



UNIVERSITI PUTRA MALAYSIA

***FORMATION, MITIGATION AND ANALYSIS OF 3-MCPD AND GLYCIDYL
ESTERS DURING DEEP-FAT FRYING OF POTATO CHIPS AND
CHICKEN BREAST MEAT USING RBD PALM OLEIN***

WONG YU HUA

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ESTERS DURING DEEP-FAT FRYING OF POTATO CHIPS AND CHICKEN
BREAST MEAT USING RBD PALM OLEIN**

By

WONG YU HUA

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

September 2016

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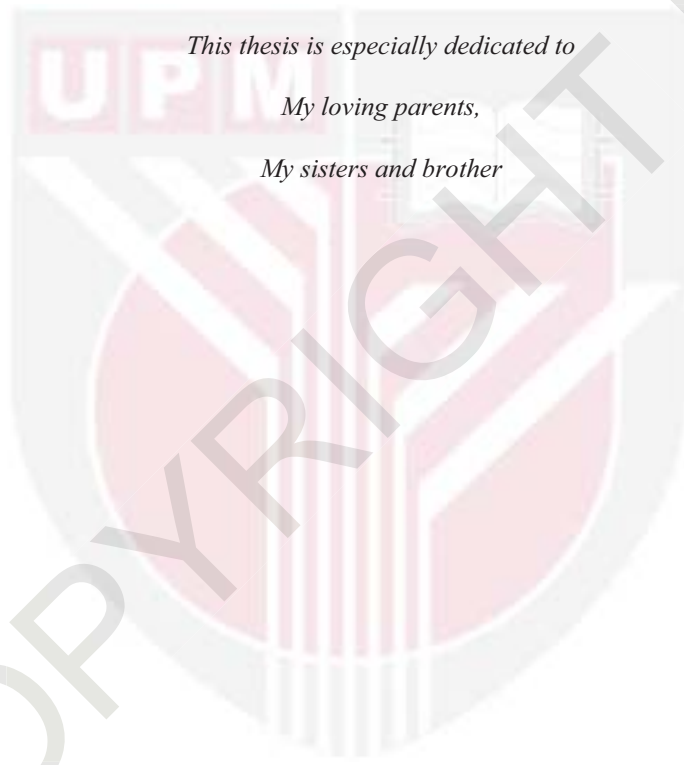
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My loving parents,

My sisters and brother



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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

FORMATION, MITIGATION AND ANALYSIS OF 3-MCPD AND GLYCIDYL ESTER DURING DEEP-FAT FRYING OF POTATO CHIPS AND CHICKEN BREAST MEAT USING RBD PALM OLEIN

By

WONG YU HUA

September 2016

Chair: Tan Chin Ping, PhD

Faculty: Food Science and Technology

3-monochloro-1,2-propanediol (3-MCPD) esters and glycidyl ester (GE) are thermally induced contaminants formed during high-temperature processes, such as deep-fat frying (DFF). In this study, the levels of 3-MCPD esters and GE formed during the frying of carbohydrate-rich and protein-rich foods in different frying systems, the effect of antioxidants, and alternative determination method of 3-MCPD esters were studied.

This study was divided into four parts. First, potato chips (a carbohydrate-rich food) were fried in palm olein under different parameters for 5 days. The applied frying temperatures used were 160 °C and 180 °C, and the concentrations of NaCl were 0%, 1%, 3% and 5%. The formation and changes of 3-MCPD esters and GE levels and the oxidative stability of palm olein during DFF were investigated. A high frying temperature resulted in a high level of 3-MCPD esters but a low level of GE. A long frying duration caused a reduction in 3-MCPD esters and an increase in GE. The addition of NaCl to the frying system significantly ($p < 0.05$) increased the level of 3-MCPD esters, but no significant difference ($p > 0.05$) was observed for the GE content. The frying oils and the oils extracted from the potato chips showed similar results. The oil qualities were within the safety limit after 5 days of frying.

Second, chicken breast meats (CBM, a protein-rich food) were fried in palm olein under the same frying conditions mentioned earlier. In general, the result showed similar trends to the frying of carbohydrate-rich food. However, the levels of 3-MCPD esters and GE were higher than those of the potato chips. This is because CBM contains transition metals, such as iron that can accumulate in frying oils, which can further enhance the degradation rate of the oils.

Third, the effect of synthetic (BHA, BHT, and TBHQ) and natural antioxidants (oleoresin rosemary, sage extract) on the oxidative stability and the levels of 3-MCPD esters and GE in palm olein was studied during DFF. The frying systems with added TBHQ and oleoresin rosemary had levels of 3-MCPD esters and GE that were significantly ($p < 0.05$) lower than those of other frying systems. In order of the inhibition ability decrease were TBHQ \approx oleoresin rosemary $>$ sage extract \approx BHA \approx BHT.

Finally, an alternative technique for the estimation of 3-MCPD esters in palm olein using a combined FTIR-chemometric analysis was successfully developed. The results obtained by using FTIR were consistent with the indirect determination method of GC-MS. Chemometric analysis was applied to correlate the content of 3-MCPD esters with the FTIR spectrum data. A partial least squares (PLS) model was able to predict the concentrations of 3-MCPD esters at a 95% confidence level with R^2 values above 0.90. In conclusion, this research can serve as a reference for the effect of frying on the levels of 3-MCPD esters and GEs, the ability of antioxidants in reducing 3-MCPD esters and GE in frying oil and the possibility of applying FTIR-chemometrics in 3-MCPD ester determination.

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

**PEMBENTUKAN, MITIGASI DAN ANALISIS 3-MCPD DAN GLISIDIL
ESTER SEMASA PROSES PENGGORENGAN JELUK TERHADAP KEREPEK
KENTANG DAN DAGING DADA AYAM DENGAN MENGGUNAKAN RBD
OLEIN SAWIT**

Oleh

WONG YU HUA

September 2016

Pengerusi: Tan Chin Ping, PhD

Fakulti: Sains dan Teknologi Makanan

Kajian telah membuktikan bahawa 3-monokloro-1,2-propanediol (3-MCPD) ester and glisidil ester (GE) adalah bahan kontaminan yang terbentuk dalam proses bersuhu tinggi, seperti proses penggorengan jeluk. Oleh itu, tahap 3-MCPD esters dan GE semasa penggorengan jeluk makanan yang kaya dengan karbohidrat dan protein dalam sistem penggorengan yang berlainan, kesan antioksidan, dan kaedah alternatif untuk menentukan kandungan 3-MCPD esters telah dikaji.

Kajian ini terbahagi kepada empat bahagian. Pertama, kerepek kentang (makanan yang kaya dengan karbohidrat) telah digoreng dalam minyak sawit olein dalam sistem yang berlainan selama 5 hari. Suhu yang digunakan adalah 160 °C and 180 °C dan kepekatan NaCl yang digunakan adalah 0%, 1%, 3% and 5%. Tahap 3-MCPD ester dan GE, serta kestabilan oksidatif minyak sawit olein semasa penggorengan jeluk telah dikaji. Suhu tinggi semasa penggorengan menyebabkan tahap 3-MCPD esters yang tinggi tetapi GE yang rendah. Tempoh penggorengan yang lebih lama menyebabkan pengurangan 3-MCPD ester dan peningkatan GE. Penambahan NaCl dalam sistem penggorengan telah meningkatkan kandungan 3-MCPD esters dengan ketara ($p < 0.05$) tetapi tiada perbezaan ketara ($p > 0.05$) bagi tahap GE. Minyak goreng dan minyak yang diekstrak daripada kerepek kentang juga menunjukkan hasil yang sama. Kualiti minyak didapati masih dalam had keselamatan selepas proses penggorengan selama 5 hari.

Kedua, daging dada ayam (makanan yang kaya dengan protein) telah digoreng dalam minyak sawit olein dengan parameter yang sama seperti yang tertera di atas. Secara amnya, keputusan menunjukkan kecenderungan yang serupa dengan makanan yang kaya dengan karbohidrat. Walau bagaimanapun, tahap 3-MCPD esters dan GE adalah lebih

tinggi berbanding dengan kerepek kentang. Ini adalah kerana daging dada ayam mengandungi logam peralihan, seperti besi yang boleh terkumpul dalam minyak goreng, yang dapat meningkatkan kadar degradasi minyak.

Ketiga, kesan antioksidan sintetik (BHA, BHT, TBHQ) dan semula jadi (oleoresin rosemary, ekstrak sage) terhadap kestabilan oksidatif dan tahap 3-MCPD esters dan GE dalam olein sawit semasa proses penggorengan telah dikaji. Sistem penggorengan yang ditambah dengan TBHQ dan oleoresin rosemary mempunyai tahap 3-MCPD ester dan GE yang lebih rendah secara ketara ($p < 0.05$) daripada sistem yang lain. Keberkesanan antioksidan tersebut berkurangan mengikut turutan yang berikut; TBHQ \approx oleoresin rosemary $>$ sage extract \approx BHA \approx BHT.

Akhirnya, satu kaedah alternatif untuk menentukan jumlah 3-MCPD esters dalam minyak sawit olein dengan menggunakan analisis FTIR-kimometrik telah berjaya dihasilkan. Keputusan 3-MCPD ester yang diperolehi daripada FTIR adalah konsisten dengan kaedah tidak langsung yang digunakan, iaitu kaedah GC-MS. Analisis kimometrik telah digunakan untuk menghubungkan kandungan 3-MCPD ester dengan maklumat daripada spektrum FTIR. Model kuasa dua terkecil separa (PLS) dapat meramalkan kandungan 3-MCPD ester dengan tahap keyakinan 95% dan nilai-nilai R^2 melebihi 0.90.

Kesimpulannya, kajian ini boleh digunakan sebagai rujukan untuk kesan penggorengan terhadap tahap 3-MCPD esters dan GE, keupayaan antioksidan dalam mengurangkan 3-MCPD esters dan GE dalam minyak goreng, serta kemungkinan untuk menggunakan FTIR-kimometrik dalam penentuan kandungan 3-MCPD esters.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Tan Chin Ping, PhD

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Lai Oi Ming, PhD

Professor
Faculty of Biotechnology
Universiti Putra Malaysia
(Member)

Faridah Abas, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Halimah binti Muhamad, PhD

Analytical and Quality Development Unit
Malaysia Palm Oil Board
(Member)

Nyam Kar Lin, PhD

Associate Professor
Faculty of Food Science with Nutrition
UCSI University
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ROBIAH BINTI YUNUS, PhD

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Signature: _____
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Committee: Tan Chin Ping, PhD

Signature: _____
Name of Member of
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Committee: Lai Oi Ming, PhD

Signature: _____
Name of Member of
Supervisory
Committee: Faridah Abas, PhD

Signature: _____
Name of Member of
Supervisory
Committee: Halimah binti Muhamad, PhD

Signature: _____
Name of Member of
Supervisory
Committee: Nyam Kar Lin, PhD

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LIST OF ABBREVIATIONS

3-MCPD	3-chloropropane-1,2-diol
ALARA	As Low As Reasonably Achievable
ATR	Attenuated total reflection
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CPO	Crude palm oil
DAG	Diacylglycerol
FFA	Free fatty acids
FFB	Fresh fruit bunches
FTIR	Fourier transform infrared spectrometer
GC	Gas chromatography
GDP	Gross Domestic Product
GE	Glycidyl ester
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MAG	Monoacylglycerol
MS	Mass spectrometry
NaCl	Sodium chloride
PCA	Principal Component Analysis
PKO	Palm kernel oil
PLS	Partial Least Squares
PMTDI	Provisional maximum tolerable daily intake
RBD	Refined, bleached, and deodorized
TAG	Triacylglycerol
TBHQ	Tert-butylhydroquinone
TDI	Tolerable daily intake
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 General Overview

Frying is a common high-temperature food processing method used throughout the world. Simultaneous heat and mass transfer processes are involved in deep fat frying (Debnath, Rastogi, Krishna, & Lokesh, 2009). The quality of the fried food depends on the frying conditions, the type of oils and the food matrix used during the process. After frying, the frying medium (oil) is partially absorbed by the fried food (5 % in frozen French fries, 15–20 % in batter-coated fish and doughnuts, and 35-40 % in potato chips) (Lin, Akoh, & Reynolds, 2001). Therefore, both consumers and researchers are concerned with the quality of frying oils.

Palm oil is the most common edible oil that being used in deep-fat frying. The palm oil industry is one of Malaysia's major agricultural enterprises. Malaysia possesses 2 % of the world's oilseed crop plantations and supplies 10 % of the world's oils and fats (Mekhilef, Siga, & Saidur, 2011). The growth of the palm oil industry is closely related to the increased demand for food and various non-food applications (Castanheira, Acevedo, & Freire, 2014).

3-MCPD esters have been found in foods for many years. More recently, high contents of 3-MCPD esters were also found in refined oils, especially in refined palm oil. It was suggested that the mechanism of formation is related to the heat treatment of the oilseeds during the refining process. Malaysia, as the world's leading palm oil-producing country, recognizes the importance of the problem and is taking steps to monitor refined oils. Much strong evidence has been reported in the scientific literature for the formation of 3-MCPD and glycidyl ester during palm oil refining. It is generally believed that the formation of 3-MCPD and glycidyl ester is due to the exposure of palm oil to high temperatures during the edible oil deodorization step. The formation mechanism of 3-MCPD esters and GE involves the acyloxonium ion as an intermediate, which is produced from partial acylglycerol. Hence, it is worth investigating the effects of synthetic and natural antioxidants on the formation of 3-MCPD esters and GE in frying oils.

The potato (*Solanum tuberosum* L.) is the top 5 most important crop in the world after sugar cane, maize, wheat and rice, with a production of more than 329 million tonnes in 2009 (FAO, 2012). According to Norimah et al. (2008), the chicken is one of the most consumed type of poultry in Malaysia in both urban and rural areas. As frying is the most

common method used in potato and chicken cooking, changes in the oil stability and the formation of hazardous compounds need to be evaluated.

The commonly use method for 3-MCPD esters and GE determination is indirect acid transesterification and derivatization followed by the use of gas chromatography with mass spectrometric detection (GC-MS). It had good repeatability and reproducibility. However, this method has complicated preparation steps and required 16 h incubation. The Fourier transform infrared spectrometer (FTIR) has been used for various food applications to monitor the quality and safety of foods by providing information on their biochemical composition (Ayvaz & Rodriguez-Saona, 2015). This analytical method requires simple or no sample preparation and minimal solvent, and the analysis process is rapid. FTIR spectroscopy is frequently combined with multivariate analysis to make this technology ideal for rapid screening, as well as the identification of various complex compounds.

1.2 Objectives

- i. To determine the oxidative stability and levels of 3-chloropropane-1,2-diol (3-MCPD) esters and glycidyl ester (GE) in palm olein during the deep-frying of potato chips.
- ii. To determine the oxidative stability and levels of 3-chloropropane-1,2-diol (3-MCPD) esters and glycidyl ester (GE) in palm olein during the deep-frying of chicken breast meat.
- iii. To determine the effects of synthetic and natural antioxidants on the oxidative stability and levels of 3-chloropropane-1,2-diol (3-MCPD) esters and glycidyl ester (GE) in palm olein during deep-frying of potato chips.
- iv. To develop an alternative technique for the estimation of the 3-monochloropropane-1,2-diol (MCPD) ester content in palm olein by using FTIR coupled with chemometric analysis.

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