



UNIVERSITI PUTRA MALAYSIA

**METHODS FOR SQUALENE CONCENTRATION FROM PALM FATTY
ACID DISTILLATE**

CAMILLA CHUA SOO LENG

FSTM 2007 13



**METHODS FOR SQUALENE CONCENTRATION
FROM PALM FATTY ACID DISTILLATE**

By

CAMILLA CHUA SOO LENG

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

November 2007



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**METHODS FOR SQUALENE CONCENTRATION
FROM PALM FATTY ACID DISTILLATE**

By

CAMILLA CHUA SOO LENG

November 2007

Chairman: Associate Professor Badlishah Sham Baharin, M.Sc.

Faculty: Food Science and Technology

Two methods for separating and concentrating of squalene from palm fatty acid distillate (PFAD) by optimizing the enzymatic hydrolysis of PFAD which has been neutralized-hydrolyzed-neutralized before being run onto adsorption column chromatography and by selection of various adsorbents used in adsorption column were being compared. Extraneous matters, especially free fatty acids (83.8%) and aclyglycerols (12.7%) in the PFAD were first neutralized and removed before being subjected to hydrolysis using commercially available immobilized *Candida antarctica* lipase at 65°C for 8 h. Neutralization followed by hydrolysis and repetition of neutralization again successfully concentrated squalene from an initial amount of 3.76% to 27.5%. Oil extracted from neutralized-hydrolyzed-neutralized PFAD (NHNPFAD) was then passed through reverse-phase adsorption chromatography using Diaion HP-20[®]. Squalene was desorbed by hexane, with a recovery of 93%.



Factors affecting the enzymatic hydrolysis and squalene concentration of extracted fraction from NHNPFAD were optimized using response surface methodology (RSM). A central composite design was employed to study the responses, namely percentage of squalene concentration (Y_1), while reaction time (X_1), water content (X_2) and enzyme concentration (X_3) were the independent variables. Results showed that the regression models generated adequately explained the data variation and significantly ($P < 0.05$) represented the actual relationships between the reaction parameters and the response. The optimum reaction parameters for maximum yield in squalene concentrations was carried out with 7.05 h, water content, 61.4% (w/w) and enzyme concentration, 2.23% (w/w).

Laboratory investigations of squalene adsorption on polyaromatic adsorbents; Diaion HP-20[®], Amberlite XAD-1180[®], Duolite XAD-761[®], SP825[®], Dowex Optipore L-285[®] (DO), SP207[®] (Sepabeads) and Florisil[®] were compared for the concentration and recovery of squalene. It was found that the Diaion HP-20[®] gave the highest concentration of squalene, 27.9% with the recovery of >90%, in comparison with that of Amberlite XAD-1180[®], DO L-285[®], SP207[®], Florisil[®], while Duolite XAD-761[®] is much lower. In terms of squalene concentration and recovery, SP825[®] is as adsorptive as Diaion HP-20, but nevertheless Diaion HP-20 was chosen as the best adsorbent due to its economical price.

Equilibrium parameters for squalene adsorption onto Diaion HP-20[®] were estimated by linear least square and a trial and error procedure of non-linear method using Langmuir, Freundlich and Redlich-Peterson isotherms. A comparison between linear and non-linear method of estimating the isotherm was reported. The best fitting isotherm was Freundlich isotherm in linear method and Langmuir and Redlich - Peterson isotherm



equation in the non-linear method. The results show that both linear and non-linear method could be use to obtain the parameters with high coefficient of determination ($R^2 > 0.90$). Redlich-Peterson isotherm is a special case of Langmuir isotherm when the Redlich-Peterson isotherm constant g was unity.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KAEDAH UNTUK PEMEKATAN SKUALIN DARIPADA
SULINGAN PENYAHBAU MINYAK KELAPA SAWIT**

Oleh

CAMILLA CHUA SOO LENG

November 2007

Pengerusi: Profesor Madya Badlishah Sham Baharin, MS

Fakulti: Sains dan Teknologi Makanan

Dua kaedah pengasingan dan pemekatan skualin daripada sulingan penyahbau minyak kelapa sawit melalui pengoptimum hidrolisis berenzim sulingan penyahbau kelapa sawit yang telah dineutrasiasi-hidrolisis-neutralisasi sebelum disalurkan ke dalam kolum kromatografi penyerapan dan juga melalui pemilihan pelbagai penyerap yang digunakan dalam kolum penyerapan telah dibandingkan. Bendasing, terutamanya asid lemak bebas (83.8%) dan asilgliserol (12.7%) di dalam sulingan penyahbau minyak kelapa sawit mula-mula dineutrasiasikan dan disingkirkan sebelum dihidrolisiskan menggunakan enzim tersekat gerak *Candida antarctica* yang boleh diperolehi secara komersial pada suhu 65°C selama 8 h. Neutralisasi diikuti dengan hidrolisis dan pengulangan neutralisasi sekali lagi telah berjaya memekatkan skualin dari amuan awal 3.76% sehingga 27.5%. Minyak yang diekstrak daripada neutralisasi-hidrolisis-neutralisasi penyahbau minyak kelapa sawit (NHNPFAD) seterusnya dilalukan melalui kromatografi penyerapan fasa berbalik menggunakan Diaion HP-20[®]. Skualin telah dinyahserap oleh heksana dengan memperoleh kembali sebanyak 93% skualin.



Faktor-faktor yang mempengaruhi hidrolisis berenzim dan kepekatan skualin daripada fraksi yang diekstrak dari NHNPFAD telah dioptimakan menggunakan Pengkaedahan Respon Permukaan (RSM). Satu rekaan komposit sentral telah diterapkan untuk mengkaji respon, khususnya peratus kepekatan skualin (Y_1), manakala masa tindak balas (X_1), kandungan air (X_2) dan kepekatan enzim (X_3) merupakan pembolehubah bebas. Keputusan menunjukkan bahawa model regresi yang dihasilkan menerangkan variasi data dengan mencukupi dan menjelaskan hubungan sebenar di antara parameter reaksi dan respon dengan signifikansi ($P < 0.05$). Parameter reaksi optima untuk hasil maksimum bagi kepekatan skualin telah dijalankan selama 7.05 jam, dengan kandungan air 61.4% (berat/berat) dan kepekatan enzim 2.23% (berat/berat).

Penyelidikan makmal bagi penyerapan skualin ke atas penyerap poliaroma Diaion HP-20[®], Amberlite XAD-1180[®], Duolite XAD-761[®], SP825[®], Dowex Optipore L-285[®] (DO), SP207[®] (Sepabeads) dan Florisil[®] telah dibandingkan bagi kepekatan dan peratus perolehan semula skualin. Didapati Diaion HP-20[®] memberikan kepekatan skualin tertinggi, 27.9% dengan peratus perolehan semula sebanyak >90%, jika dibandingkan dengan Amberlite XAD-1180[®], DO L-285[®], SP207[®], Florisil[®], sementara Duolite XAD-761[®] memberikan nilai yang lebih rendah. Bagi syarat kepekatan dan pemulangan skualin, kebolehan penyerapan SP825[®] adalah hampir sama dengan Diaion HP-20[®], namun demikian Diaion HP-20[®] telah dipilih sebagai penyerap terbaik disebabkan harganya yang lebih rendah.

Parameter keseimbangan bagi penyerapan skualin ke atas Diaion HP-20[®] telah dianggar melalui pengurangan kuasa linear dan kaedah tak linear secara percubaan menggunakan isoterma Freundlich dan Redlich-Peterson. Satu perbandingan di antara

kaedah linear dan tak linear untuk menganggar isoterma telah dilaporkan. Isoterma yang dipadankan paling baik ialah isoterma Freundlich bagi kaedah linear dan persamaan isoterma Redlich-Peterson bagi kaedah tak linear. Keputusan menunjukkan bahawa kedua-dua kaedah linear dan tak linear dapat digunakan untuk mendapatkan parameter dengan koefisien hubung kait yang tinggi ($R^2 > 0.90$). Isoterma Redlich-Peterson merupakan satu kes khusus bagi isoterma Langmuir apabila pekali isoterma Redlich-Peterson g adalah 'unity'.



ACKNOWLEDGEMENTS

Grateful acknowledgement is made for the encouragement and advice given by the chairman of my supervisory committee, Assoc. Prof. Badlishah Sham Baharin, and other members of my committee, Prof. Dr. Yaakob Che Man and Dr. Tan Chin Ping.

My gratitude is extended to the laboratory staffs and numerous friends for their unfaltering support and understanding while I was completing this thesis.

Lastly, I would like to dedicate this thesis to my family, who gave me an appreciation of learning and taught me the value of perseverance and endurance.



I certify that an Examination Committee has met on 12 November 2007 to conduct the final examination of Camilla Chua Soo Leng on her Master of Science thesis entitled 'Methods for Squalene Concentration from Palm Fatty Acid Distillate' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Act recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Sharifah Kharidah Syed Muhamad, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Hasanah Mohd. Ghazali, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Azis Ariffin, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Milton T.W. Hearn, PhD
Professor
School of Chemistry
Monash University
Australia
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 April 2008



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

Badlishah Sham bin Baharin, MS

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

Yaakob Che Man, PhD

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

Tan Chin Ping, PhD

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

AINI IDERIS, PhD

Professor/Dean
School of Graduate School
Universiti Putra Malaysia

Date: 8 May 2008



DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at UPM or at any other institutions.

CAMILLA CHUA SOO LENG

Date: 8 May 2008



TABLE OF CONTENTS

		Page
	ABSTRACT	ii
	ABSTRAK	v
	ACKNOWLEDGEMENTS	viii
	APPROVAL	ix
	DECLARATION	xi
	LIST OF TABLES	xv
	LIST OF FIGURES	xvi
	LIST OF ABBREVIATIONS	xviii
	LIST OF NOTATIONS	xx
	CHAPTER	
I	INTRODUCTION	1
II	LITERATURE REVIEW	5
	Palm Fatty Acid Distillate	5
	Squalene	7
	Nomenclature, Properties and Natural Sources of Squalene	7
	Functional and Nutritional Aspects of Squalene	9
	Utilization of Squalene	12
	Methods for Analysis of Squalene	13
	Chromatographic Methods	14
	Supercritical Carbon Dioxide Extraction	17
	Other Analyses	20
	Enzymatic Hydrolysis of Lipid	22
	Structural and Characterization of <i>Candida</i> Lipase	22
	Interfacial Activation of Lipase-Catalysis	23
	Parameters Effecting on the Degree of Hydrolysis	25
	Adsorption as a Separation Method for Squalene	27
	Definition, Characteristics and Applications of Adsorption	27
	Adsorbent Diaion HP-20®	29
	Mechanism and Kinetics of Adsorption	31
	Equilibrium of Adsorption	34
III	ENZYMATIC HYDROLYSIS AS A CONCENTRATION STEP FOR SEPARATION OF SQUALENE FROM PFAD	41
	Introduction	41
	Materials and Methods	45
	Materials	45
	Determination of Lipase Activity	45
	Method of Concentration of Squalene	46
	Method 1: PFAD	46



Method 2: Neutralized -Hydrolyzed-Neutralized PFAD (NHNPFAD)	46
Method 3: Hydrolyzed-Neutralized PFAD (HNPFAD)	48
Effect of Time on Enzymatic Hydrolysis	48
Optimization of Enzymatic Hydrolysis of NPFAD using Response Surface Methodology	49
Recovery of Squalene-Rich Fraction by Adsorption Chromatography	50
Determination of Squalene	50
Determination of the Acylglycerol Composition	51
Determination of Slip Melting Point	51
Determination of Free Fatty Acids	52
Determination of FFA for Neutralized-Hydrolyzed PFAD	52
Determination of Total Fatty Components	53
Determination of Iodine Value	53
Determination of Saponification Value	53
Determination of Unsaponifiable Matter	53
Statistical Analyses for Response Surface Methodology (RSM)	54
Statistical Analysis	55
Results and Discussion	55
Physicochemical Characteristics of PFAD after Neutralization and Hydrolysis	55
Time Course Study of Enzymatic Hydrolysis	59
One Factor Study	60
Effect of Temperature	61
Effect of Water Content	63
Effect of Lipase Content	63
Regression Model of the Enzymatic Hydrolysis	64
Interaction of Reaction Variables	66
Response Surface Plots	67
Optimization of Enzymatic Hydrolysis	69
Verification of the Regression Models	70
Conclusion	72
IV SELECTION OF ADSORBENTS AND EQUILIBRIUM STUDY ON THE ADSORPTION OF SQUALENE	73
Introduction	73
Materials and Methods	75
Materials	75
Preparation of the Adsorption Column	76
Adsorption Chromatography	77
Preparation of Squalene Solution	77
Adsorption Profiling	77
Adsorption Equilibrium Experiments	78
Models of Adsorption Equilibrium Isotherms	78
The Langmuir Isotherm	79
The Freundlich Isotherm	80
The Redlich-Peterson Isotherm	81
Determination of Squalene	82
Model Fitting and Statistical Analyses	82



Results and Discussion	83
Selection of Adsorbents	83
Adsorption Profiles	89
Profiles of Equilibrium Isotherm	90
Equilibrium Adsorption Isotherm Using Linear and Non-linear Method	91
Linear Method	91
Non-linear Method	95
Conclusion	97
V	
SUMMARY, CONCLUSION AND RECOMMENDATION	98
Summary	98
Conclusion and Recommendation	100
BIBLIOGRAPHY	102
BIODATA OF THE AUTHOR	117
LIST OF PUBLICATIONS	118



LIST OF TABLES

Table		Page
1	Composition of Palm Fatty Acid Distillates	6
2	Squalene Content in Palm Oil Products	6
3	Structure and Nomenclature of Squalene and Squalane	7
4	Potential Availability of Squalene from PFAD in Malaysia	9
5	Squalene Found in Varying Amounts from Other Sources	10
6	Physical Properties of Diaion HP Series	31
7	Common Isotherm Adsorption Models Based on the Thermodynamic Equilibrium	35
8	Independent Variables and Their Levels for Central Composite Rotatable Design in Optimization of Squalene Concentration by <i>Candida antarctica</i> Lipase-Assisted Hydrolysis of NPFAD	50
9	Physico-Chemical Properties of PFAD, HNPFAD, NPFAD, NHPFAD and NHNPFAD	56
10	Experimental Design of 5-level, 3-Variable Central Composite Rotatable design (CCRD)	65
11	Coefficients and Its Probability of a Larger F Value (Prob>F) of the Models	67
12	Optimal Conditions of Squalene Concentration from Enzymatic Hydrolysis of NPFAD by RSM	70
13	Predicted and Actual Percentage of Squalene Concentrated Extracted from Enzymatic Hydrolysis of PFAD	71
14	Properties and Characteristics of Adsorbents	76
15	Recoveries of Oil and Squalene by Adsorption Chromatography on Various Adsorbents	84
16	Isotherm Parameters for Squalene Adsorption onto Diaion HP-20 [®] Using Linear Method	95
17	Isotherm Parameters for Squalene Adsorption onto Diaion HP-20 [®] Using Non-Linear Method	97



LIST OF FIGURES

Figure		Page
1	Drawing of the protein showing a side view of the 11-stranded mixed β sheet	22
2	Graphical representation of isotherm models	40
3	Method development for separation of squalene from palm fatty acid distillate (PFAD)	47
4	Time course profile of the hydrolysis of 1:2 (w/w) ratio of neutralized palm fatty acid distillate and water mixture at $65 \pm 1^\circ\text{C}$ with 1.0% w/w of commercially immobilized <i>Candida antarctica</i> lipase	60
5	Changes in percentage of squalene extracted from NHPFAD and NHNPFAD	62
6	Response surface plot of squalene contents (%) as the function of the interaction between two variables with the other held constant at low value: (A) interaction between water content and reaction time with 2.5% w/w lipase concentration; (B) interaction between lipase concentration and reaction time in 60% w/w water content; and (C) interaction between lipase concentration and water content over 8 h reaction time	68
7	Reversed-phase high-performance liquid chromatograms for squalene concentration (%) obtained from first elution-methanol at RT9.912 by adsorption chromatography on various adsorbents: a) Diaion HP-20 [®] , b) Amberlite XAD-1180, c) Duolite XAD-761, d) Dowex Optipore L-285, e) Polyaromatic SP207, f) SP825 and g) Florisil	87
8	Reversed-phase high-performance liquid chromatograms for squalene concentration (%) obtained from second elution-hexane at RT9.912 by adsorption chromatography on various adsorbents: a) Diaion HP-20 [®] , b) Amberlite XAD-1180, c) Duolite XAD-761, d) Dowex Optipore L-285, e) Polyaromatic SP207, f) SP825 and g) Florisil	88
9	Milligram squalene adsorbed as a function of time for adsorbent Diaion HP-20 [®]	90
10	Adsorption isotherm of squalene onto Diaion HP-20 [®]	91
11	(a) Langmuir, (b) Freundlich, and (c) Redlich-Peterson isotherm linear plots for the sorption of squalene onto Diaion HP-20 [®]	93



- 12 Equilibrium adsorption profiles of squalene onto adsorbent Diaion HP-20 using linear method. Experiments were carried out by agitating 1 g Diaion HP-20 with 50 mL squalene solution at 180 rpm. Abbreviations: C_e , squalene concentration in the solution at equilibrium; q_e , amount squalene adsorbed per g Diaion HP-20[®] at equilibrium 94
- 13 Equilibrium adsorption profiles of squalene onto adsorbent Diaion HP-20 using non-linear method. Experiments were carried out using the same condition as described in Figure 12. For abbreviations, see Figure 12 96



LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	American Oil Chemists' Society
BET	Brunauer-Emmet-Teller
CCRD	Central composite rotatable design
cGC	Capillary gas chromatography
DEAG	Diesterified alkoxyglycerols
DG	Diacylglycerols
DO	Dowex Optipore
DVB	Styrene-divinyl benzene
ELSD	Evaporative light scattering detectors
FAD	Fatty acid distillates
FFA	Free fatty acids
GC	Gas chromatography
HPLC	High performance liquid chromatography
HRGC	High resolution gas chromatography
HSCCC	High-speed counter-current chromatography
IV	Iodine value
LC	Liquid chromatography
LC-GC	Liquid chromatography-gas chromatography
MG	Monoacylglycerols
NPFAD	Neutralized palm fatty acid distillate
NHPFAD	Neutralized hydrolyzed palm fatty acid distillate
NHNPFAD	Neutralized-hydrolyzed-neutralized palm fatty acid distillate



PFAD	Palm Fatty Acid Distillate
PKO	Palm kernel oil
PORIM	Palm Oil Research Institute Malaysia
PUFA	Polyunsaturated fatty acid
RBD	Refined, bleached and deodorized
RP	Reversed-phase
R-P	Redlich-Peterson
RSM	Response surface methodology
SCCO ₂	Supercritical fluid carbon dioxide
SCF	Supercritical fluid
SMP	Slip melting point
SP	Sepabeads
SPE	Solid phase extraction
SSE	Sum squares of errors
TAG	Triacylglycerols
TG	Triglycerides
TLC	Thin-layer chromatography
USM	Unsaponifiable matter
UV-vis	Ultra-violet vis
VLDL	Very low density lipid

LIST OF NOTATIONS

α_L	Constant in Langmuir equation (mL mg^{-1})
α_R	Constant in Redlich-Peterson equation (mL mg^{-1})
a_R	Redlich-Peterson isotherm constant ($\text{mg g}^{-1} \text{dm}^{-3}$)
β	Constant in Redlich-Peterson equation (mL mg^{-1})
β_0	The intercept in Eqn [6] (dimensionless)
β_i	First-order model coefficient in Eqn [6] (dimensionless)
β_{ii}	Quadratic coefficient for the i th variable in Eqn [6] (dimensionless)
β_{ij}	Interaction coefficient for the interaction of variables i and j in Eqn [6] (dimensionless)
b	Constant in Langmuir equation (mL mg^{-1})
b'	Redlich-Peterson isotherm exponent (dimensionless)
B	Energy-adsorption constant (dimensionless)
c_e	Equilibrium liquid phase solute concentration (mg mL^{-1})
C	Concentration (mg dm^{-3})
C_e	Initial concentration of adsorbate in the solution/at time 0 (mg mL^{-1})
C_s	Solute concentration at the particle surface (mg dm^{-3})
$\bar{d}q$	Average concentration of solute in particle (mg g^{-1})
D_{eff}	Effective diffusion coefficient (dimensionless)
k_f	Freundlich isotherm constant ($\text{dm}^3 \text{g}^{-1}$)
k_i	Rate constant of intraparticle diffusion ($\text{mg g}^{-1} \text{min}^{0.5}$)
K_L	Constant in Langmuir equation (mL mg^{-1})
K_F	Constant in Freundlich equation (mL mg^{-1})
K_p	Partition coefficient of the adsorbate



K_R	Constant in Redlich-Peterson equation (mL mg^{-1})
n	Freundlich isotherm constant (dimensionless)
n_f	Freundlich isotherm constant (dimensionless)
n_R	Redlich-Peterson isotherm constant (dimensionless)
Q	Concentration of adsorbed adsorbate per unit mass of adsorbent at time t (mg g^{-1})
Q_e	Amount of adsorbed adsorbate per unit mass of adsorbent at equilibrium (mg g^{-1})
Q_o	Theoretical monolayer saturation capacity derived from Langmuir equation (mg g^{-1})
R^2	Coefficient of determination (dimensionless)
R_L	Separation factor (dimensionless)
t	Time (min)
X_1, X_2, X_3	Independent variables of regression model for Response Surface Methodology representing reaction time, water content and enzyme concentration (h, % v/w, % w/w)
X_i, X_j	Independent variables of regression model for Response Surface Methodology (units are depending on the variables)
Y_1	Response, namely percentage of squalene concentration of regression model for Response Surface Methodology (%)

CHAPTER I

INTRODUCTION

The present study relates to a method developed for the extraction and concentration of squalene from palm oil by-products such as palm fatty acid distillate (PFAD). Squalene is a triterpene primarily known as an intermediate in the biosynthesis of sterols in plant and animal world (Psomiadou and Tsimidou, 2003). It is widely distributed in nature existing in large quantities in shark liver oil, while reasonable amounts are found in wheat germ oil, palm oil, amaranth oil and rice bran oil (Smith, 2000). Squalene is also present in lower concentrations in some natural plant oils such as olive, corn, peanut and rapeseed oils (Catchpole and Kamp, 1997).

Squalene is a very useful substance exhibiting strong anti-carcinogenic activity. It plays an important role as a dietary cancer chemo-preventive agent and act as chain-breaking antioxidants by scavenging chain-carrying peroxy radicals of the chain propagation. Trials have shown that where squalene is taken as a dietary supplement, evidence has shown that it has preventative effects against carcinogenesis (Choo *et al.*, 2003). Therefore, squalene which is present as one of the minor components in palm oil, could be recovered as a valuable antioxidant if present in high concentration.

PFAD is a by-product obtained from palm oil refining. Deodorized distillate obtained from the deodorization process of vegetables oils consists of many components including free fatty acids, tocopherols, sterols, squalene and neutral oil (Verleyen *et al.* 2001). High concentrations of tocopherols, tocotrienols and hydrocarbons are not



easily obtained by concentration of PFAD, because the amounts of these components in PFAD are very low compared to soyabean, rapeseed and similar raw materials (Top *et al.*, 1993). Squalene and hydrocarbons are two important components in PFAD with concentrations of 2404-13504 and 4000-8000 ppm, respectively.

With the recent recognition of enzyme hydrolysis in the modification of fats, the application of the lipase from *Candida antartica* has opened interesting research opportunity with an important role of hydrolyzing esters of long-chain aliphatic acids from glycerol at oil/water interfaces. Lipases have the important physiological role of preparing the fatty acids of water-insoluble TG for adsorption into and transportation through membranes by converting the TG to the more polar DG, MG, FFA and glycerol (Jensen, 1983). Al-Duri *et al.* (1995) demonstrated that lipase from *Candida cylindracea* was the most active on olive oil for the modification of triacylglycerols by splitting the triglycerides into their constituent fatty acid and glycerol. Studies have shown that the specificity of lipases leads to a purer product, minimizes the production of undesirable by-products, and provides a wide range of new products that have both useful and marketable applications (Al-Duri *et al.*, 1995).

A possible process for the separation of squalene is mainly adsorption and desorption operating at isocratic conditions. According to Chu *et al.* (2004a), adsorption involves separation of a substance (i.e. adsorbate) from one phase, accompanied by its accumulation or concentration on the surface of the adsorbing phase (i.e. adsorbent). Adsorption of the concentrate of squalene obtained was conducted using adsorbents such as normal-phase silica, reversed-phase resin or neutral alumina in a non-polar solvent. Palaniappan and Proctor (1990), Toro-

Vazquez and Rocha-Urbe (1993) and Toro-Vazquez and Mendez-Montealvo (1995) reported on the applications of adsorption including removal of free fatty acids and other oxidation products from various vegetable oils.

In this research, HPLC was used to identify and determine squalene from PFAD. Much efforts have been made on the isolation of non-glyceride components particularly squalenes, carotenes, vitamin E, sterols from palm oils and by-products (Choo *et al.*, 2005). Choo *et al.* (2005) reported the chromatographic isolation of squalene from palm oil using supercritical fluid in combination with adsorbents such as silica gel. Fractional crystallization was used by Nenadis and Tsimidou (2002) to obtain squalene in the liquid fraction of virgin olive oil prior to HPLC determination of squalene. Meanwhile, Vidal-Escales and Borros (2004) used HPLC coupled with a light scattering detector to separate squalene from its by-products after vulcanization from rubber samples.

It has been disclosed lately that squalene as the major hydrocarbon in non-glyceride components further possess an antioxidizing action for oil or fat derived foods as well as medicinal action of preventing diseases, and their demand for use as pharmaceuticals, nourishing foods and cosmetics has increased sharply (He and Corke, 2003). The current research seeks to provide better quality and yield of squalene obtained from PFAD in a complete and economic way. It is, therefore, essential to develop a novel and efficient method for the concentration of squalene from PFAD. The objectives of this work were to: