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CHLORINATED PRECURSORS OF 3-MONOCHLOROPROPANEDIOL ESTERS IN PALM OIL SUPPLY CHAIN

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CHLORINATED PRECURSORS OF 3-MONOCHLOROPROPANEDIOL (3-MCPD) ESTERS IN PALM OIL SUPPLY CHAIN

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3-MCPD esters are present in most fat-containing food products to exert toxicity as free 3-MCPD, a non-genotoxic carcinogen after hydrolysis in the human digestive system. 3-MCPD esters form from the reaction of lipid with chlorine sources such as organochlorine compounds found in crude palm oil (CPO) to act as chlorinated precursors. Therefore, this study evaluated organochlorine compounds in palm fruit and oil. In addition to known sphingolipid-based organochlorine compounds, this study also identified several new organochlorine compounds as wax ester-, fatty acid- and diacylglycerol-based organochlorine compounds through liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis of CPO.

Organochlorine compounds were established for the first time as ubiquitous in all vegetable oils in this study. Changes of organochlorine compounds content in oil palm mesocarp from 14 to 22 weeks after pollination (WAP) also were showed for the first time in this study to support organochlorine compounds as natural chemical constituents that are produced in oil palm fruits. Sphingolipid-based organochlorine compounds showed the highest reduction (>75%) before to after deodorization of bleached palm oil with minimal distillation into palm fatty acid distillate. Besides, the highest formation of 3-MCPD esters (19.00 mg/kg) were found in fraction (F5) containing sphingolipid-based organochlorine compounds obtained from silica column chromatography of CPO. Thus, supporting sphingolipid-based organochlorine as the main chlorine source for 3-MCPD esters formation among organochlorine compounds identified in this study.

Sphingolipid based-organochlorine compounds in oil palm mesocarp increase by two folds within the first 6 hours and remain unchanged between 6 to 96

hours after harvest reported for the first time in this study. Loose fruits of oil palm that experience more environmental degradation also showed higher content of sphingolipid-based organochlorine than fresh fruits. Subsequently, this study also showed sterilizer condensate oil and empty fruit bunch oil produced in oil palm mill have higher content sphingolipid-based organochlorine compound than CPO by about 10 and 3-folds respectively for the first time. Inclusion of sterilizer condensate dilution instead of clean water dilution showed an increment in the content of sphingolipid-based organochlorine (>70%) and formation of 3-MCPD esters (>60%) after refining of CPO.

This study also pioneering evaluation of changes in sphingolipid-based organochlorine content during physical, chemical refining and water washing of CPO. Chemical refining showed about 82% reduction of sphingolipid-based organochlorine content due to neutralization while physical refining showed about 42% reduction only due to the degum-bleaching approach. Meanwhile, water washing of CPO reduced 3-MCPD ester formation by removing only the water-soluble chlorinated compounds; the insoluble organochlorine compounds were not removed.

This study provides new perspectives and understanding of organochlorine compounds as a chlorinated precursor for 3-MCPD ester formation in palm oil where organochlorine compounds in oils should be considered to produce 3-MCPD ester-free oil for human consumption. A more thorough and comprehensive identification of organochlorine compounds could be developed in the future to support further research on organochlorine compounds in palm oil.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

REAKTAN PEMULA BERKLORIN UNTUK PENGHASILAN ESTER 3-MONOKLOROPROPANEDIOL (3-MCPD) DALAM RANGKAIAN BEKALAN MINYAK SAWIT

Oleh

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Ester 3-MCPD terdapat di dalam hampir semua produk makanan yang mengandungi lemak dan menunjukkan ketosikan sebagai 3-MCPD bebas, karsinogen bukan tosik genetik selepas hidrolisis dalam sistem pencernaan manusia. Ester 3-MCPD terbentuk daripada tindak balas lipid dengan sumber klorin seperti organoklorin yang terdapat dalam minyak sawit mentah (CPO) dan berfungsi sebagai reaktan pemula berklorin. Justeru, kajian ini telah menyelidik sebatian organoklorin sebagai reaktan pemula berklorin untuk pembentukan ester 3-MCPD di dalam buah dan minyak sawit. Selain daripada organoklorin berasaskan sfingolipid yang telah diketahui, kajian ini juga telah mengenalpasti beberapa organoklorin baru iaitu organoklorin berasaskan ester lilin, asid lemak dan diasilgliserol di dalam sample CPO melalui analisis kromatografi cercair-spektrometri jisim resolusi tinggi (LC-HRMS).

Sebatian organoklorin juga telah dikenalpasti untuk kali pertama sebagai sebatian umum yang boleh dijumpai dalam semua minyak sayur mentah dalam kajian ini. Perubahan kandungan sebatian organoklorin di dalam sabut kelapa sawit dari minggu ke-14 hingga ke-22 selepas pendebungaan (WAP) juga dikenalpasti dalam kajian ini untuk kali pertama bagi menyokong sebatian organoklorin sebagai konstituen kimia semulajadi yang dihasilkan dalam buah kelapa sawit. Organoklorin yang berasaskan sfingolipid menunjuk penurunan terbesar (>75%) sebelum dan selepas penyahbauan daripada minyak sawit terluntur dengan penyulingan yang minima ke dalam distilat asid lemak sawit. Selain daripada itu, pembentukan ester 3-MCPD yang tertinggi (19.00 mg/kg) juga didapati dalam pecahan (F5) yang mengandungi organoklorin yang

berasaskan sfingolipid yang diperolehi melalui kromatografi turus silika menggunakan CPO. Penemuan ini menyokong bahawa organoklorin berasaskan sfingolipid merupakan punca utama klorin untuk pembentukan ester 3-MCPD di kalangan organoklorin yang telah dikenalpasti dalam kajian ini.

Kepekatan organoklorin yang berasaskan sfingolipid juga dilaporkan meningkat sebanyak dua kali ganda dalam sabut kelapa sawit selepas 6 jam pertama dituai namun tidak berubah kandungan dari 6 hingga 96 jam selepas dituai dan pencerapan ini juga dilaporkan untuk kali pertama dalam kajian ini. Leraian buah sawit yang mengalami lebih kemerosotan daripada kesan persekitaran berbanding dengan buah segar juga didapati mengandungi lebih banyak organoklorin berasaskan sfingolipid. Seterusnya, minyak kondensat pengsterilan dan minyak tandan kosong kepala sawit yang diperolehi selepas pemprosesan buah sawit dalam kilang juga menunjukkan kandungan organoklorin berasaskan sfingolipid yang lebih tinggi iaitu disekitar 10 dan 3 kali ganda masing-masing berbanding dengan CPO. Penggunaan kondensat pengsterilan untuk pencairan minyak berbanding dengan pencairan dengan air bersih juga menunjuk peningkatan dalam kandungan organoklorin berasaskan sfingolipid (>70%) dan pembentukan ester 3-MCPD (>60%) selepas proses penulenan CPO.

Kajian ini juga mempelopori penilaian dalam perubahan kandungan organoklorin berasaskan sfingolipid semasa penulenan dan pembersihan CPO dengan air. Penulenan secara kimia menunjukkan penurunan kandungan organoklorin berasaskan sfingolipid pada sekitar 82% akibat proses penuetralan manakala penyulingan secara fizikal hanya menunjukan penurunan di sekitar 42% disebabkan oleh proses penyahgam dan pelunturan. Sementara itu, pembersihan CPO dengan air mengurangkan pembentukan ester 3-MCPD melalui pembuangan sebatian berklorin yang larut air. Akan tetapi, organoklorin yang tidak larut dalam air tidak dapat dibuang daripada CPO melalui pembersihan dengan air.

Kajian ini telah mengemukakan pemahaman melalui perspektif baru berkenaan dengan sebatian organoklorin sebagai reaktan pemula berklorin untuk pembentukan ester 3-MCPD dalam minyak sawit di mana sebatian organoklorin patut dikurangkan untuk menghasilkan minyak yang bebas daripada ester 3-MCPD bagi kengunaan manusia. Kaedah pengenalpastian organoklorin yang lebih teliti dan menyeluruh perlu dibangunkan dalam penyelidikan lanjutan sebatian organoklorin dalam minyak sawit pada masa hadapan.

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LIST OF ABBREVIATIONS

3-MCPD	3-Monochloropropandiol
AP	Ascorbyl Palmitate
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CONTAM	European Food Safety Authority Panel on Contaminants in the Food Chain
CPO	Crude Palm Oil
DAG	Diacylglycerol
EFSA	European Food Safety Authority
FFB	Fresh Fruits Bunch
LC-HRMS	Liquid Chromatography-High Resolution Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MAG	Monoacylglycerol
PFAD	Palm Fatty Acid Distillate
PFO	Palm Fiber Oil
PG	Propyl Gallate
SPD	Short Path Distillation
TAG	Triacylglycerol
TBHQ	tert-Butylhydroquinone
VE	Vitamin E
WAP	Weeks After Pollination

CHAPTER 1

INTRODUCTION

1.1 Research background on 3-MCPD esters in palm oil

Palm oil is a major vegetable oil that has been widely used in food applications such as in cooking, margarine, spreads, confectionary fats, ice cream, emulsifiers, and vanaspati (Pande et al., 2012). Overall, each person is estimated to consume between 3.5 to 4.3 kg of palm oil every year in developing and historically high-income countries (Chen et al., 2011). However, every vegetable oil including palm oil contains various hazardous contaminants such as 3-MCPD esters that need to be eliminated from food products due to food safety concerns. The content of 3-MCPD esters in refined bleached deodorized palm oil has been reported to be the highest among all vegetable oils (Weißhaar, 2011).

3-MCPD esters and their related compounds are found in most processed food as free, mono-, di-chloro substituted bound esters on a glycerol backbone (Figure 1.1) (Hamlet et al., 2002; Zelinková et al., 2006). 3-MCPD esters ingested along with foods are reported to be 86% bioavailable as free 3-MCPD (Abraham et al., 2013). Free 3-MCPD is a possible carcinogen that is found to be responsible for infertility in rats and suppresses the immune function (CONTAM, 2016; Hamlet et al., 2011; Hoogenboom, 2016). The German Institute for Risk Assessment and the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (COMTAM panel) have assumed 100% release of free 3-MCPD from its esters in our digestive system (EFSA, 2008). As a result, EFSA had set a tolerable daily intake for 3-MCPDs in January 2018 at 2 µg/kg body weight/day (CONTAM, 2018).

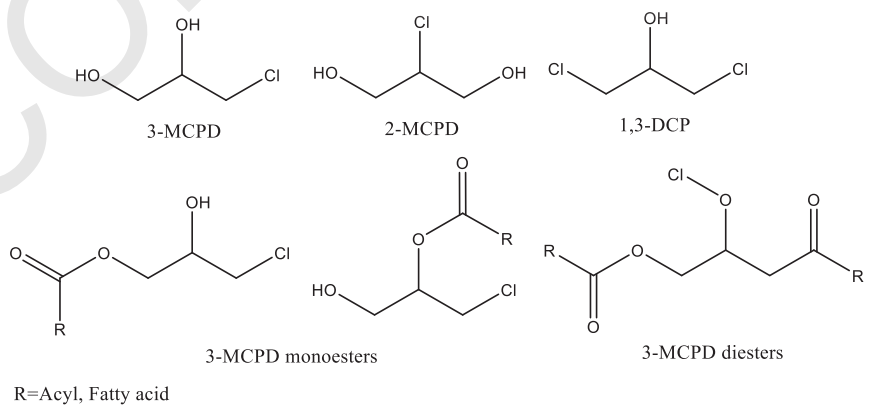

Figure 1.1: 3-MCPD esters and related compounds.

The formation of 3-MCPD esters can be attributed to high-temperature conditions during deodorization in palm oil refining (Matthäus et al., 2011) under the presence of precursors contributing the glycerol and chlorine source. The mechanism for the formation of 3-MCPD esters has shown the involvement of triacylglycerol, diacylglycerol and monoacylglycerol as glycerol precursors (Rahn et al., 2011; Zhang et al., 2013). This is supported by several experimental studies (Ermacora et al., 2014; Šmidrkal et al., 2016).

However, chlorine precursors present in palm oil were not well known until the discovery of more than 200 monochlorinated organic compounds along with some inorganic chlorine compounds in palm oil by Nagy et al. in 2011 (Nagy et al., 2011). Organochlorine was shown to be present in oil extracted from oil palm mesocarp and decreased as 3-MCPD esters increased during *in-vitro* heat treatment, thus suggesting organochlorine as an endogenous compound produced by oil palm that is involved in 3-MCPD ester formation by contributing chlorine (Nagy et al., 2011).

1.2 Problem Statements

Although several organochlorine compounds had been identified as phytosphingosine derivatives in CPO, there are many more organochlorine compounds that remain unknown in palm oil. In addition, the occurrence of organochlorine compounds was only known in CPO as other crude vegetable oils were never investigated for occurrence of organochlorine. Despite the presence of organochlorine compounds in oil palm mesocarp, the information about organochlorine compounds in oil palm mesocarp during fruit development to maturity is unavailable that can reinforce the suggestion of organochlorine compounds as an endogenous compound produced by oil palm. The role of organochlorine compounds to the formation of 3-MCPD esters have not been evaluated under deodorization condition that reflects the realistic condition where 3-MCPD esters are formed almost exclusively during deodorization. (Nagy et al., 2011) The changes undergone by organochlorine compounds within the palm oil supply chain from oil palm fruits after harvest to the production of refined deodorized palm oil have not been elucidated.

The gap in knowledge of chlorinated precursors especially organochlorine compounds for 3-MCPD esters formation in palm fruit and oil had steered understanding on this matter to rely on circumstantial observation. Analysis of organochlorine compounds in palm fruit and oil complement with total chlorine content would provide solid evidence to complete the understanding of chlorinated precursors for 3-MCPD esters formation in palm oil. Therefore, the interest of this study is to provide more understanding on organochlorine compounds for 3-MCPD esters formation in palm fruits and oil.

1.3 Research objectives

This study aims to investigate the presence of organochlorine compounds in vegetable oils, especially palm oil, to gain a better understanding of their characteristics, occurrence and contribution as a precursor of 3-MCPD esters. Ultimately, the knowledge obtained can guide relevant industries to adopt effective strategies for the mitigation of 3-MCPD ester formation in fat and oil products. Therefore, this research project evaluates organochlorine compounds as 3-MCPD esters precursors in palm oil.

Firstly, this study identified the organochlorine compounds that occur in crude palm oil (CPO) based on chlorine isotope characteristics, accurate mass and mass fragmentation exhibited in LC-HRMS analysis. Subsequently, other crude vegetable oils besides CPO were evaluated for the presence of organochlorine compounds. This was related to total chlorine content and 3-MCPD ester formation to provide new evidence to support the occurrence of organochlorine compounds in all vegetable oils.

Secondly, this study evaluated the identified organochlorine compounds in the mesocarp of oil palm fruits during development toward maturity to validate organochlorine compounds as endogenous metabolites produced in plants. The role of organochlorine compounds as precursors of 3-MCPD ester formation was also evaluated through a comparison of the changes undergone by organochlorine compounds during deodorization. This can assist in establishing the availability and feasibility of organochlorine compounds to act as precursors for 3-MCPD ester formation. In order to evaluate the role of organochlorine as a chlorine precursor for 3-MCPD ester formation, the effect of introducing palm oil fractions with concentrated organochlorine into model oil was investigated.

Thirdly, this study looked into the changes of the sphingolipid-based organochlorine in oil palm fruits as a precursor of the 3-MCPD ester from the harvest process to the milling process. The abundance of sphingolipid-based organochlorine compounds in oil palm fruits after harvest in different fruit grades or types such as fresh bunch fruits and loose fruits was also determined. As oil palm fruits are processed in a mill, the effects of processing on sphingolipid-based organochlorine content in CPO production were also investigated in this study.

Lastly, sphingolipid-based organochlorine compounds in CPO going through various refining steps were also evaluated. Physical and chemical refining of CPO was compared for efficiency in removal of sphingolipid-based organochlorine compounds at pre-deodorization. Furthermore, the effects of water washing of CPO in reducing 3-MCPD ester formation and chlorine source were investigated. This study also evaluated the effects of CPO water washing

on sphingolipid-based organochlorine compounds. Ultimately, this research study on organochlorine compounds as precursors of 3-MCPD esters in the palm oil supply chain from fruits to oils would provide new knowledge and perspective on the effects of organochlorine compounds on the formation of 3-MCPD esters in palm oil products.

The main objectives of this study are as follows:

a. To identify organochlorine compounds in palm oil and occurrence of organochlorine compounds in other vegetable oils;

b. To validate organochlorine compounds as endogenous compounds produced by oil palm and role as precursors for 3-MCPD ester formation in palm oil production;

c. To evaluate sphingolipid-based organochlorine compounds in oil palm fruits harvested from estate to the production of CPO in oil palm mills;

d. To determine the effects of refining and water washing of CPO to sphingolipid-based organochlorine compounds to affect the formation of 3-MCPD esters.

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