents (Tres *et al.*, 2013).

Moreover, G. P. P. Kamatou and A. M. Viljoen (2017) compared CPO and PKO from different country in Africa which are Ghana, Nigeria and Cameroon. They analyzed fatty acid composition by transesterification reaction for gas chromatography analysis (GC). The results show that hexadecanoic acid methyl ester, Stearic acid methyl ester, (Z)-9-Octadecadienoic acid methyl ester, (Z, Z)-9-12-Octadecadienoic acid methyl ester were found and perform as the major components of fatty acid methyl esters (FAMEs) in CPO from different country. Also, 2-Pentanoic acid-2-methoxy-3-methyl-methyl ester, 6-Octadecenoic acid methyl ester, 2,6-Dimethyl-8-oxoocta-2,6-dienoic acid methyl ester and Unknown 2 could be distinguished crude palm oil of Cameroon from Ghana and Nigeria. When focusing on the results of Cameroon CPO extracted from different method, it was found that Unknown 2 and Tetradecanoic acid,2-oxo-, methyl ester could be differentiated raw

palm oil from refined palm oil. Unknown 4 was found only in Ghana, not in Nigeria and Cameroon. Tetradecanoic acid methyl ester, Hexadecanoic acid methyl ester, Dodecanoic acid methyl ester and (Z)-9-Octadecanoic acid methyl ester were the major esters of fatty acid and found in both of commercially available and raw palmist oil while (Z, Z, Z)-9,12,15-Octadecadienoic acid methyl ester, Tetradecanoic acid,12-methyl, methyl ester and unknown 4 could be identified and distinguish crude palm oil in both methods.

2.2.2 Volatile Organic Compounds in Palm Oil

Volatile Organic compounds (VOCs) are organic chemical compounds that easy to evaporate at room temperature. There are few studies about VOCs in crude palm oil. Dirinck *et al.* (2006) studied VOCs of CPO using GC-FID and GC-MS. They found that palm oil volatiles are given in Error! Reference source not found.**Figure 2.5.** the important compounds are alkanals, 2-alkenals and 2,4-alkadienals which were typical oxidation products of triglycerides. It was oxidized by free radical auto-oxidation of unsaturated fatty acids chains such as oleic, linoleic and linolenic acid as shown in **Figure 2.4**. Similarly, Jayasena *et al.* (2013) describes that VOCs could be formed by lipid oxidation. For example, nonanal could be derivatized by C18:1 n9 (oleic acid) or C18:2 n6 (linoleic acid). Moreover, C18:2 n6 (linoleic acid) could be oxidizing to hexanal, 2,4, -decadienal, 2-octenal and nonanal. Moreover, β-ionone is probably formed by oxidation of carotene which is accountable for the deep red color of the palm oil. They identified C5-9 alkanals, C7,9,11, alkenals, C7,9,10 alka-2,4-dienals, C7,9 alka-2-ones, α and β- ionone, and γ-heptalactone in the steam distillate of palm oil. If it is possible, the compound responsible for the odor is indicated.

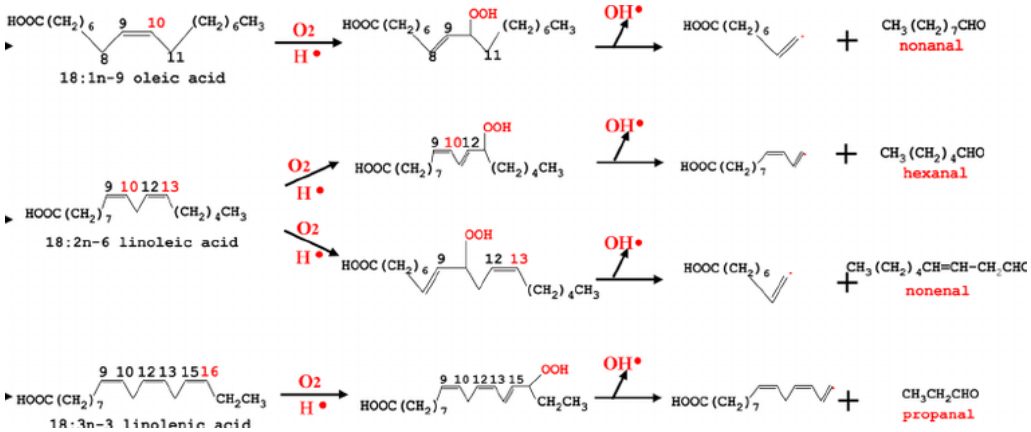


Figure 2.4 Products from oxidation of fatty acid (Cao *et al.*, 2014).

Table 1—Volatile compounds identified in palm oil

Ipr SE$_{30}$	Compounds	Concentration[b]
	Dichloromethane (S)	
600	Ethyl acetate	S
674	n-Pentanal	S
778	n-Hexanal	M
800	Butyl acetate	S
861	Xylene (mixture of m and p)	S
871	2-Heptanone	S
882	o-Xylene	S
	n-Heptanal	S
935	*trans*-2-Heptenal	M
937	Benzaldehyde	M
965	2-Methyl-2-hepten-6-one	M
977	*trans,cis*-2,4-heptadienal	M
984	n-Octanal	M
991	*trans,trans*-2,4-heptadienal	M
1037	*trans*-2-octenal	L
1060	Decalin[a]	S
1073	2-Nonanone	S
1086	n-Nonanal	L
1090	Linalol	M
1098	Oxygenated monoterpene (M.W. 154)	M
1137	p-Cymeen[a]	S
1142	*trans*-2-Nonenal	M
1184	α-Terpineol	S
1195	*trans,trans*-2,4-Nonadienal	M
1209	Oxygenated monoterpene (M.W. 152)	M
1242	*trans*-2-Decenal	L
1250	Oxygenated monoterpene (M.W. 152)	M
1275	*trans,cis*-2,4-Decadienal	L
1295	*trans,trans*-2,4-Decadienal	L
1331	γ-Heptalactone	M
1343	*trans*-2-Undecenal	L
1418	α-Ionone	M
1475	β-Ionone	L
1520	Unknown (sesquiterpene hydrocarbon)	L
1681	2-Pentadecanone	M
—	Ethyl myristate	M
	Methyl pentadecanoate	M

[a] Tentative identifications
[b] Concentration: arbitrary peak height scale: L = large, M = medium, S = small.

Figure 2.5 Volatile compounds identified in palm oil (Dirinck *et al.*, 2006).

Kuntom *et al.* (1989) were studied palm oil oxidation using the headspace technique of adsorption on Tenax to monitor the development of volatile compounds in palm oil samples. The result found that the most important compounds in the volatile compounds of oxidized palm oil were C4-9alkanals, C5-8 trans-2-

alkenals, C1,2,4,5 2-alkyls furans, and aliphatic and aromatic hydrocarbons. The dominant aldehyde was n-hexanal (Sambanthamurthi *et al.*, 2000). Also, they studied crude and oxidized palm oil were steam-distilled in vacuum to obtain volatile aroma compounds. The results showed that the steam distillate from CPO contained compounds such as 2,2,6-trimethylcyclohexanone, 3,3,5-trimethylcyclohex-2-enone, nonanone, nonanal, ethyl benzoate, linalol, trans-allo-ocimene, β-cyclocitral and ionol.

Tres *et al.* (2013) studied VOCs profile of headspace samples of three CPO in continental scales (Southeast Asia VS Africa VS South America) using proton transfer reaction – mass spectrometry (PTR-MS). **Figure 2.6** show that the mass to charge ratio (m/z) of CPO were different and intensity in different continental scales, but PTR-MS is not proposed to identify the volatile compounds which was due to several compounds might yields the same m/z signal because it is probable that m/z intensities might be uncertainly corresponds to other compounds. Most of abundant m/z found in CPO samples were m/z 33, m/z 43, m/z 59, m/z 61, m/z 73, m/z 75 and m/z 83 which are highly probable related to a fragment of larger compounds, aliphatic alcohols, a fragment of hexenol or propanal, acetates, butanal or butanone, propanoates and the main ion formed from hexanal and hexenol or hexenyl acetate, respectively.

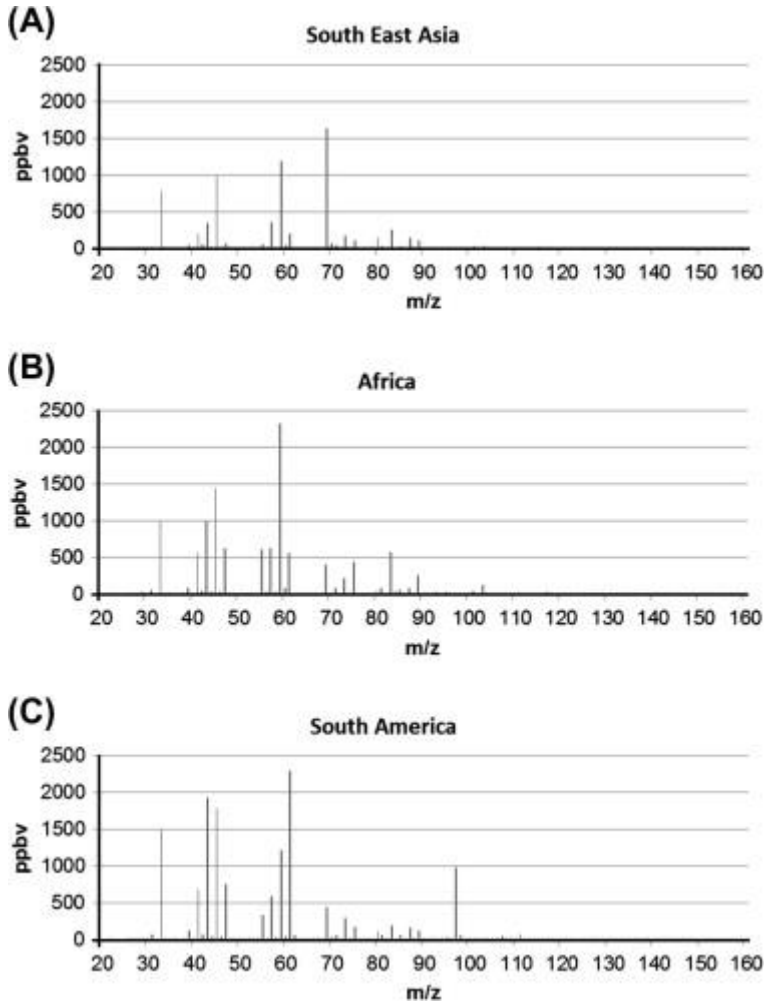


Figure 2.6 Volatile profile of three crude palm oil samples originating from (A) South East Asia, (B) Africa and (C) South America, assessed by PTR-MS (Tres *et al.*, 2013).

Furthermore, Goggin *et al.* (2020) used Gas Chromatography - Ion Mobility Spectrometry (GC-IMS) to investigate VOC fingerprinting of CPO samples in Malaysia (Peninsular and Sabah). The results as shown in **Figure 2.7**. 3D nature of each spectrum contains around 11 million data points that were difficult and unproductive. Likewise, it has less powerful to differentiate CPO by their visual. There was no correlation between specific features and individual spectral peaks because important signals were not possible apparent above the noise which making the identification of compounds and picking peaks were difficult. Nevertheless, GC-

IMS technique combined with chemometrics was feasible to be fingerprinting for discriminating CPO samples from Sabah and Peninsular Malaysia.

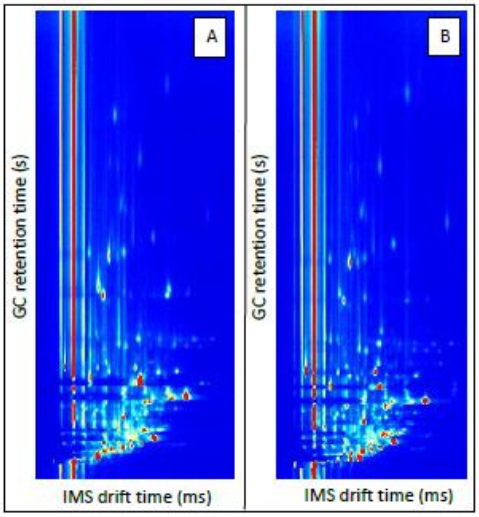


Figure 2.7 VOCs profiles of typical GC-IMS spectra from (a) Sabah and (b) Peninsular Malaysia crude palm oil samples (Goggin *et al.*, 2020).

2.2.3 Carotenoids

Carotenoids are the major pigment in the living organism with red to orange colors (May, 1994). The structure of carotenoids are unsaturated tetraterpenes (C40) biosynthesized from eight isoprene units. All trans were their favored state. Carotenoids are divided into two main classes which are carotenes and xanthophylls. Carotenes are strictly polyene hydrocarbons, and xanthophylls containing oxygen which may be in the form of hydroxy (e.g., zeaxanthin and lutein), keto, epoxy, or carboxyl groups. The chromophore group in carotene structure is characterized by conjugated double bonds which is accountable for light absorption. The total carotenoids in palm oil are usually determined by ultraviolet-visible spectroscopy at 446 nanometers as ppm of β-carotene. **Figure 2.8** shows some of carotenoid's structure.

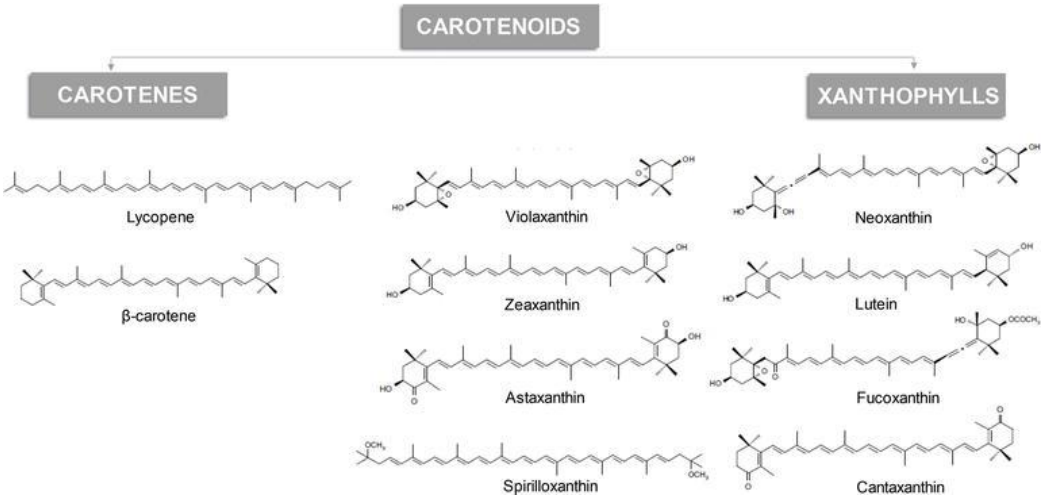


Figure 2.8 The chemical structure of carotenoids and xanthophylls (Fernandes *et al.*, 2018).

CPO has a rich orange-red color due to its high carotene contents. CPO extracted commercially by sterilization and press containing 500–700 ppm of carotenoids. The variation of carotenoids contents is due to process conditions, species of oil palm and level of oxidation.

Clegg (1973) reported carotenoid content of palm oil varied between countries as shown in **Table 2.6** and **Table 2.7** Composition of crude palm oil carotenoids in different countries (Clegg, 1973). It is feasible that part of this variation is genetic variety and the ripeness standards for harvesting within and between the different countries, although climate and other geographical factors are also be affected.

Table 2.6 Carotenoids contents in different countries (Clegg, 1973)

Country of origin	**Carotenoids, ppm**
Plantation of oil	
Malaysia	500-700
Indonesia	400-600
Zaire	500-700
Ivory coast	390-610
Palm grove oils	
Nigeria	800-1600
Ivory coast	790-1400
Togo	1310-1480
Dahomey	910-1520

Table 2.7 Composition of crude palm oil carotenoids in different countries (Clegg, 1973)

Carotenoids	**Percentages of total carotenoids**		
	Zaire	Dahomey	Togo
α-carotene	36.2	85	87
β-carotene	54.4		
γ-carotene	3.3	15	13
Lycopene	3.8		
Xanthophyll	2.2		

The types and composition of carotenoids extracted from different species of CPO were studied by Yap *et al.* (1991) as shown in **Table 2.8**. They found 13 types of carotenoids. β - and α -carotenes were the major components which account for 90% of the total carotenoids. The types of carotenoids were found in oil palm species (E. oleifera and E. guineensis, and their hybrids and backcrosses to E. guineensis) were similar.

Table 2.8 The composition of carotenoids in palm oil (Yap *et al.*, 1991)

Types	**E. guineensis (Eg)**	**E. oleifera (Muhammad *et al.*, 2018a)**	**Eg x Eo hybrids**
Phytoene	1.27	1.12	1.83
Cis-β-carotene	0.68	0.48	0.38
Phytofluene	0.06	Trace	Trace
β-carotene	56.02	54.08	60.53
α-carotene	35.06	40.38	32.78
Cis-α-carotene	2.49	2.30	1.37
ξ-carotene	0.69	0.36	1.13
γ-carotene	0.33	0.08	0.23
δ-carotene	0.83	0.09	0.24
Neurosporene	0.29	0.04	0.23
β-zeacarotene	0.74	0.57	1.03
α-zeacarotene	0.23	0.43	0.35
Lycopene	1.30	0.07	0.05
Total (ppm)	500-700	4300-4600	1250-1800

Peng (2006) studied profile of carotenoid extracts from different part of palm by HPLC-UV techniques, it was found that palm carotenes elution sequence to be lutein, neurosporene (trans), neurosporene (cis), α-zeacarotene (cis), α-zeacarotene (trans), α-zeacarotene (cis), phytoene; phytofluene; β-zeacarotene; 13 and 13’ cis α-carotene; 13 cis β-carotene, trans α-carotene; 9 cis α-carotene, trans β-carotene, δ-carotene a (cis), δ-carotene b (cis), δ-carotene (trans); γ-carotene a (cis); γ-carotene (trans); γ-carotene b (cis), lycopene (cis) and lycopene (trans) as shown in **Figure 2.9** and **Figure 2.10**. The highest peak is trans β-carotene and α-carotene which represent the amount and correspond to previous work.

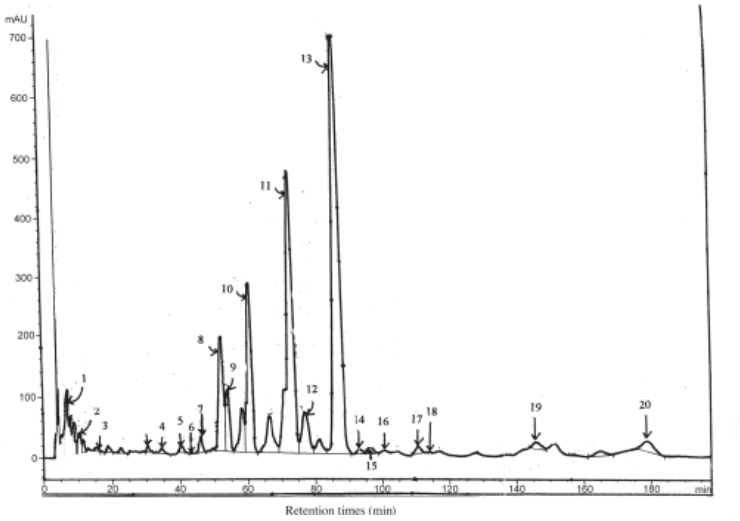


Figure 2.9 C30 Chromatographic profile of carotenoids extracts from palm pressed fibers: 1) lutein, 2) neurosporene (trans), 3) neurosporene (cis), 4) α-zeacarotene (cis), 5) phytoene, 6) phytofluene, 7) β-zeacarotene, 8) 13 cis α-carotene, 9) 13' cis α-carotene, 10) 13 cis β-carotene, 11) trans α-carotene, 12) 9 cis α-carotene, 13) trans β-carotene, 14) δ-carotene a (cis), 15) δ-carotene b (cis), 16) δ-carotene (trans); γ-carotene a (cis), 17) γ-carotene b (cis), 18) γ-carotene (trans), 19) lycopene (cis) and 20) lycopene (trans) (Peng, 2006).

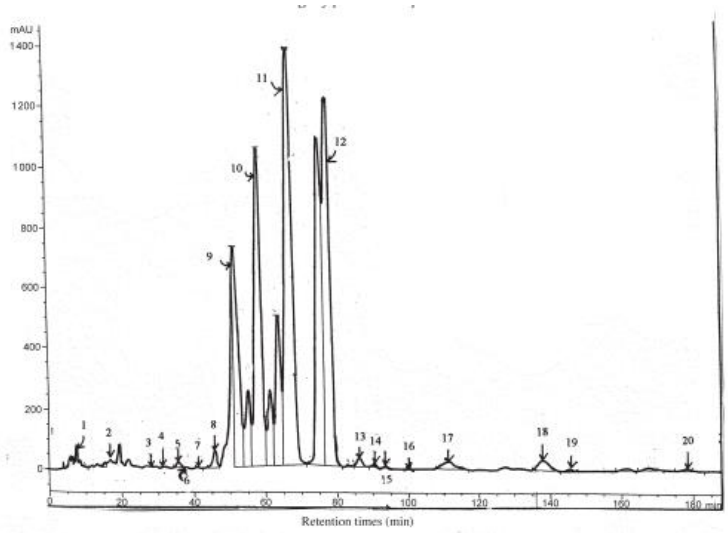


Figure 2.10 C30 Chromatographic profile of CPO extracts: 1) lutein, 2) neurosporene (cis), 3) α-zeacarotene (cis), 4) α-zeacarotene (trans), 5) α-zeacarotene (cis), 6) phytoene, 7) phytofluene, 8) β-zeacarotene, 9) 13 cis α-carotene, 10) 13 cis β-

carotene, 11) trans α-carotene, 12) trans β-carotene, 13) 9 cis β-carotene, 14) δ-carotene a (cis), 15) δ-carotene b (cis), 16) δ-carotene (trans), 17) γ-carotene a (cis), 18) γ-carotene (cis), 19) lycopene (cis) and 20) lycopene (trans) (Peng, 2006).

On the other hand, Santos *et al.* (2015) studied carotenoid contents in palm oils from different types of oil palm in Brazilian amazon showed highly variable amounts, with pupunha, buriti and tucumã containing the highest contents from the analyzed species in their work.

In addition, carotenes are sensitive to oxygen and light. It might have degraded during palm oil extraction and storage, producing more volatile degradation compounds (Tres *et al.*, 2013). Zou *et al.* (2012) reported that carotenoids degradation in palm oil occurred by oxidation when they were under 100°C because oxidation of carotenes is accelerated by hydroperoxides generated from lipid oxidation, leading to discoloration and bleaching. When temperature is higher, heat is more influential affected to degradation. Heating palm oil at 160, 180 and 200°C for 20 minutes cause the loss of β-carotene more than 35, 50 and 85%, respectively. However, the stability of β-carotene storages can persist for 48 hours in the dark because ultraviolet and visible light destroyed β-carotene while they were contacted with oxygen in air. The among of products formed from the oxidative deterioration of carotenoids are α- and β-ionones, β-13 and β-14-apocarotenals and β-13-apocrotenone as shown in **Figure 2.11** (Sambanthamurthi *et al.*, 2000; YCW, 2014).

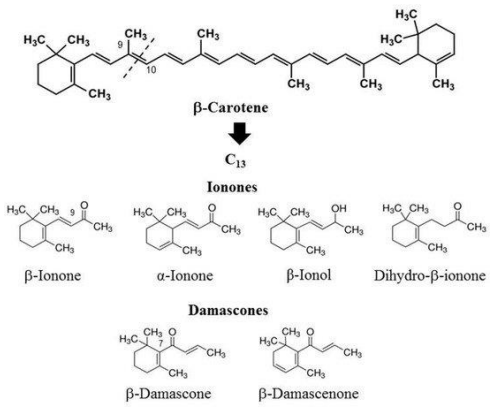


Figure 2.11 Example products of β -carotenes oxidation (Popova *et al.*, 2019).

2.3 Crude Palm Oil Identification and Characterization

Crude palm oil fingerprinting is used for finding the geographical origin region. Nowadays, there are few studies that show different techniques to analyze fingerprinting of crude palm oil, for example, gas chromatography – flame ionization detector (GC-FID), gas chromatography – mass spectrometry (GC-MS), high performance chromatography (HPLC) and ultra violet (UV) spectroscopy (Goggin *et al.*, 2020; Obisesan *et al.*, 2017; Pérez-Castaño *et al.*, 2015; Ruiz-Samblás *et al.*, 2013; Tres *et al.*, 2013). However, the most extensively techniques are GC technique to find the fatty acid composition.

2.3.1 GC-FID

Chromatography is an analytical technique to separate a compound mixture into their individual components according to their volatility. There are many types of chromatography such as liquid chromatography (LG), gas chromatography (GC), ion-exchange chromatography, but all of these operate with same principles. The gas chromatography (GC) is the most important one for oil fingerprinting since it has many advantages. For example, the result of GC show both qualitative (identify component) and quantitative (measuring amount of sample component).

The gas chromatography (GC) is one of chromatography techniques in which the component of a sample separates between two phases (the stationary phase and the mobile phase). Normally, GC has three main parts, which are injector, column, and detector. The sample solution is injected into instrument by injector and volatilized into vapor phase. Then, the sample stream is carried to column by carrier gas such as Helium or Nitrogen in order to separate various components. After that, the separated components are detected and measured by detector (Moustafa and Morsi, 2012). **Figure 2.12** shows a photograph of a typical GC.

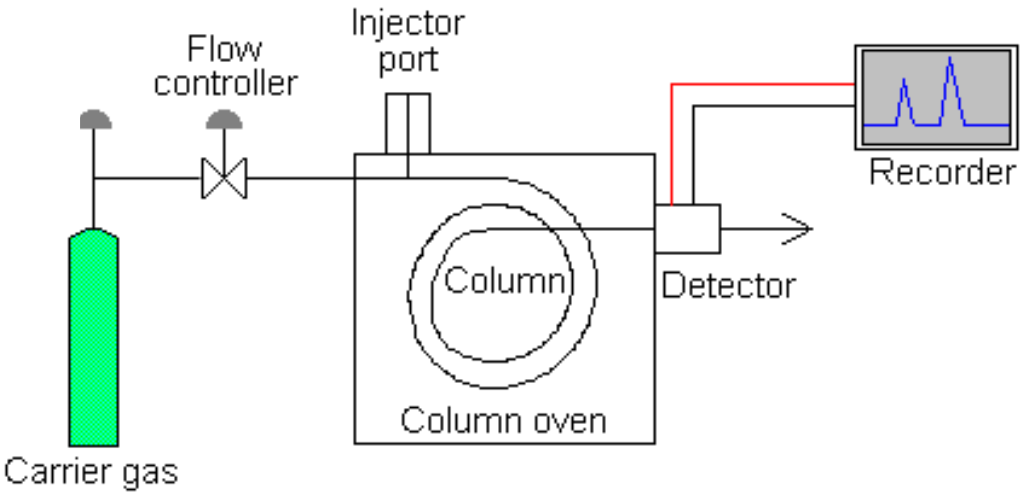


Figure 2.12 Typical Gas chromatography (GC) instrument (Moustafa and Morsi, 2012).

The heart of this system is column where the separation of various component take place. In column, there are stationary phase and packed material, which influence to the separation and affect the time of separation (retention time). Columns are classified as either capillary or packed columns. Generally, the capillary columns give better fine structure chromatographic fingerprinting, and it is vastly used in environmental forensic than packed column. The column is placed in an oven where temperature can be controlled precisely over a wide range of temperature. The photograph of capillary and packed column is shown in **Figure 2.13** and **Figure 2.14** For the detector of GC, there are many types of detectors and the choice is based on application. Most of detectors that use in GC were especially invented for this technique. Mass spectroscopy is one of detector, which is widely used in GC technique. It provides the most information, both qualitative identification of various compound and quantitative analysis, with only few micrograms of sample. When GC link with MS, Electron ionization (EI) or chemical ionization (CI) will ionizes the compounds that eluted from GC column into charge. Then, charge fragments are accelerated into mass analyzer. The different of mass to charge ratio will generate different signals. Thus, the various compound that produces ions within the range of mass analyzer will be detected. However, in complex sample sometimes their

distribution pattern quite difficult to detect since co-eluted interfering of component could obstruct the accurate information from GC. Thus, there are many research which develop GC-MS. In this research will focus on GCxGC-TOFMS because it significantly reduces the interference of some component in same retention time (Piantanida and Barron, 2014).

Figure 2.13 Capillary column of gas chromatography (Piantanida and Barron, 2014).

Figure 2.14 Packed column of gas chromatography (Piantanida and Barron, 2014).

2.3.2 GCxGC-TOFMS

In the past few years, two-dimensional gas chromatography (GCxGC) has been estimated for oil identification. The advantage of TOFs (time of flight) is good separation and high sensitivity for the detection and identification. Moreover, TOFMS detector will be complementary to conventional GC-FID. GCxGC consists of two columns, which in the first column is a non-polar column, and the second one is a polar column. However, some researches are claimed that the opposite sequence allow a better result of non-polar component. The use of two columns of GCxGC expands the chromatogram distribution. The key to these is thermal modulator, which place between two columns. It ensures that all of stream from the first column is properly reach temperature before release into the second column. Figure 2.16 shows diagram of GCxGC-TOFMS. For the part of detector, analytical bands that eluted from the first column are significantly sharpen before it is released into the second column. The narrow peak requires a detector that can collect data at rate 100 Hz. Hence, TOFMS is suitable since it provides mass spectral acquisition rate up to 500 Hz (LECO, 2014).

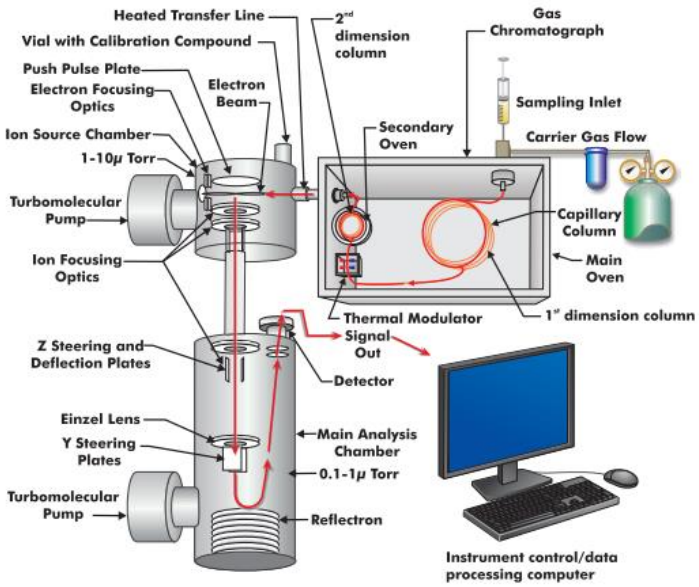


Figure 2.15 Diagram of GCxGC-TOFMS (LECO, 2014).

2.3.3 HPLC-DAD

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. HPLC operate under the same basic principle of chromatography as GC. The separation involves a stationary phase (solid, or liquid supported on a solid) and a mobile phase (liquid or gas). It works by mobile phase flow through the stationary phase and carries the component of the mixture with it. Sample components that have similar polar to stationary phase will have stronger interactions with stationary phase and move more slowly to the column than components with weaker interactions. The types of HPLC depend on the stationary phase in the process such as Normal phase, Reverse phase, Size exclusion and Ion exchange HPLC. Normally, the instrument includes a pump, injector, column, detector, and acquisition and display system as shown in **Figure 2.16**.

Column represents the heart of HPLC system when separation occurs, A pump aspirates the mobile phase contained in a glass reservoir, and then forces it through the column and detector. When the sample injector injects the sample into the column, it enters into the detector to detect the analytes that elute from chromatographic column. Commonly, detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors. Data collection from the detector are collected as signals on chart recorders (Team, 2020).

UV detector for HPLC measures the absorbance of monochromatic light of fixed wavelength in the UV or visible wavelength range. Typically, between 190 nm and 400 nm. Analytes suitable for UV detection contain unsaturated bonds, aromatic groups or functional groups containing heteroatoms. Polychromatic light from a deuterium (UV) or tungsten (visible) lamp is focused onto the entrance slit of a monochromator which selectively transmits a narrow band of selected wavelength. Using photodiode array with reference photodiode which known as a variable wavelength detector. **Figure 2.17** shows the diagram of UV detector. In diode-array detection (DAD), radiation is dispersed via a holographic grating into individual wavelength of light. Each photodiode receives a different narrow wavelength band, and a complete spectrum may be obtained for any point within the chromatogram because of a single or narrow range of wavelengths. So, targeted analysis, component identification, and the simultaneous quantitative analysis of signals at several discrete

wavelengths may be achieved and using reference wavelength in diode-array mode can help to remove signal contributions (noise and baseline drift) (Taylor, 2015).

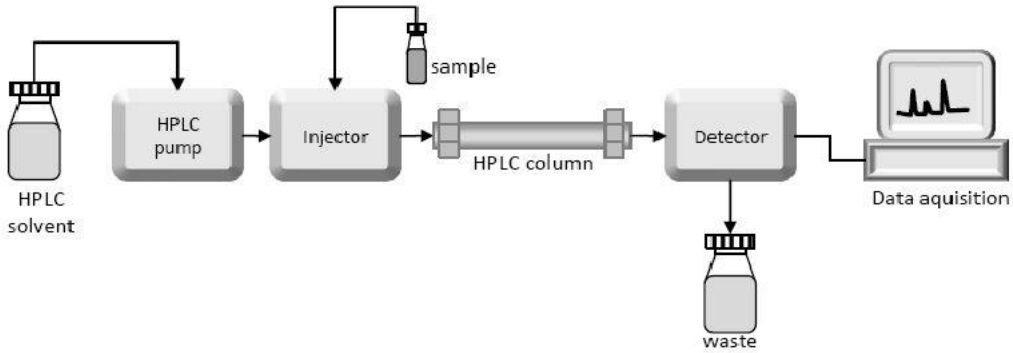


Figure 2.16 Schematic diagram of HPLC (Team, 2020).

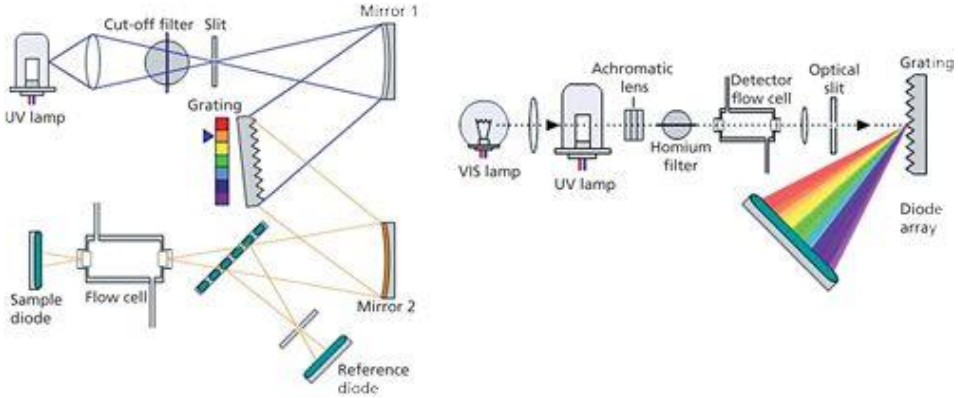


Figure 2.17 The diagram of UV detector (Taylor, 2015).

CHAPTER 3
METHODOLOGY

3.1 Objectives

1. To study the distribution of chemical fingerprinting in crude palm oil from different parts of Thailand.

2. To differentiate crude palm oil using fatty acid composition, volatile organic compounds (VOCs) and carotenoids by GC-FID, GC-MS, and HPLC-DAD, respectively.

3.2 Scope of research

To obtain the objectives of research, the following scope of work is proposed:

1. Crude palm oils were obtained from the Department of Business Energy, Ministry of Energy.
2. Testing crude palm oil
   - Transesterification of CPO to be fatty acid methyl esters (FAMEs) before testing with gas chromatography equipped with FID detector (GC-FID.
   - Headspace sample of CPO before testing with 2-dimensional gas chromatography equipped with a time-of-flight mass spectroscopy (GCxGC TOFMS).
   - Carotenoids dissolve in mobile phase before testing with high performance liquid chromatography with a diode array detector (HPLC-DAD).
   - Using GC-FID to provide the information about fatty acid distributions.
   - Using GCxGC TOF MS to provide the information about VOCs.
   - Using HPLC-DAD to analyze carotenoids.
3. Collecting and analyzing data with ChromaTOF program, LC solution, Chem station, and Microsoft excel.

3.3 Materials and Equipment

3.1.1 Sources of Oil Samples

The oil samples that use in this research are list in **Table 3.1**. All crude palm oils are obtained from Department of Energy Business, Ministry of Energy.

Table 3.1 Information of eleven crude palm oil samples

No	Code	Sample Sources	Description
1	CPO1	Maung, Sarat Thani	Pago Trading Co., Ltd
2	CPO2	Phra Saeng, Surat Thani	S.P.O Agro Industry Co., Ltd
3	CPO3	Phra Saeng, Surat Thani	Bang Sawan Palmoil Co., Ltd
4	CPO4	Ao luek, Krabi	Univanich Palm Oil Public Co., Ltd
5	CPO5	Ao luek, Krabi	Siam Modern Palm Co., Ltd
6	CPO6	Khao Phanom, Krabi	Phattara Palm OIL Co., Ltd
7	CPO7	Tha Sae, Chumphon	Mitcharoen Palm Oil Co., Ltd
8	CPO8	Muang, Chumphon	Jareon Palm Oil Co., Ltd
9	CPO9	Bang Saphan, Prachuap Khiri Khan	Thongmongkol Palm Oil Industry Co., Ltd
10	CPO10	Sam Roi Yot, Prachuap Khiri Khan	Palm Thong Thai Co., Ltd
11	CPO11	Tha Maka, Kanjanaburi	Sathina Palm Co., Ltd

3.1.2 Gases

1. The ultra-high purity (UHP) nitrogen for GC-FID and GCxGC TOFMS.
2. The ultra-high purity (UHP) hydrogen is used for FID detector.
3. The high purity (HP) helium 99.995 % for GC-FID and GCxGC TOFMS.
4. The zero-grade air is used for FID detector.

3.1.3 Chemicals

1. Sodium hydroxide pellet from Merck, Germany
2. n-Haxane with 95% from Fisher Chemical
3. Methanol, AR grade from RCI Labscan
4. Methanol, HPLC grade from RCI Labscan

5. Acetonitrile, isocratic grade from Supelco.
6. Supelco 37 Mix FAMEs standard
7. Beta-carotene standard from Sigma-Aldrich

3.1.4 Equipment

1. Gas chromatography with a flame ionization detector (GC-FID)
2. Agilent© 7890 comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS)
3. High Performance Liquid Chromatography with a diode array detector (HPLC-DAD)

3.2 Methodology

3.2.1 Preparation of Fatty Acid Methyl Esters (FAMEs)

CPO samples were methylated to convert fatty acids to (FAMEs) which adapted from G. P. P. Kamatou and A. M. Viljoen (2017). A 10 mL of hexane was added to 1 mL of CPO. After that, a 10 mL of sodium methoxide was mixed (1.55 g of NaOH in 50 mL of methanol) by a vortex mixer for 30 second. Then the solution was centrifuged with 1,200 rpm and incubated at room temperature for 10 min. FAMEs containing in the upper layer.

3.2.2 Analysis of FAMEs Using GC-FID

FAMEs samples were analyzed by an Agilent 6890A gas chromatograph coupled with a flame ionization detector (GC-FID) and select FAMEs capillary column (50 m x 0.25 mm i.d and 0.25 µm film thickness). The samples were injected in the spit mode with the split ratio of 1:30. The GC-FID conditions follow the published article (Tres *et al.*, 2013). The GC oven was programmed as follows: initial temperature at 100 °C, increased at 5°C/min to 230°C and held at 230°C for 9 min. Helium was used as the carrier gas. The GC injector and detector were set at 250 °C and 280°C, respectively. Fatty acids were identified by their retention times according to those found in the FAME standard mixture.

3.2.3 VOCs Preparation in CPO

No derivatization is required. This method was adapted from Goggin *et al.* (2020). CPO samples were melted at 45°C to enable aliquoting of 0.036 g to a 2 mL glass headspace vial. Vials were secured with a magnetic screw cap, sealed with a

PTFE/silicon septum. Samples were pre-conditioned at 60°C and 275 rpm for at least 30 min. 1 mL of headspace sample was directly injected into GCxGC-TOFMS system via 5 mL Hamilton syringe with a 51 mm needle.

3.2.4 CPO dilution

A 0.25 mL of CPO samples were diluted with 5 mL of n-hexane and injected into GCxGC-TOFMS.

3.2.5 Analysis of VOCs in Oil Sample Using GCxGC-TOFMS

The VOCs of CPO samples were analyzed by a LECO Pegasus 4D GC×GC-TOFMS. The first dimension (1D) chromatographic column was performed on the capillary Rxi-PAH column (60 m long × 0.25 mm internal diameter, and 0.25 μm film thickness) and for the second dimension (2D) column was performed by a capillary Rxi-1HT column (1 m long × 0.25 mm internal diameter, and 0.25 μm film thickness). GCxGC columns is manufactured from Restek. The 1D oven was held isothermally for 1 min at 100°C, operated from 100°C to 270°C with ramped at 4°C/min and held at that temperature for 5 min. The samples were injected in the spit mode with injection ratio equal 5:1 split. Helium was used as a carrier gas with 1 mL/min constant flowrates. Moreover, the temperature of injector, transfer line, and MS source was 300°C, 300°C and 250°C, respectively.

3.2.6 Analysis of Carotenoids in Oil Samples Using HPLC-DAD

CPO samples were dissolved in the HPLC mobile phase to make into concentration of 2,000 ppm and were analyzed using Shimadzu, SPD-20A HPLC model with a stainless-steel column packed with a Venusil AQ-C18 column (5μm, 100Å, 2.1x150 mm). Using the following conditions adapted from Ng and Choo (2016). The mobile phase was acetonitrile/dichloromethane (98.5/1.5, v/v). The separation was performed using a low-pressure gradient at total pump flow rate 0.75 mL/min for 20 min at the wavelength 450 nm.

3.3 Software

3.3.1 Chem Station

Data of FAMEs is acquired and processed using Chem Station program for calculating area.

3.3.2 Pegasus Program

Data of VOCs is acquired and processed using Pegasus program. The absorbance mode and number of scans was set up in this program. For calculating area and height, the data was calculated from zero baseline method.

3.3.3 LC Solution

Data of carotenoids is acquired and processed using LC solution for calculating area.

3.3.4 Microsoft Excel

Microsoft Excel will provide the plotting and calculation of normalization of signal by GC to comparison between different CPOs.

CHAPTER 4
RESULTS AND DISCUSSION

This chapter illustrates the results of eleven crude palm oils (CPO) from different parts of Thailand. the appearances of the CPO samples were observed. The results show distribution of fatty acid methyl esters (FAMEs) using GC-FID as well as volatile organic compounds (VOCs) distribution of CPO by GC-MS. In addition, carotenoids of CPO will be also discussed in this chapter.

4.1 Physical Appearance

The appearance of the fresh CPOs were observed. **Figure 4.1** shows the physical appearance of different fresh CPOs at room temperature. Fresh CPO from Maung, Surat Thani (**Figure 4.1**(a)) was dark red liquid with a little bit of yellow wax floating on their surface. Fresh CPO from Phra Saeng, Surat Thani (**Figure 4.1**(b)) was clear dark red liquid in the upper layer and dark orange semi-solid in the lower layer. Fresh CPO from Phra Saeng, Surat Thani (**Figure 4.1**(c)) was dark orange semi-solid with dark red liquid on the surface. Fresh CPO from Ao Luek, Krabi (**Figure 4.1**(d)) was clear light dark red with dark orange semi-solid in the lower layer. Fresh CPO from Ao Luek, Krabi (**Figure 4.1**(e)) was clear dark red liquid in the upper layer and dark orange semi-solid in the lower layer. Fresh CPO from Khao Phanom, Krabi (**Figure 4.1**(f)) was clear dark red liquid in the upper layer and dark orange semi-solid in the lower layer. Fresh CPO from Tha Sae, Chumphon (**Figure 4.1**(g)) was thick orange-yellow semi-solid. Fresh CPO from Muang, Chumphon (**Figure 4.1**(h)) was clear dark red liquid in the upper layer and dark orange semi-solid in the lower layer. Fresh CPO from Bang Saphan, Prachuap Khiri Khan (**Figure 4.1**(i)) was dark orange semi-solid. Fresh CPO from Sam Roi Yot, Prachuap Khiri Khan (**Figure 4.1**(j)) was dark orange semi-solid with dark red liquid on the surface. Fresh CPO from Tha Maka, Kanchanaburi (**Figure 4.1**(k)) was dark yellow orange semi-solid. All CPOs were slightly different in physical appearance.

(a) CPO1

(b) CPO2

(c) CPO3

(d) CPO4

(e) CPO5

(f) CPO6

(g) CPO7

(h) CPO8

(i) CPO9

(j) CPO10

(k) CPO11

Figure 4.1 Physical appearance of all fresh CPO samples.

4.2 Analysis of Fatty Acid Compounds

The fatty acid composition obtained in different CPOs determined by FAMEs. The FAMEs standard mixture and previous report values were used to identify fatty acid composition (G. P. P. Kamatou and A. M. Viljoen, 2017; Tres *et al.*, 2013).

4.2.1 Standard Analysis

The 1 µL of FAMEs mixed standard were analyzed by GC-FID in order to confirm the retention time of fatty acids. **Figure 4.2** shows the chromatogram of FAMEs mixed standard which contains 35 peaks, which are listed in **Table 4.1**.

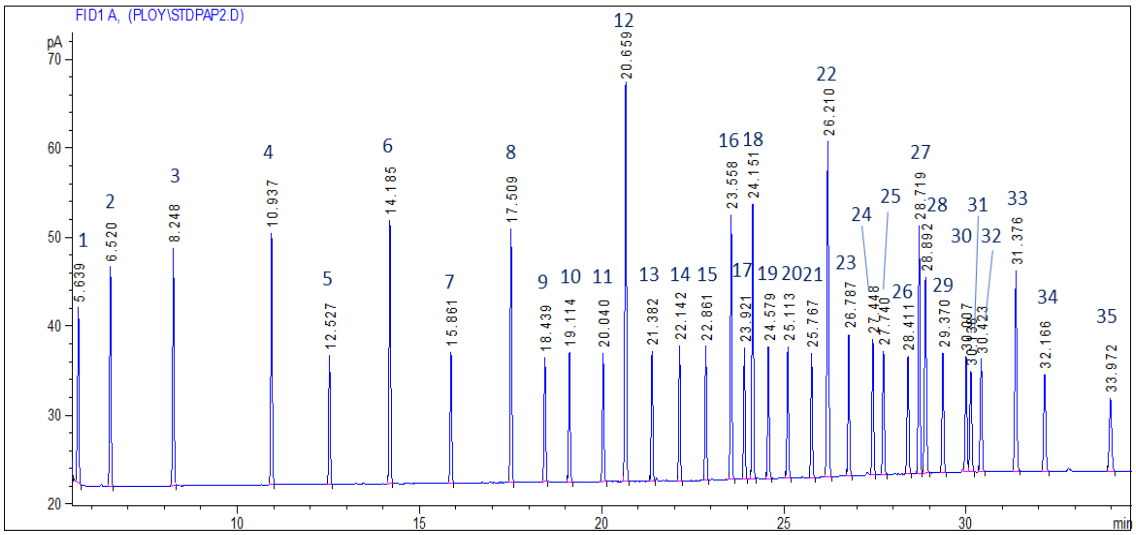


Figure 4.2 GC-FID chromatogram of FAMEs mixed standard.

Table 4.1 The components of FAMEs mixed standard

peak	R.T.	Compound	Name
1	5.744	C4:0	Butyric acid
2	6.638	C6:0	Caproic acid
3	8.385	C8:0	Caprylic acid
4	11.094	C10:0	Capric acid
5	12.691	C11:0	Undecanoic acid
6	14.358	C12:0	Lauric acid
7	16.039	C13:0	Tridecanoic acid
8	17.691	C14:0	Myristic acid
9	18.626	C14:1	Myristoleic acid
10	19.302	C15:0	Pentadecanoic acid
11	20.232	C15:1	cis-10-Pentadecanoic acid

Table 4.1 (cont.) The components of FAMEs mixed standard

peak	R.T.	Compound	Name
12	20.851	C16:0	Palmitic acid
13	21.577	C16:1	Palmitoleic acid
14	22.339	C17:0	Heptadecanoic acid
15	23.062	C17:1	cis-10-Heptadecanoic acid
16	23.760	C18:0	Stearic acid
17	24.125	C18:1 n9 t	Elaidic acid
18	24.354	C18:1 n9 c	Oleic acid
19	24.786	C18:2 n6 t	Linoleic acid
20	25.320	C18:2 n6 c	Linolelaidic acid
21	25.977	C18:3 n6	Gamma-Linolenic acid
22	26.423	C20:0	Arachidic acid
23	27.001	C21:0	Henicosanoic acid
24	27.675	C20:2	Cis-11,14-Eicosenoic acid
25	27.971	C20:3 n6	Cis-8,11,14-Eicosatrienoic acid
26	28.651	C20:1	Cis-11-Eicosenoic acid
27	28.961	C20:3 n3	Cis-11,14,17-Eicosatrienoic acid
28	29.139	C20:4 n6	Arachidonic acid
29	29.624	C18:3 n3	Alpha-Linoleic acid
30	30.273	C22:0	Beheric acid
31	30.405	C22:1	Erucic acid
32	30.693	C20:5	Cis-5,8,11,14,17-Eicosapentaenoic acid
33	31.664	C24:0	Lignoceric acid
34	32.474	C24:1 n9	Nervonic acid
35	34.324	C22:6 n3	Cis-4,7,10,13,16,19-Docosahexaenoic

4.2.2 Analysis of FAMEs

The GC-FID chromatogram of FAMEs from different CPOs are shown in **Figure 4.3**, it can be seen that the chromatogram of all CPO samples was similar. The dominant peaks were C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 n9 (oleic acid) and C18:2 n6 (gamma-linoleic acid). It was noticed that the dominant peaks were the major compounds which could be found in all CPO samples and could not be differentiated CPO. Therefore, minor peaks of FAMEs were observed. The amplification of GC-FID chromatograms of different CPOs in Thailand which divided by the retention time is shown in **Figure 4.4**.

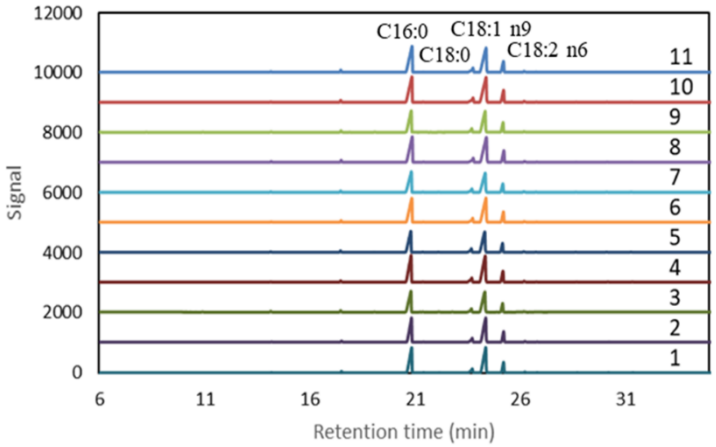


Figure 4.3 GC-FID chromatogram of CPOs from different parts in Thailand.

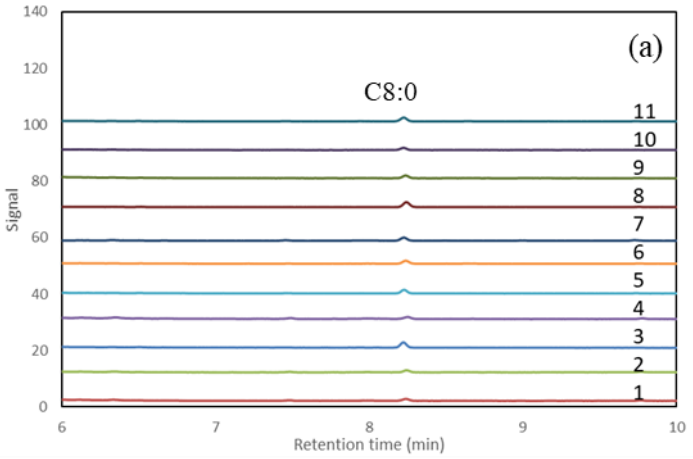


Figure 4.4 The amplification of GC-FID chromatogram of different CPOs in Thailand divided by the retention time in (a) 6-10 min, (b) 10-15 min, (c) 15-20 min, (d) 20-25 min, (e) 25-30 min, and (f) 30-35 min.

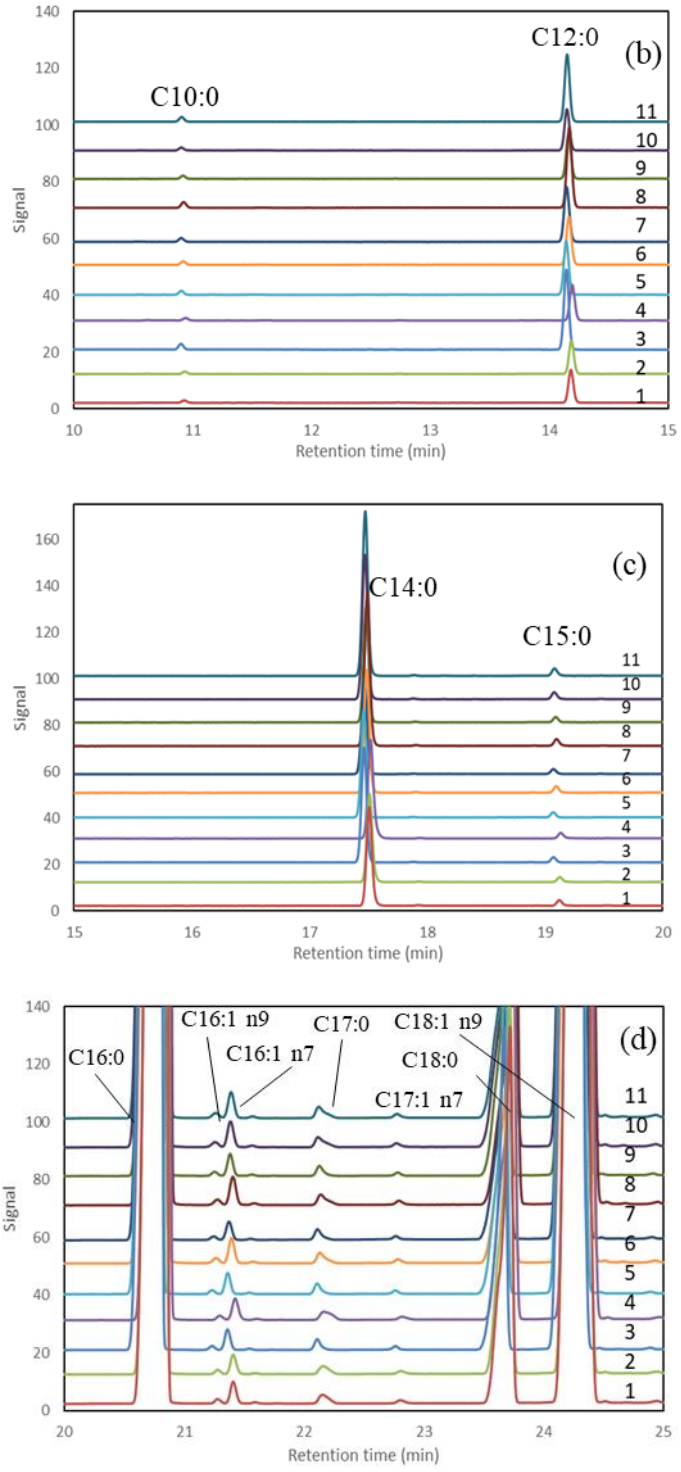


Figure 4.4 (cont.) The amplification of GC-FID chromatogram of different CPOs in Thailand divided by the retention time in (a) 6-10 min, (b) 10-15 min, (c) 15-20 min, (d) 20-25 min, (e) 25-30 min, and (f) 30-35 min.

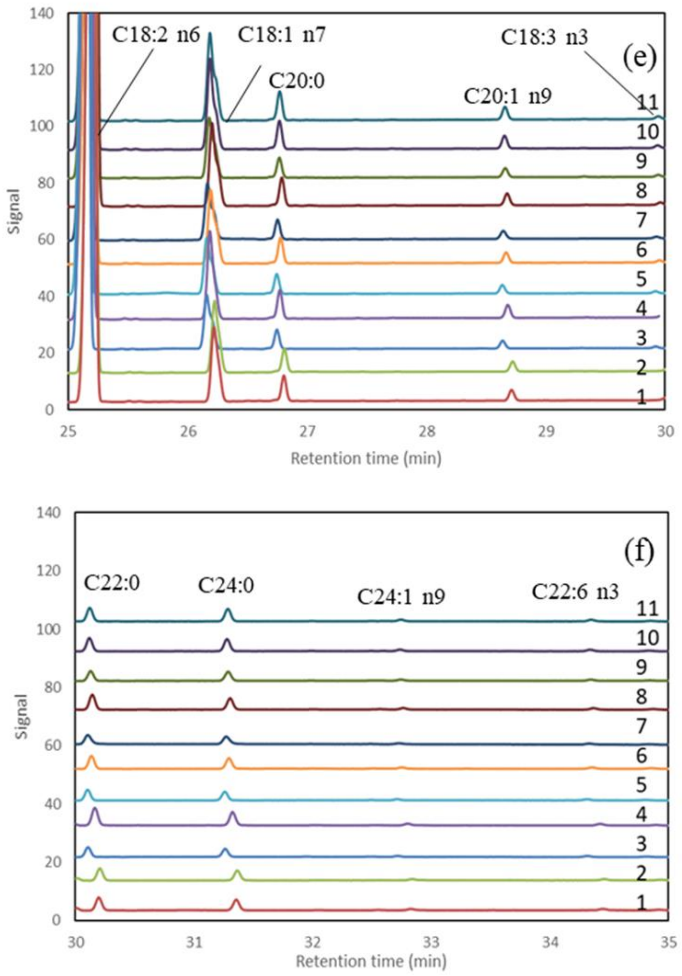


Figure 4.4 (cont.) The amplification of GC-FID chromatogram of different CPOs in Thailand divided by the retention time in (a) 6-10 min, (b) 10-15 min, (c) 15-20 min, (d) 20-25 min, (e) 25-30 min, and (f) 30-35 min.

Figure 4.4 The amplification of GC-FID chromatogram of different CPOs in Thailand divided by the retention time in (a) 6-10 min, (b) 10-15 min, (c) 15-20 min, (d) 20-25 min, (e) 25-30 min, and (f) 30-35 min.it can be seen that the chromatograms of all CPO samples were similar. Similarly, GC-FID chromatograms of different CPOs in Thailand as shown in **Figure 4.4** The amplification of GC-FID chromatogram of different CPOs in Thailand divided by the retention time in **(a**) 6-10 min, (b) 10-15 min, (c) 15-20 min, (d) 20-25 min**, (**e) 25-30 min, and (f) 30-35 min.Therefore, the amount of FAMEs components expressed as percentage peak area (%area) which refer to weight percentage (wt%) were discussed. **Table 4.2** shows the weight percentage (wt%) of FAMEs component which have wt% greater than 0.01

and divided into 3 parts. The major components of FAMEs were greater than 5 wt%, the minor components of FAMEs were between 0.01 wt% – 5.0 wt%, and the trace components were less than 0.01 wt%. It can be seen that the major components of FAMEs were C16:0 (palmitic acid), C18:0 (Stearic acid), C18:1 n9 (oleic acid) and C18:2 n6 (gamma-linoleic acid). The minor components of FAMEs were C8:0, C10:0, C12:0, C14:0, C15:0, C17:0, C17:1 n7, trans18:1, C20:0, C20:1 n9, C18:3 n3, C22:0 and C24:0. Moreover, the trace components of FAMEs were C24:1 n9, C22:6 n3 and all of unknowns. It can be seen that the weight percentage (wt%) of FAMEs component were related to the peak area of GC-FID chromatogram.

Unfortunately, this method could not distinguish CPO from different parts of Thailand because the fatty acid composition in different CPO were similar. Nevertheless, it is worth to investigate CPOs from neighboring countries compared with CPOs in Thailand.

Table 4.2 Fatty acid compositions (wt%) of different CPOs

		%AREA										
No	FAMEs component	CPO1	CPO2	CPO3	CPO4	CPO5	CPO6	CPO7	CPO8	CPO9	CPO10	CPO11
1	C8:0	0.01	0.01	0.04	0.01	0.02	0.02	0.02	0.03	0.02	0.01	0.02
2	C10:0	0.02	0.02	0.04	0.01	0.03	0.02	0.03	0.03	0.02	0.02	0.02
3	C12:0	0.21	0.22	0.65	0.21	0.42	0.30	0.43	0.45	0.33	0.22	0.36
4	C14:0	0.87	0.82	1.20	0.81	1.09	1.00	0.99	1.11	1.12	1.04	1.16
5	Unknown1	<0.01	<0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01	0.01	0.01	0.01
6	C15:0	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
7	C16:0	39.97	38.19	40.59	39.85	40.61	40.46	42.39	40.39	40.85	39.86	41.98
8	C16:1 n9	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.04	0.04
9	C16:1 n7	0.16	0.15	0.18	0.15	0.18	0.17	0.16	0.18	0.17	0.16	0.16
10	Unknown2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
11	C17:0	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.11
12	C17:1 n7	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
13	C18:0	4.56	4.73	4.27	4.78	4.34	4.44	4.52	4.34	4.49	4.86	4.59
14	C18:1 n9	42.93	43.87	42.16	42.88	42.18	42.62	40.78	41.98	41.21	41.58	40.75
15	Unknown3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 4.2 (cont.) Fatty acid compositions (wt%) of different CPOs

		%AREA										
No	FAMEs component	CPO1	CPO2	CPO3	CPO4	CPO5	CPO6	CPO7	CPO8	CPO9	CPO10	CPO11
16	trans18:1	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02
17	C18:2 n6	9.76	10.47	9.43	9.74	9.66	9.53	9.22	10.08	10.33	10.83	9.57
18	Unknown4	0.01	0.01	0.01	0.01	0.01	0.01	0.01	<0.01	0.01	0.01	0.01
19	Unknown5	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
20	Unknown6	<0.01	<0.01	0.01	<0.01	0.06	<0.01	0.02	<0.01	0.01	<0.01	<0.01
21	C18:1 n7	0.68	0.69	0.66	0.68	0.65	0.68	0.65	0.66	0.46	0.68	0.67
22	C20:0	0.17	0.17	0.16	0.17	0.16	0.16	0.15	0.16	0.10	0.15	0.16
23	C20:1n9	0.07	0.07	0.07	0.08	0.07	0.06	0.07	0.07	0.07	0.07	0.07
24	C18:3 n3	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
25	C22:0	0.10	0.10	0.09	0.12	0.09	0.09	0.09	0.09	0.08	0.08	0.08
26	C24:0	0.08	0.08	0.07	0.09	0.08	0.07	0.08	0.07	0.08	0.07	0.08
27	C24:1 n9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
28	C22:6 n3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
29	Unknown7	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

4.3 VOCs Distribution

Volatile organic compounds (VOCs) of CPO were the oxidation product of fatty acids and other compounds in CPO (Dirinck *et al.*, 2006; Kuntom *et al.*, 1989; Tres *et al.*, 2013). Mostly, VOCs in CPO are aldehydes such as hexanal, octanal, nonanal, 2,4-decadienal etc. and ketones such as hexanone, nonanone, and beta-ionone etc.

Figure 4.5 shows the total ion chromatogram of (a) the first and (b) second analysis of headspace CPO sample from Muang, Surat Thani (CPO1). The results show that the pattern at 2100 s were different between two analysis. Similarly, **Figure 4.6** shows the total ion chromatogram (TIC) of (a) the first and (b) second analysis of the headspace CPO sample from Phra Saeng, Surat Thani (CPO2). The results show that the peak around 1400 s, 2100 s, 2600 s were different between two analysis. Moreover, the VOCs component were also different, the first analysis has heptanal, octanal, nonanal,2-decenal, 2-octanal, and 2,4-decadienal but the second analysis has octanal, nonanal, and 2-octanal.

Unsuccessfully, this method could not differentiate CPOs from different parts of Thailand. Since the VOCs might be lost before being manually injected into the GC because of its high volatility.

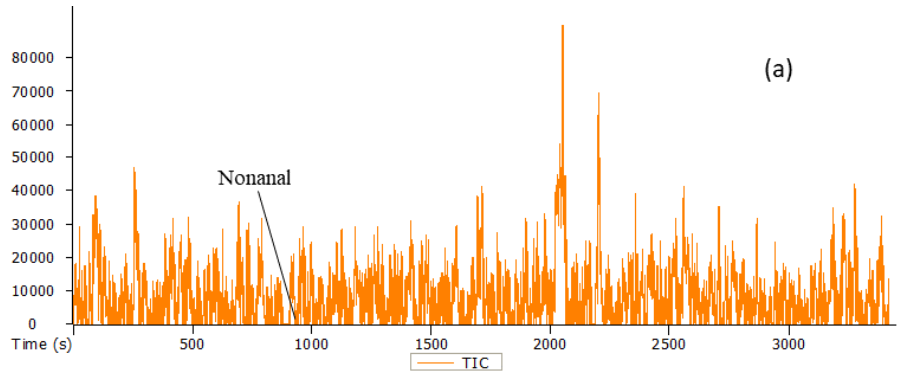


Figure 4.5 The comparison of total ion chromatogram of sample headspace VOCs of CPO1 between (a) the first analysis and (b) the second analysis.

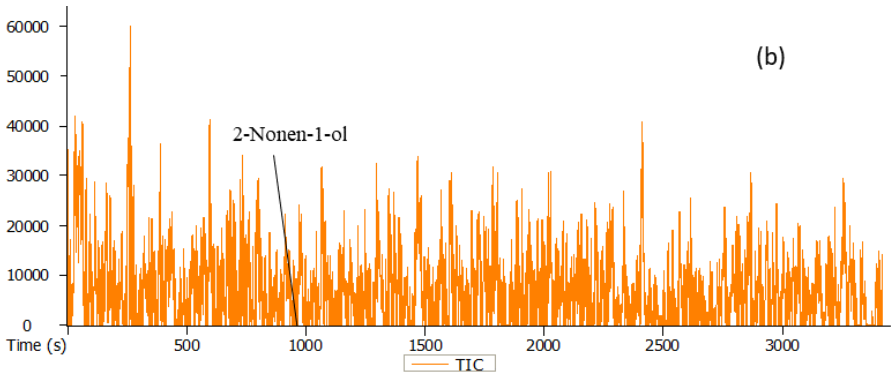


Figure 4.5 (cont.) The comparison of total ion chromatogram of headspace of CPO1 between (a) the first analysis and (b) the second analysis.

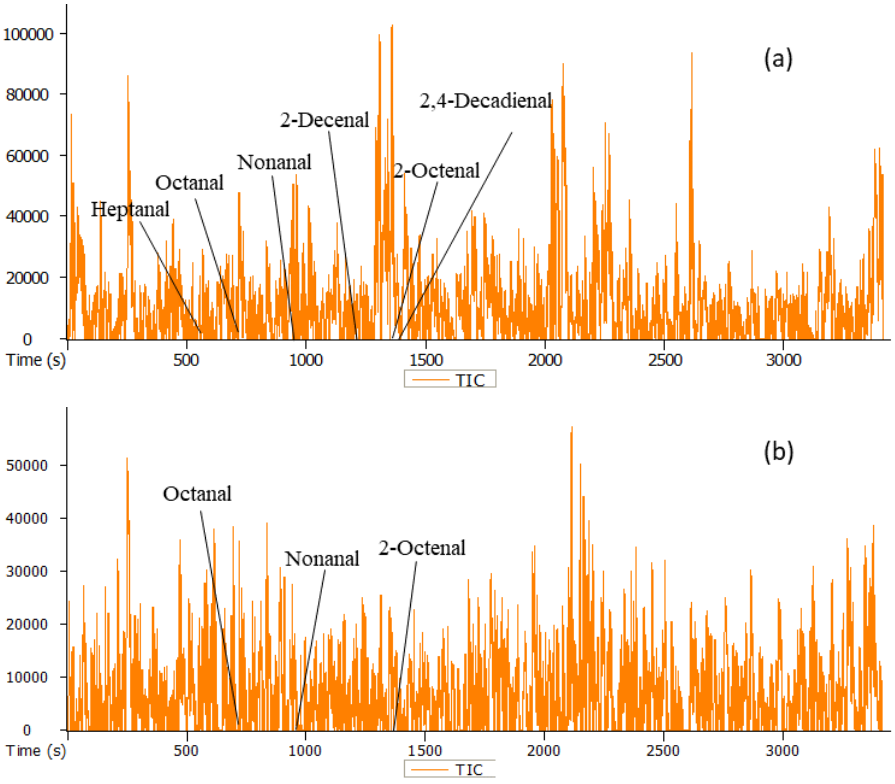


Figure 4.6 The comparison of total ion chromatogram (TIC) of headspace of CPO2 between (a) the first analysis and (b) the second analysis.

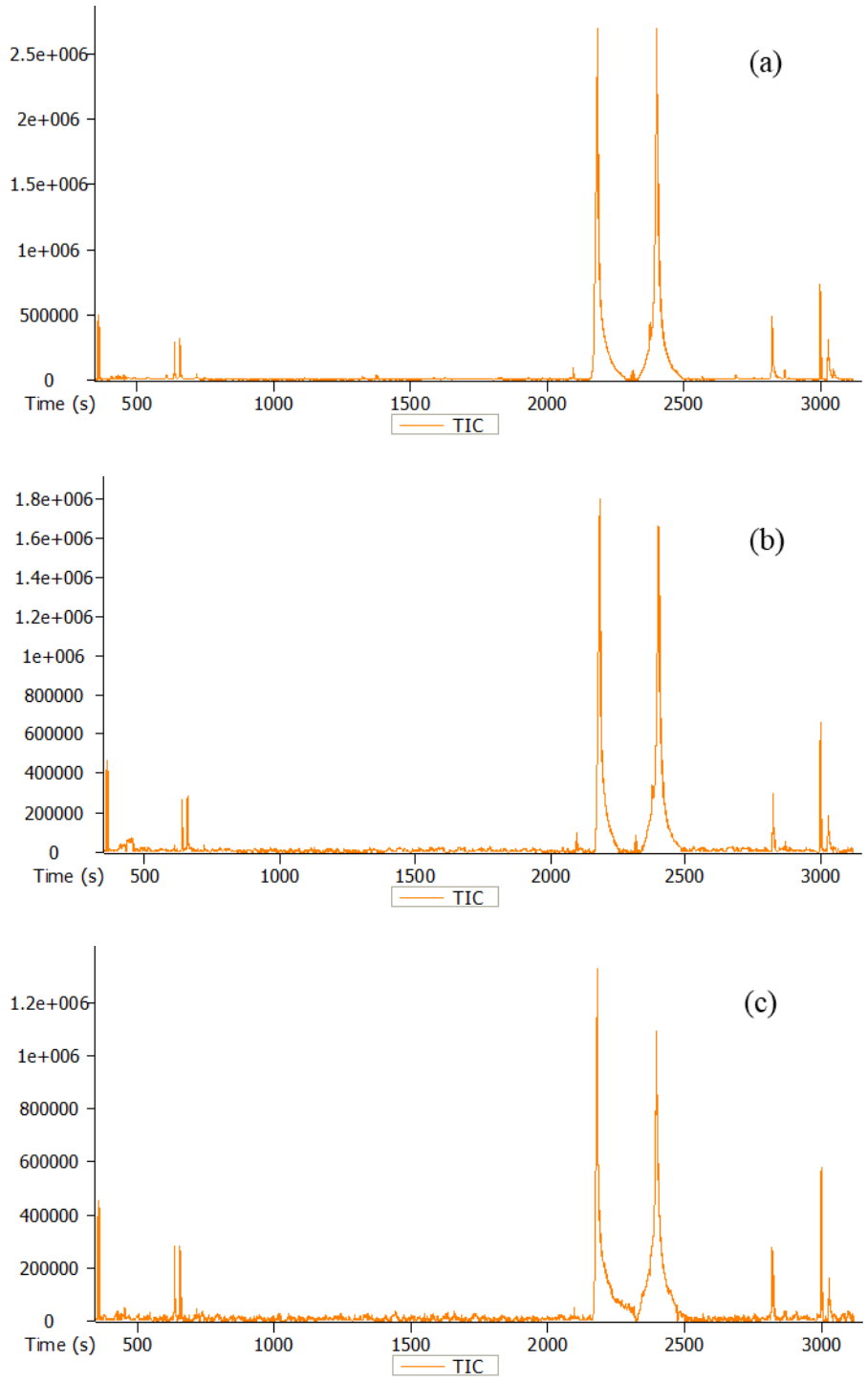


Figure 4.7 Total ion chromatogram (TIC) of VOCs from liquid CPO between (a) CPO1, (b) CPO2, (c) CPO4, and (d) CPO7.

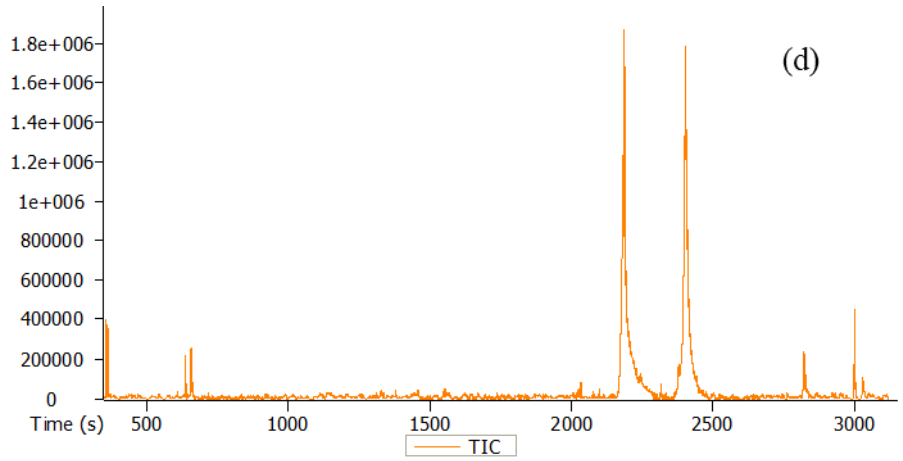


Figure 4.7 (cont.) Total ion chromatogram (TIC) of VOCs from liquid CPO between (a) CPO1, (b) CPO2, (c) CPO4, and (d) CPO7.

Due to the instability of volatiles analyzed by vapor phase. The analysis of volatile compound using liquid sample were also performed. The liquid CPO diluted with hexane were investigated. **Figure 4.7**(a) – (d) show the chromatograms of liquid samples of CPO1 (Muang, Surat Thani), CPO2 (Phra Saeng, Surat Thani), CPO4 (Ao Luek, Krabi), and CPO7 (Tha Sae, Chumphon), respectively. The analysis results of VOCs were comparable for each sample. However, the chromatograms of four CPO samples were similar which could not distinguish CPOs from different parts of Thailand.

4.4 Analysis of Carotenoids

The different color level of CPO is depended on the amount of carotenoids in CPO. The chromatogram of carotenoids was investigated by HPLC-DAD.

4.4.1 Standard Analysis

The beta-carotene standard was analyzed by HPLC-DAD at the wavelength 450 nm in order to confirm the retention time before CPO sample analysis. **Figure 4.8** shows HPLC chromatogram of beta-carotene standard which appears the retention time at 8.514 min.

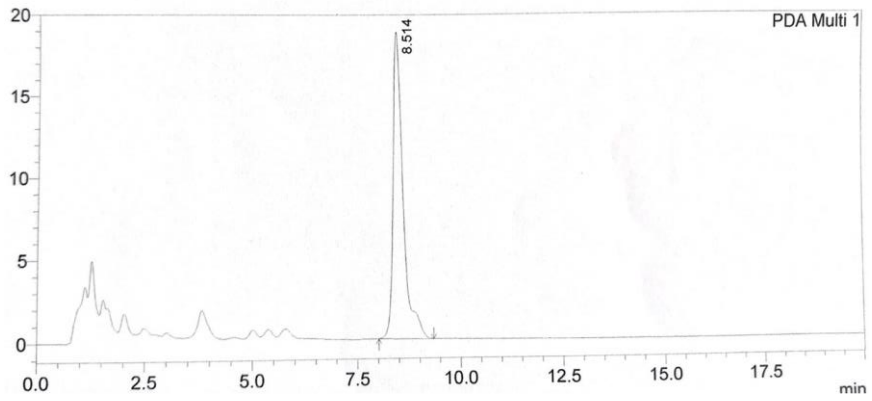


Figure 4.8 HPLC chromatogram of beta carotene standard.

4.4.2 Carotenoids Analysis of CPOs

HPLC chromatogram of carotenoids in CPO1 is shown in **Figure 4.9**. It was found that the highest peak of CPO sample appeared at around 8.5 min, which can be refer to beta-carotene. The left hand side peaks of beta-carotene were alpha and cis-alpha-carotenes which followed by Ng and Choo (2016).

Figure 4.9 - **Figure 4.12** show HPLC chromatogram for the first month in the blue line and third month in the orange line of CPO1, CPO2, CPO4, and CPO7, respectively. It can be seen that the pattern of chromatogram between the first and third month were similar, but the proportion were different. It can be seen that the chromatogram of the third month were decreased in four CPOs. Due to the degradation of carotenoids, it can be oxidized by light, temperature, or moisture during storage which cause the content of carotenoids (Zou *et al.*, 2012). Therefore, all signals were normalized with the highest peak at retention time 8.5 min. After that, the alpha to beta-carotenes ratio of CPO between the first and third month were investigated. The results from **Table 4.3** were in line with **Figure 4.9** - **Figure 4.12**. It could be indicated that after three months of the first-time analysis, the ratio of alpha to beta-carotenes of CPO1 was increased from 0.85 to 0.87, CPO2 was decreased from 0.80 to 0.79, CPO4 was increased from 0.88 to 0.90, and CPO7 was increased from 0.82 to 0.85. It could be noticed that each CPO have approximately different 0.02 between first and third month. Also, the standard variation of each CPO was not excess 0.01 which is an acceptable value and stable to be considered as a characteristic of CPO.

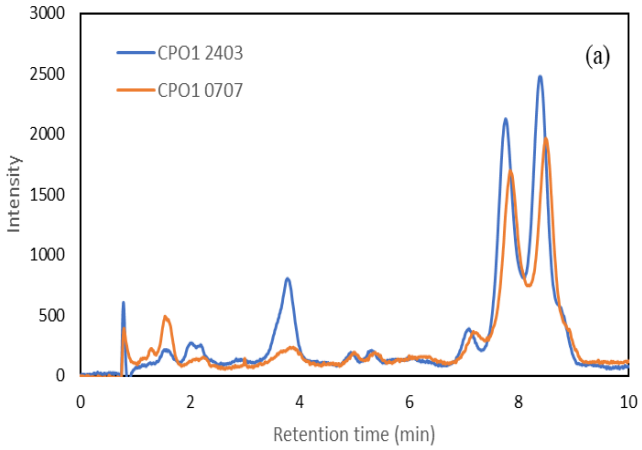


Figure 4.9 The comparison between the first and second-time analysis of (a) HPLC chromatogram in CPO1.

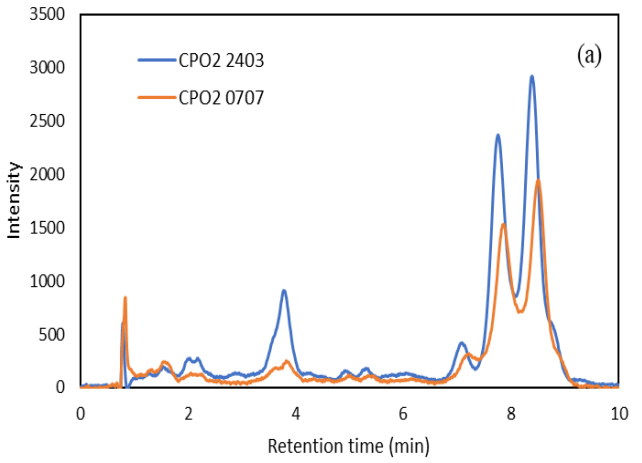


Figure 4.10 The comparison between the first and second-time analysis of HPLC chromatogram in CPO2.

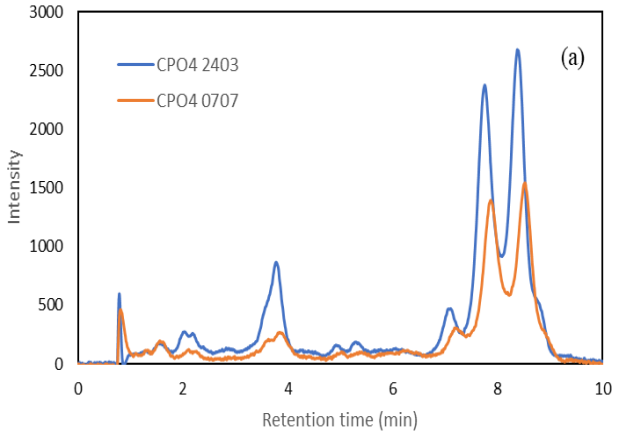


Figure 4.11 The comparison between the first and second-time analysis of (a) HPLC chromatogram in CPO4.

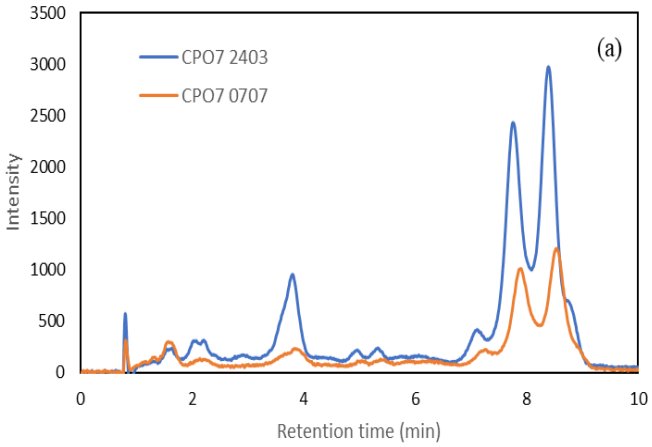


Figure 4.12 The comparison between the first and second-time analysis of (a) HPLC chromatogram in CPO7.

Table 4.3 The ratio of alpha to beta-carotenes of four different CPOs between first month and third month

Alpha to beta carotene ratio						
Oil samples	Analyzed date	Repetition			Avg	SD
		1	2	3		
CPO1	24032021	0.86	0.85	0.85	0.85	0.01
	07072021	0.87	0.87	0.88	0.87	0.01
CPO2	24032021	0.81	0.81	0.79	0.80	0.01
	07072021	0.79	0.78	0.79	0.79	0.01
CPO4	24032021	0.89	0.88	0.87	0.88	0.01
	07072021	0.90	0.91	0.90	0.90	0.00
CPO7	24032021	0.82	0.82	0.83	0.82	0.01
	07072021	0.84	0.84	0.86	0.85	0.01

Furthermore, the HPLC chromatograms of eleven CPO samples were investigated as shown in **Figure 4.13**. it indicates that all of CPO samples have the similar component, but the proportion were different. After all signals were normalized with the highest peak at retention time 8.5 min. The ratio of alpha to beta-carotenes was observed as shown in **Table 4.4**. It can be seen that the ratio of alpha to beta-carotenes of CPO from Surat Thani was different. CPO1, CPO2, and CPO3 which was 0.89, 0.79, and 0.81, respectively. The ratio of alpha to beta-carotenes of CPO4 from Krabi was different from CPO5 and CPO6. Similarly, the ratio of alpha to beta-carotenes of CPO7 and CPO8 from Chumphon was different. Also, the alpha to beta-carotenes ratio of CPO9 and CPO10 from Prachuap Khiri Khan was different. Even though, the ratio of alpha to beta carotene between CPO6 from Krabi and CPO8 from Chumphon was similar.

Consequently, the chromatograms of carotenoids were similar in all CPO samples but the ratio of alpha to beta-carotenes of each CPO was unique. The alpha to beta-carotenes ratio were in the range of 0.70 - 0.91 with ±0.02 tolerance. Therefore, this method can be considered as a characteristic of each CPO. However, more CPO

samples must be further investigated to improve the identification of CPO from different sources.

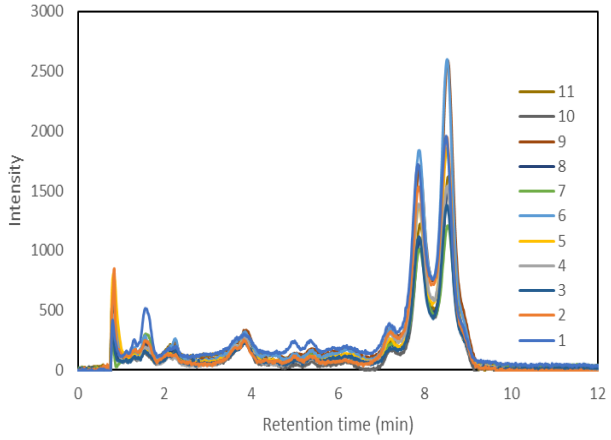


Figure 4.13 HPLC chromatogram of carotenoids in different CPO samples.

Table 4.4 The ratio of alpha to beta carotenes of different CPOs

Alpha to beta carotenes ratio					
Oil samples	**Repetition**			**Avg**	**SD**
	1	2	3		
CPO1	0.87	0.87	0.88	0.87	0.01
CPO2	0.79	0.78	0.79	0.79	0.01
CPO3	0.81	0.81	0.81	0.81	0.00
CPO4	0.90	0.91	0.90	0.90	0.00
CPO5	0.75	0.75	0.75	0.75	0.00
CPO6	0.71	0.70	0.71	0.71	0.01
CPO7	0.84	0.84	0.86	0.85	0.01
CPO8	0.71	0.72	0.71	0.71	0.01
CPO9	0.66	0.66	0.66	0.66	0.00
CPO10	0.73	0.73	0.73	0.73	0.00
CPO11	0.75	0.74	0.74	0.74	0.01

Regarding the analysis results of fatty acid compositions, volatile organic compounds (VOCs) and carotenoids. It can be seen that the composition of fatty acids and carotenoids were similar in all CPOs. Moreover, VOCs of headspace samples were not stable in the same CPO samples, but the chromatograms of VOCs of liquid CPO samples in CPOs were similar. The ratio of alpha to beta-carotenes can be considered as a characteristic of a CPO. However, some different samples exhibited the same value. Hence, more CPO samples with different seasons, location and species must be investigated to further develop the identification of CPO from different sources.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this work, eleven CPO samples from five provinces in Thailand were investigated for their composition of fatty acids, VOCs, and carotenoids. The results showed that the distributions of fatty acids and VOCs in all CPOs were insignificantly different. Moreover, the distributions of carotenoids were similar for all CPO samples. After three months of storage, the chromatograms of carotenoids were slightly decreased while the ratios of alpha to beta-carotenes were unchanged. It was found that the ratio of alpha to beta-carotenes was unique for each CPO which can be considered as a characteristic of the CPO. Hence, more CPO samples with specified species, locations, and seasons must be investigated to further develop the identification of CPO.

5.2 Recommendations

The CPO samples from neighboring countries are worth to compare with CPO samples from Thailand. Moreover, CPO was affected by their various species. Thus, species data collection of crude palm oil for each source is crucial to identify crude palm oil from different sources.

APPENDICES

Appendix A Graphical Abstract

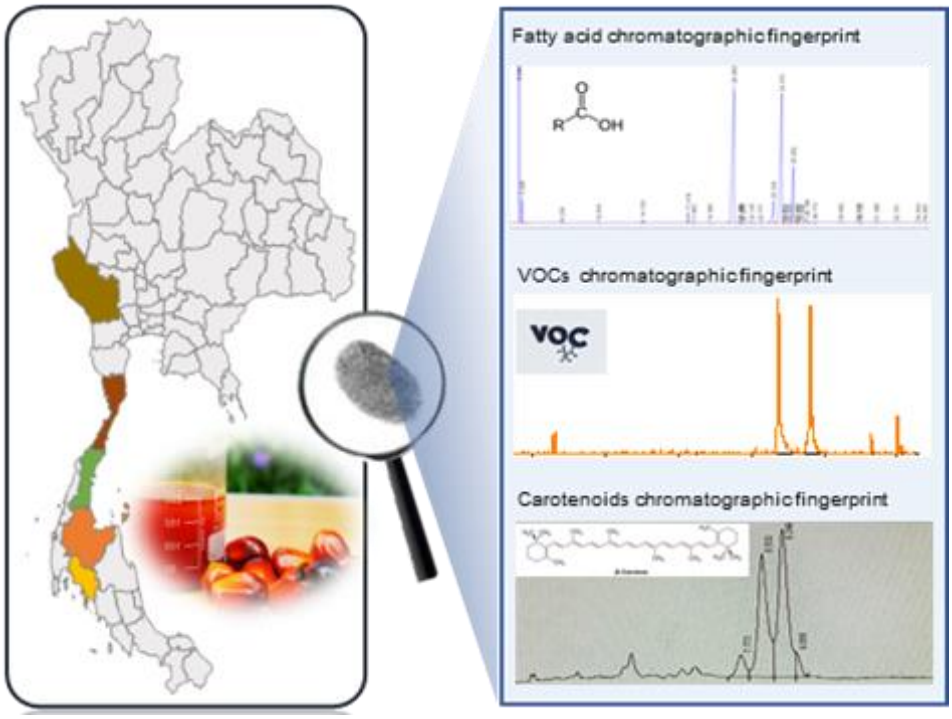


Figure A1 Graphical Abstract.

Appendix B GC-FID Chromatogram Results

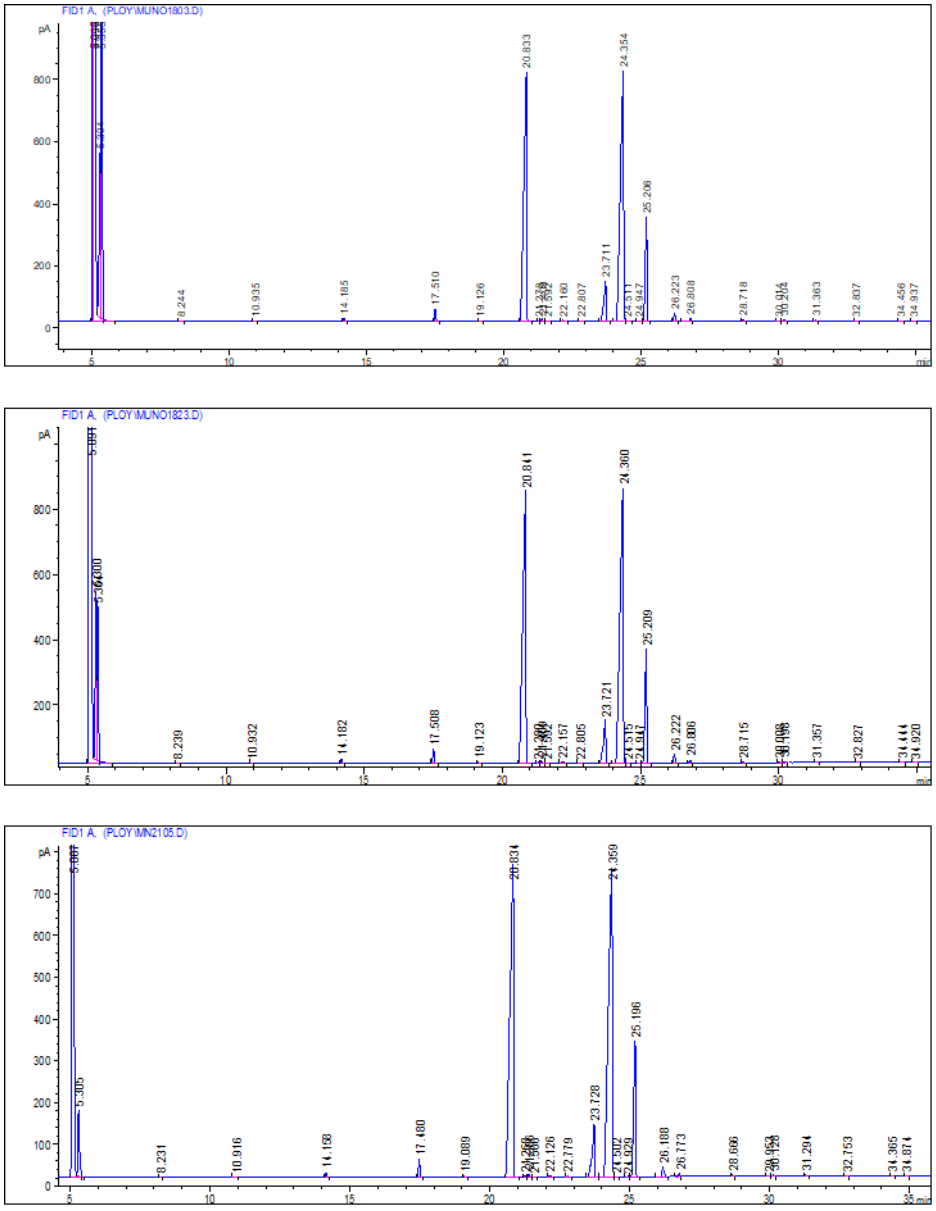


Figure B1 GC-FID Chromatogram of CPO1 with 3 repetitions.

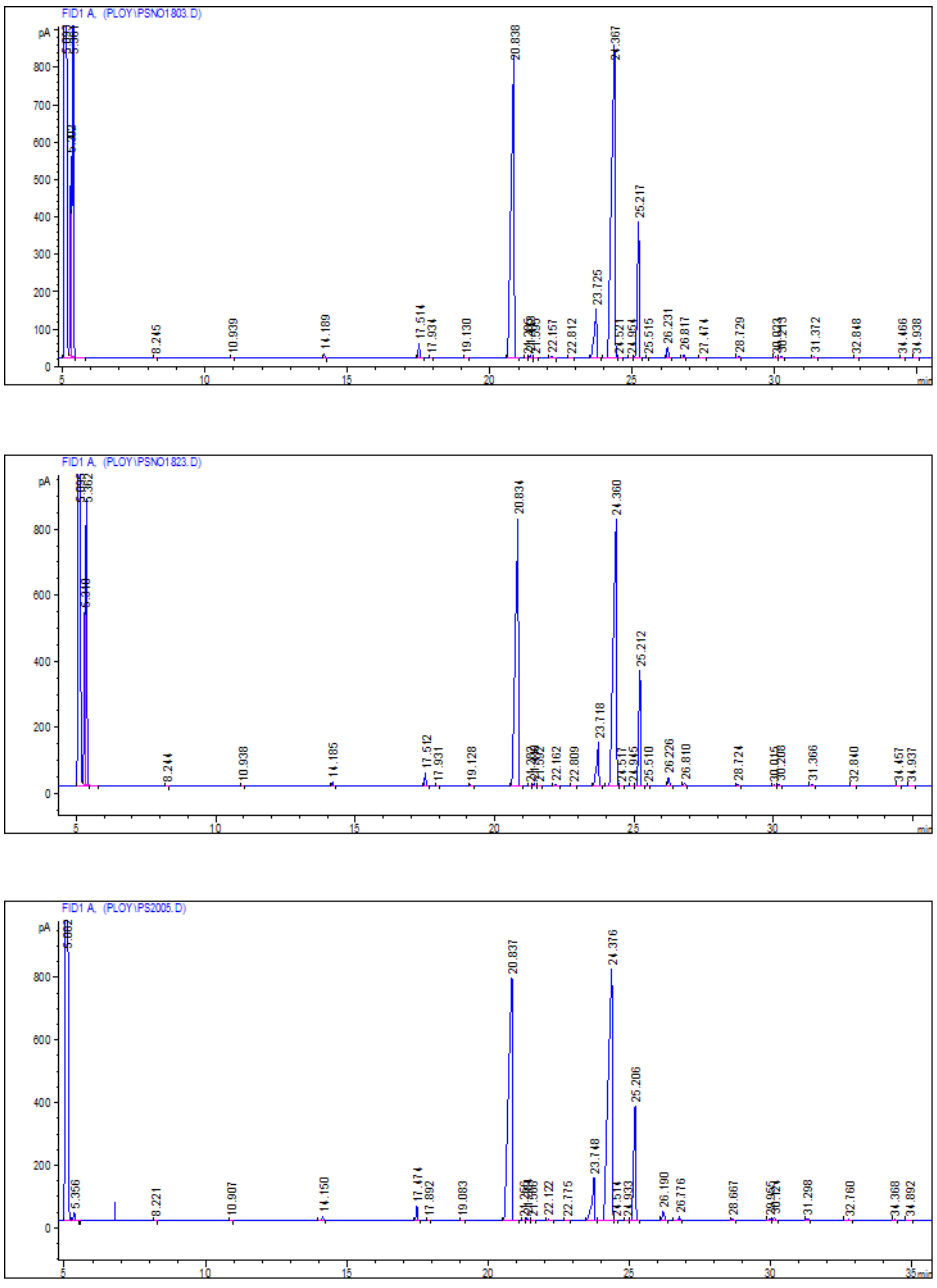


Figure B2 GC-FID Chromatogram of CPO2 with 3 repetitions.

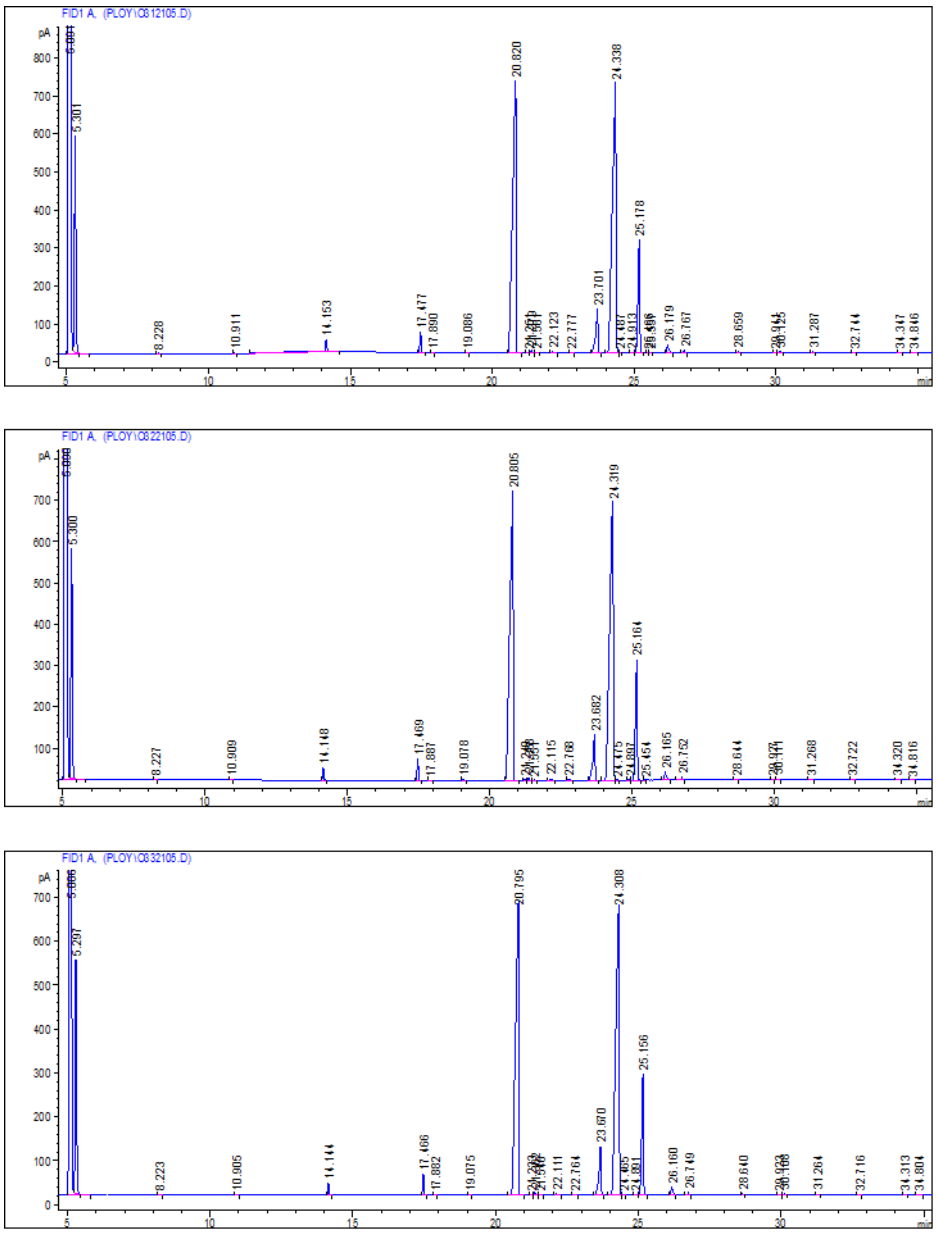


Figure B3 GC-FID Chromatogram of CPO3 with 3 repetitions.

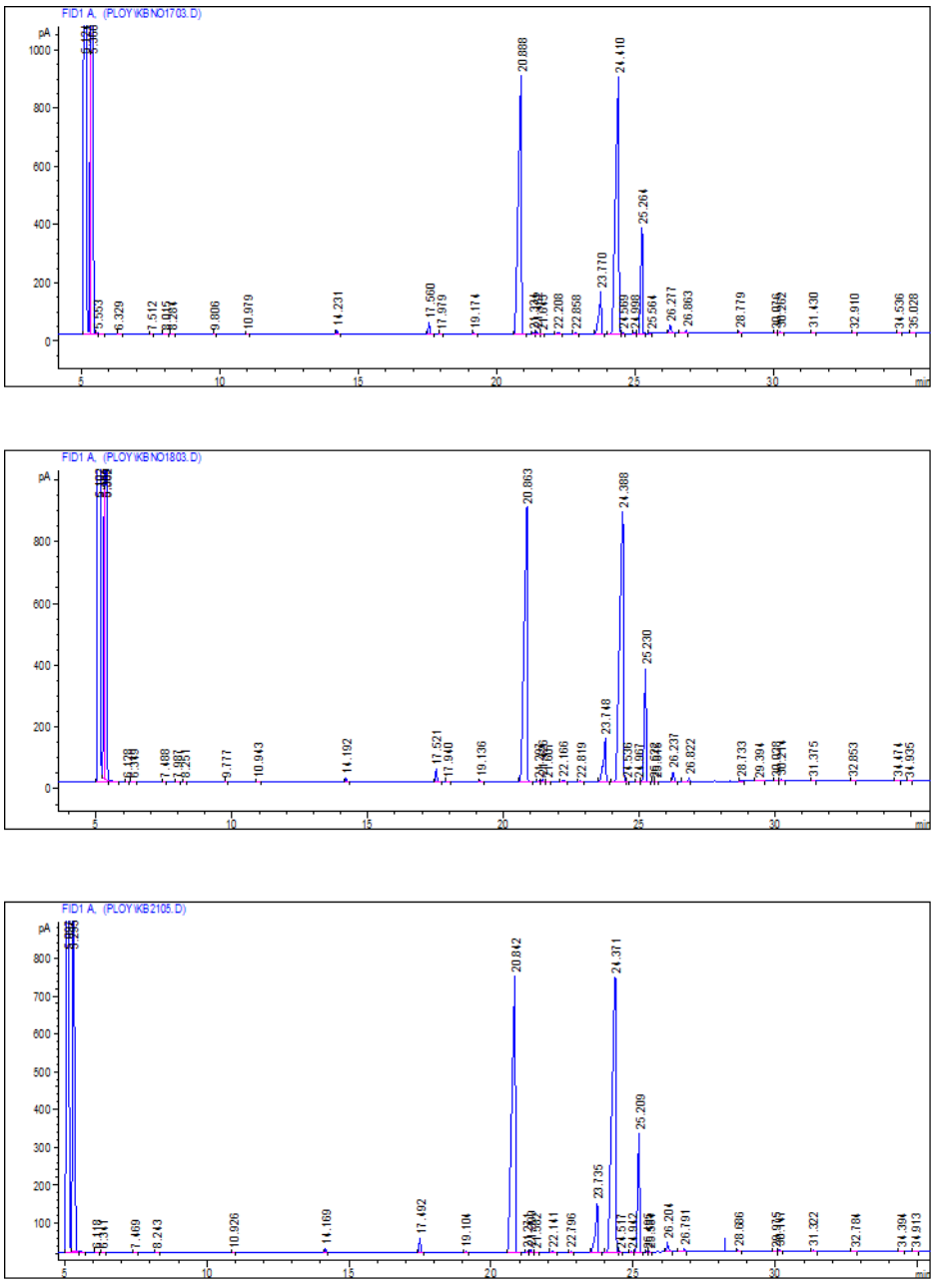


Figure B4 GC-FID Chromatogram of CPO4 with 3 repetitions.

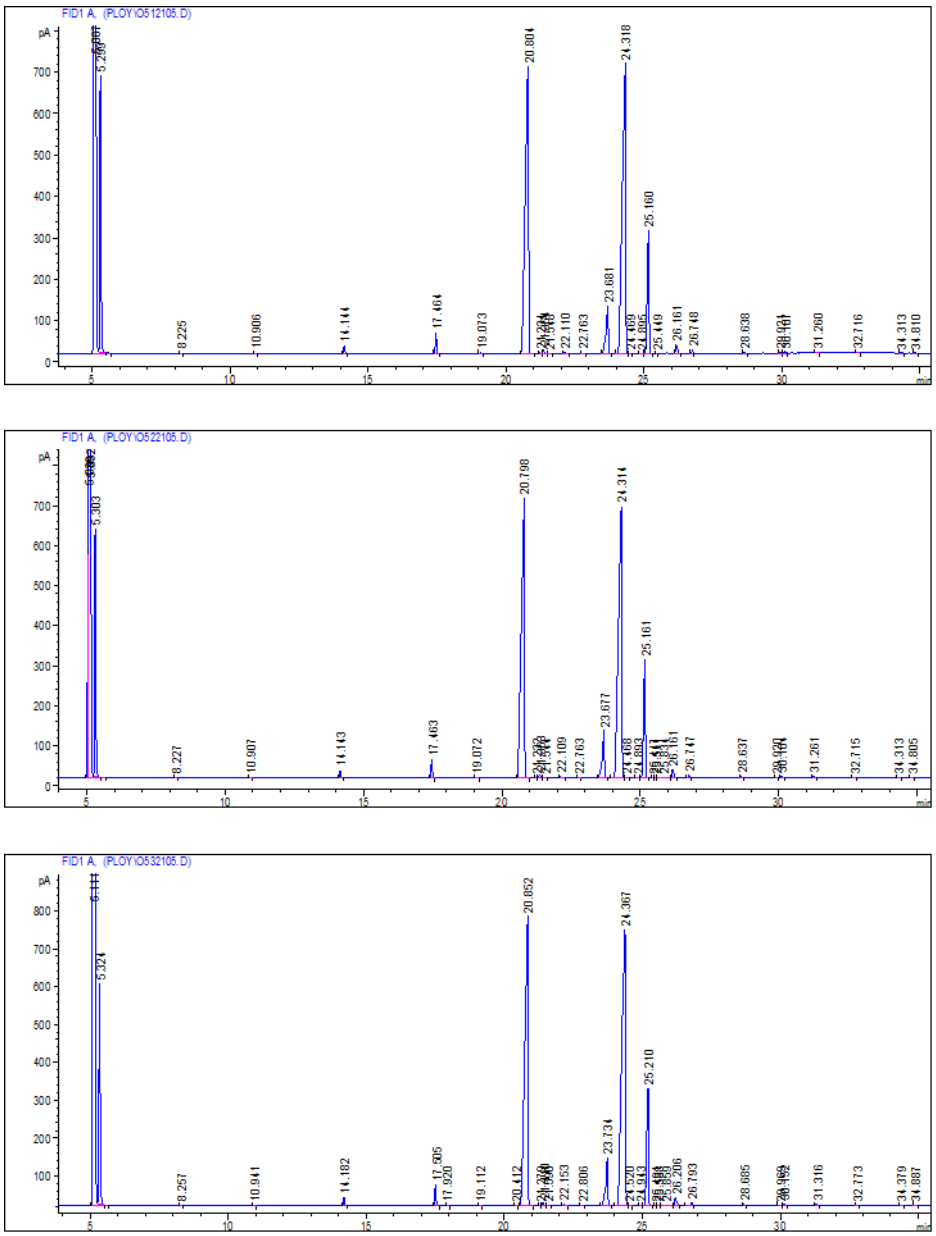


Figure B5 GC-FID Chromatogram of CPO5 with 3 repetitions.

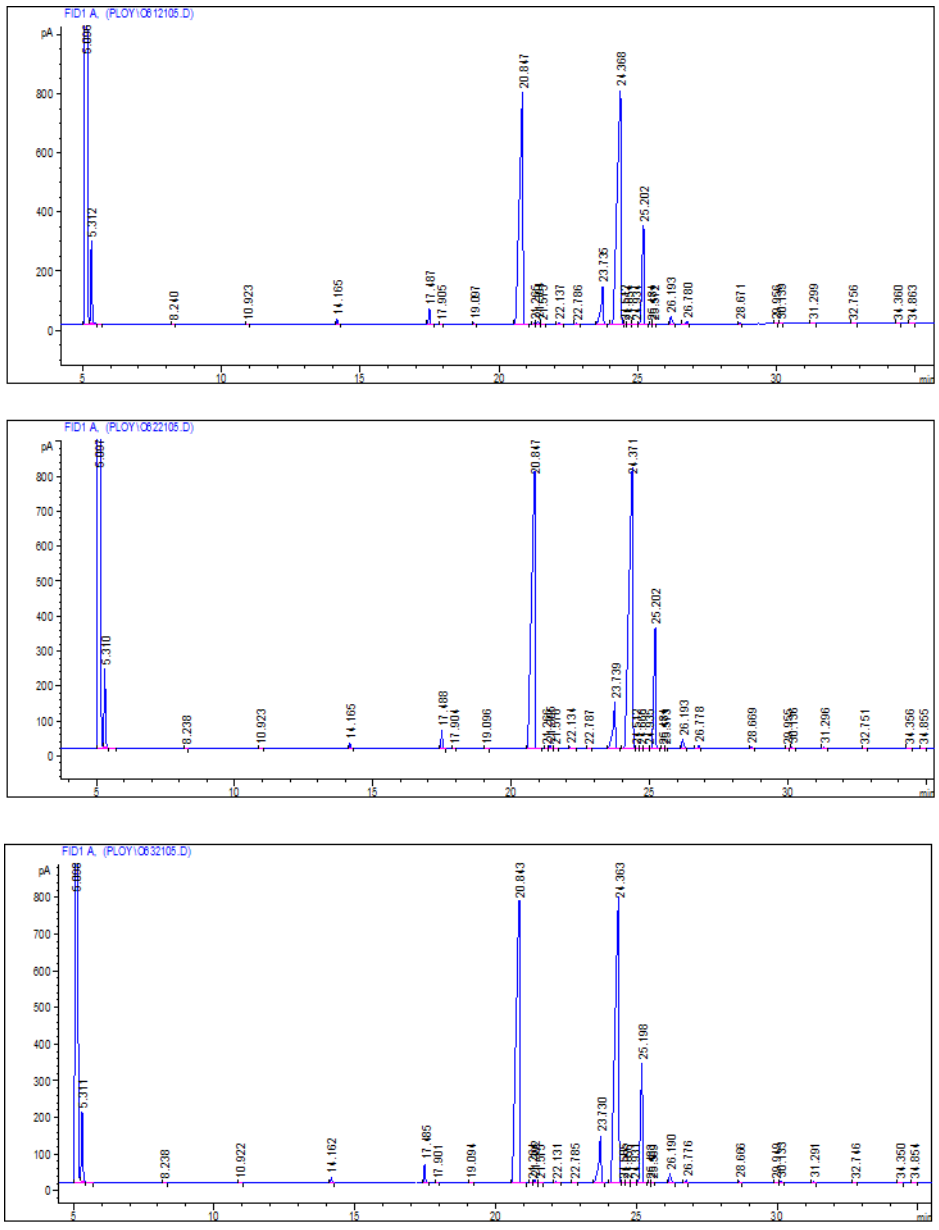


Figure B6 GC-FID Chromatogram of CPO6 with 3 repetitions.

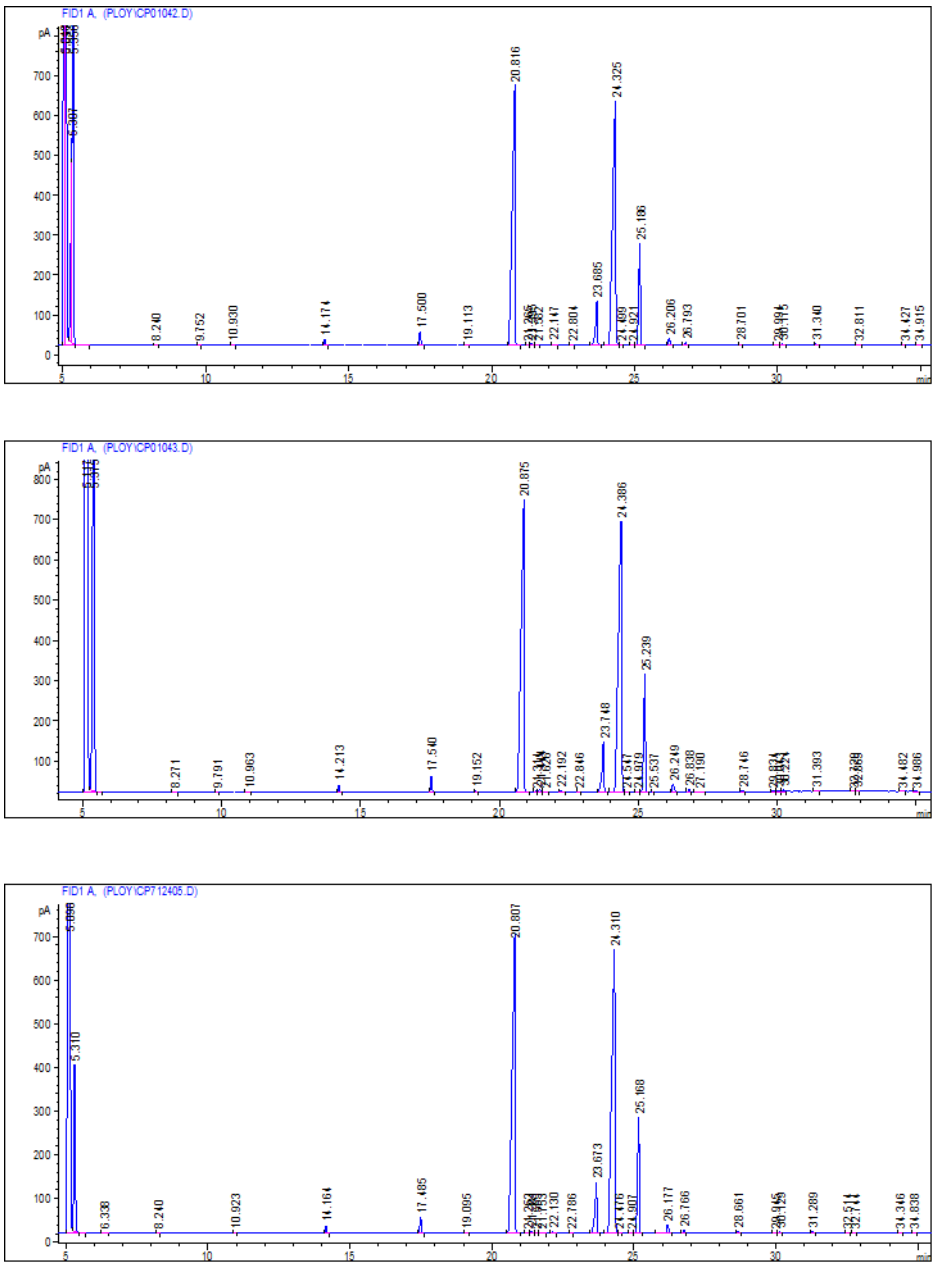


Figure B7 GC-FID Chromatogram of CPO7 with 3 repetitions.

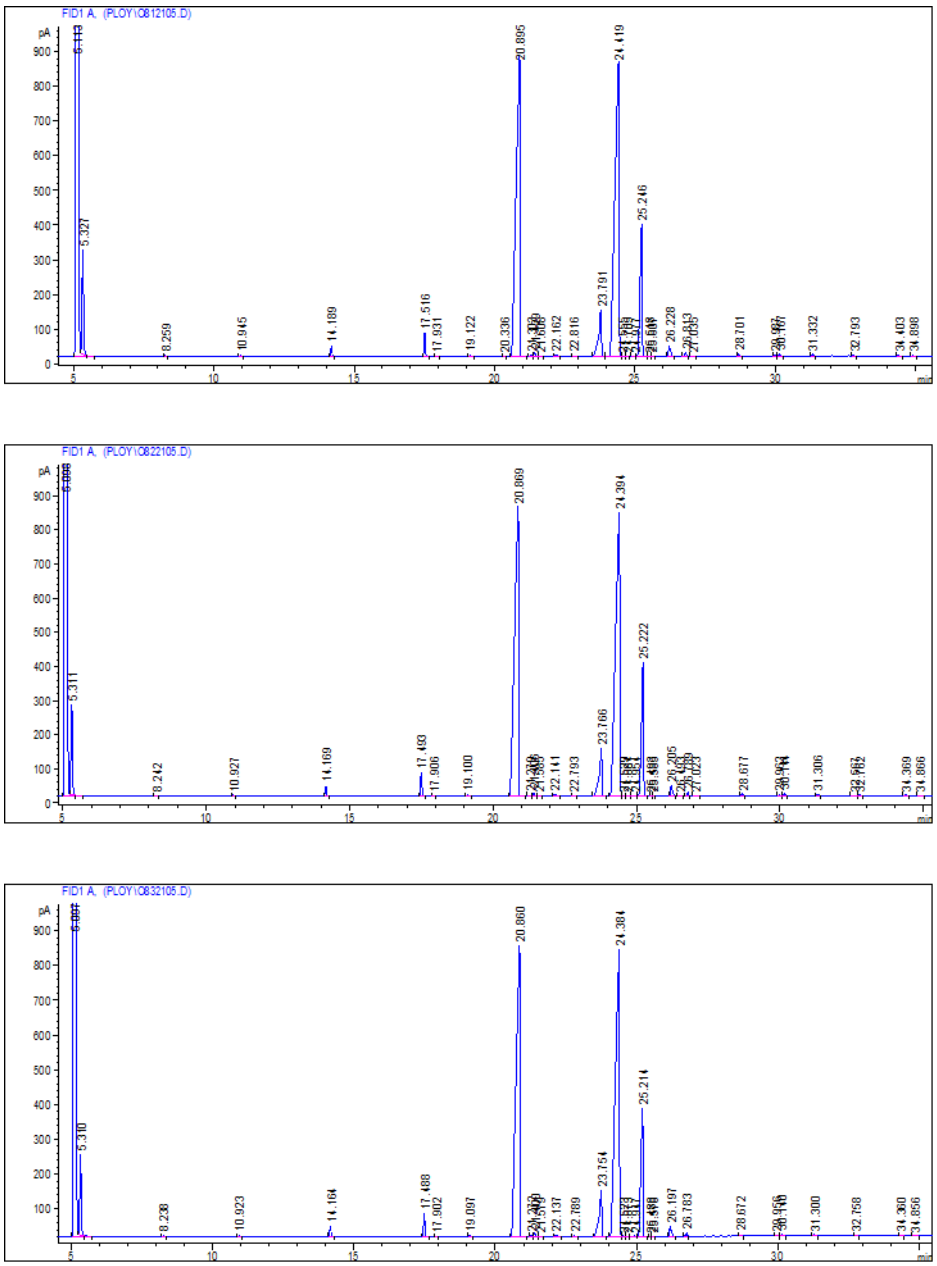


Figure B8 GC-FID Chromatogram of CPO8 with 3 repetitions.

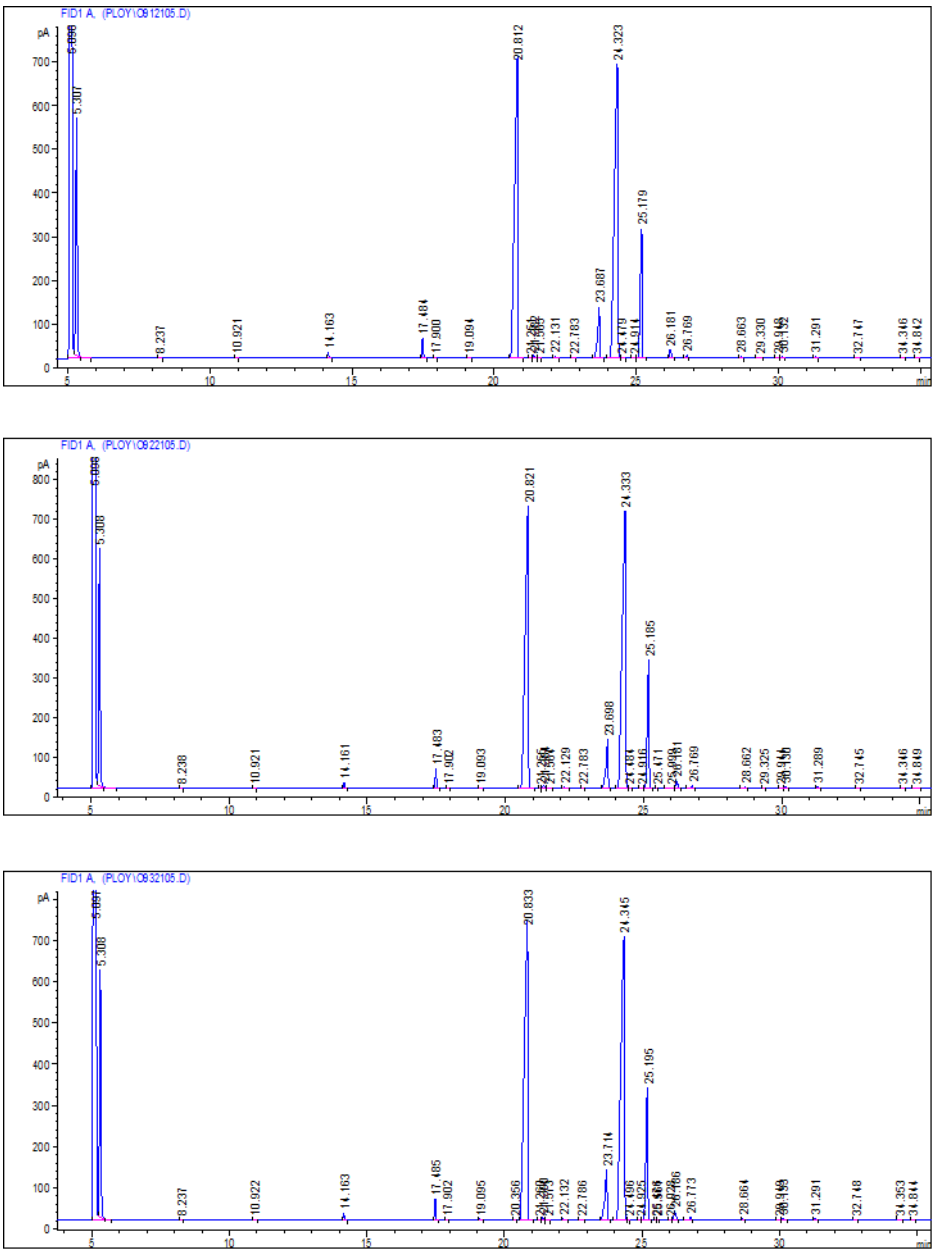


Figure B9 GC-FID Chromatogram of CPO9 with 3 repetitions.

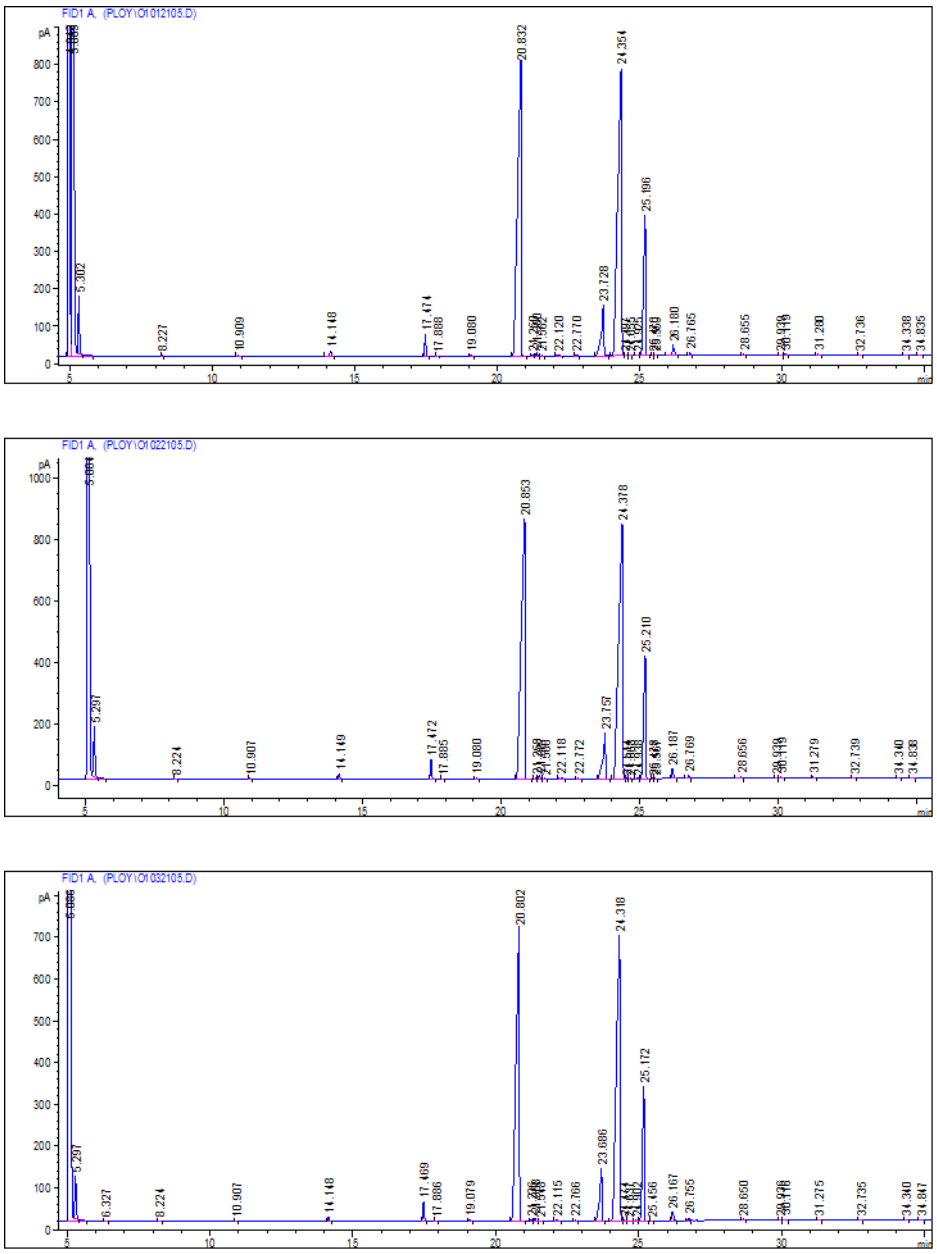


Figure B10 GC-FID Chromatogram of CPO10 with 3 repetitions.

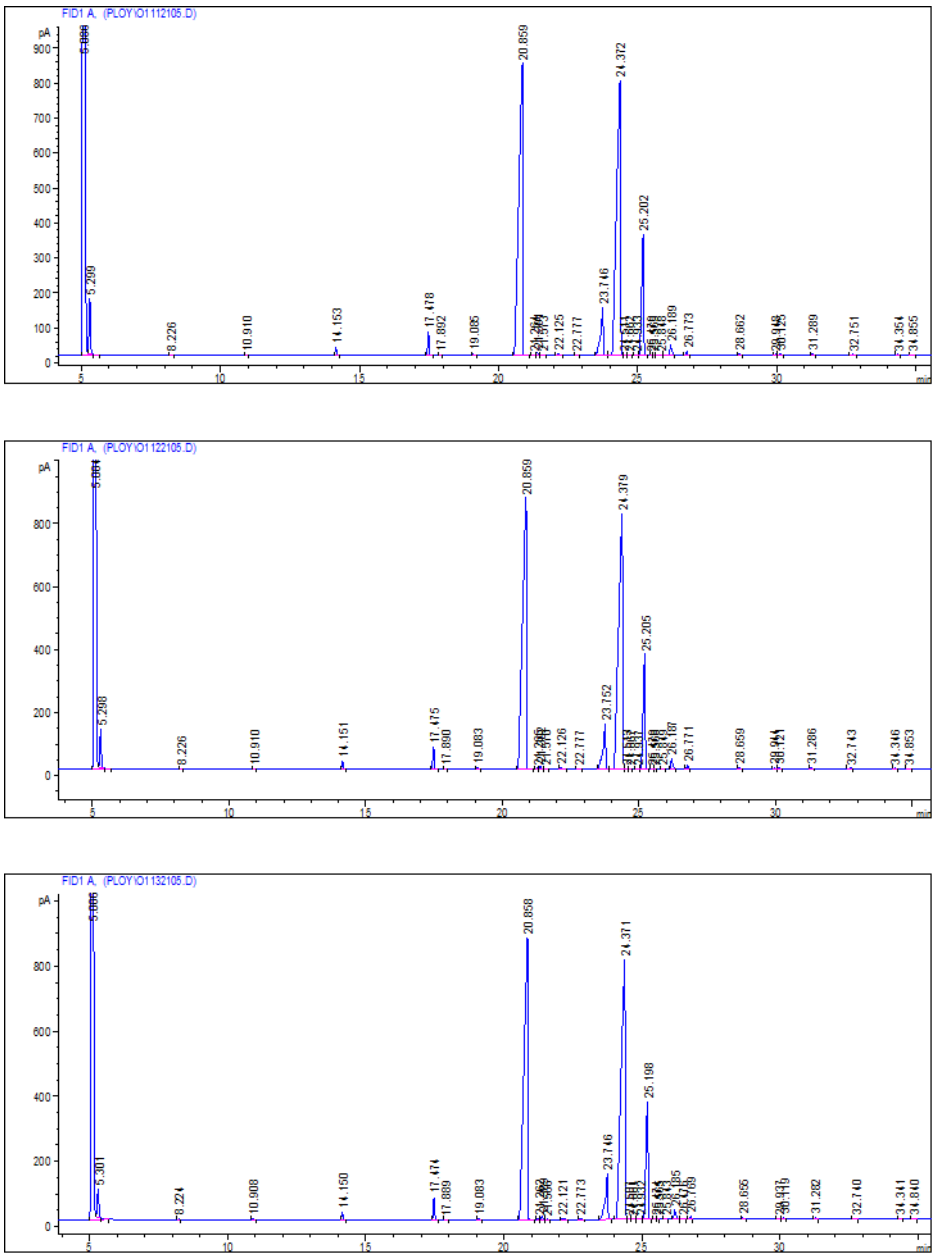


Figure B11 GC-FID Chromatogram of CPO11 with 3 repetitions.

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