RECOVERY OF CAROTENES AND TOCOPHEROLS FROM PALM OIL MILL EFFLUENT VIA EXTRACTION AND CHROMATOGRAPHY

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by

CHAN CHOI YEE

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LIST OF SYMBOLS

		Unit
A	EFB ash percentage	%
A_{max}	Maximum wavelength	nm
A_{min}	Minimum wavelength	nm
A_T	Constant for Temkin isotherm	L/g
a_b	Cuvette error	-
a_s	Absorbance	-
B	Constant for Temkin isotherm	-
b	Monolayer capacity of the adsorbent	mg/g
C_A	Parameter concentration in EFB ash	mg/kg
C_e	Concentration of adsorbate at equilibrium	mg/L
$C_{extract}$	Carotenes concentration in the extracted oil	ppm
C_i	Carotenes concentration in oil eluted in respective fraction	ppm
C_{oil}	Carotenes concentration in oil loaded onto the column	ppm
C_{POME}	Carotenes concentration in oil from POME	ppm
$C_{predicted}$	Predicted concentrations in sludge-ash mixtures	mg/kg
C_S	Parameter concentration in POME sludge	mg/kg
C_t	Concentration of adsorbate at time, t	mg/L
C_0	Initial adsorbate concentration	mg/L
K_F	Freundlich adsorption isotherm constant	mg/g (L/mg)$^{1/n}$
K_L	Langmuir adsorption equilibrium constant	L/mg
k_p	Intraparticle diffusion rate constant	mg/g h$^{1/2}$
k_1	Adsorption rate constant for pseudo-first-order kinetic model	1/h
k_2	Adsorption rate constant for pseudo-second-order kinetic model	g/mg h
n	Constant for Freundlich isotherm	-
q_e	Amount of adsorbate adsorbed per unit mass of adsorbent at equilibrium	mg/g
$q_{e,cal}$	Calculated amount of adsorbate adsorbed at equilibrium	mg/g
$q_{e,exp}$	Experimental amount of adsorbate adsorbed at equilibrium	mg/g

q_t	Amount of adsorbate adsorbed per unit mass of adsorbent at time, t	mg/g
R	Universal gas constant	8.314 J/mol K
R_L	Separation factor	-
S	POME sludge percentage	%
T	Temperature	K
t	Time	h
V	Volume	L or mL
W	Weight	g
$W_{extract}$	Weight of oil extracted	mg
W_i	Weight of oil eluted in respective fraction	mg
W_{oil}	Weight of oil loaded onto the column	mg
W_{POME}	Weight of oil contains in POME	mg
x	Experimental variables	-

Greek letters

ΔG	Free energy change	J/mol
ΔH	Enthalpy change	J/mol
ΔS	Entropy change	J/mol K
β	Regression coefficient	-
μ	Dipole moment	D
ε	Relative permittivity	-
δ	Solubility parameter	$(J/mL)^{1/2}$
λ_{max}	Maximum absorption	nm

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometry
ANOVA	Analysis of variance
APHA	American Public Health Association
AV	Anisidine value
B	Boron
BJH	Barrett-Joyner-Halenda
BOD	Biochemical oxygen demand
Ca	Calcium
CCD	Central composite design
C:N	Carbon to nitrogen
COD	Chemical oxygen demand
CPO	Crude palm oil
CSTR	Continuous stirred-tank reactor
CV	Coefficient of variation
DF	Degrees of freedom
DOBI	Deterioration of bleachability index
DoE	Design of experiment
DSM	Department of Standards Malaysia
EFB	Empty fruit bunch
Em	Emission
EPA	Environmental Protection Agency
EQA	Environmental Quality Act
Ex	Excitation
Fe	Iron
FFA	Free fatty acid
FFB	Fresh fruit bunch
H-E	*n*-Hexane:ethanol
HPLC	High performance liquid chromatography
IV	Iodine value
K	Potassium
K_2O	Potassium oxide

KOH	Potassium hydroxide
Mg	Magnesium
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Council
MSDS	Material safety data sheet
N	Nitrogen
NPLC	Normal phase liquid chromatography
OCC	Open column chromatography
OMW	Olive mill wastewater
OSRMA	Official, Standardised and Recommended Methods of Analysis
P	Phosphorus
PE	Petroleum ether
P-E	Petroleum ether:ethanol
PFAD	Palm fatty acid distillate
P_2O_5	Phosphorus pentoxide
PO_4	Phosphate
POME	Palm oil mill effluent
ppm	Part per million
PV	Peroxide value
R^2	Correlation coefficients
rpm	Rotation per minute
RSM	Response surface methodology
SMP	Slip melting point
SPO	Sludge palm oil
T	Tocopherols
T3	Tocotrienols
TAG	Triacylglycerols
TOC	Total organic carbon
TOTOX	Total oxidation value
UF	Ultrafiltration
UV	Ultraviolet
VAD	Vitamin A deficiency
WHO	World Health Organization

II	Middle absorption peak
III	Longest-wavelength absorption peak

PEROLEHAN SEMULA KAROTENA DAN TOKOFEROL DARIPADA KUMBAHAN KILANG MINYAK KELAPA SAWIT MELALUI PROSES PENYARIAN DAN KROMATOGRAFI

ABSTRAK

Karotena dan tokoferol mempunyai banyak faedah kesihatan dan penting dalam industri makanan, kosmetik dan farmasi. Disebabkan oleh permintaan yang meningkat untuk produk semulajadi ini, maka penyelidikan ini bertujuan untuk mengkaji kemungkinan perolehan semula karotena dan tokoferol daripada air buangan pertanian yang banyak didapati di Malaysia, iaitu kumbahan kilang minyak kelapa sawit (POME). Proses penyarian pelarut digunakan untuk mendapatkan semula minyak daripada POME manakala penjerapan kromatografi digunakan untuk mendapatkan semula karotena dan tokoferol daripada minyak yang diekstrak. Komponen utama pengekstrakan minyak daripada POME didapati menyerupai minyak sawit mentah yang mengandungi α-karotena, β-karotena, α-tokoferol, γ-tokoferol dan β-tokoferol. Keputusan eksperimen menunjukkan bahawa nisbah pelarut:POME dan kadar campuran memainkan peranan penting dalam perolehan semula minyak dan karotena daripada POME dengan menggunakan proses penyarian pelarut. Keadaan-keadaan optimum yang diperolehi untuk pengekstrakan minyak dan karotena daripada POME adalah nisbah 8:10 *n*-heksana:POME; 500 rpm kadar campuran dan 25 min masa campuran. Keupayaan penjerapan β-karotena meningkat dengan peningkatan kepekatan awal, masa sentuh dan suhu. Penjerapan β-karotena pada gel silika dan florisil adalah bersesuaian dengan model isoterma Langmuir dan model kinetik pseudo tertib kedua. Proses penjerapan berlaku secara spontan dan endoterma di bawah keadaan-keadaan kajian. Gel silika menunjukkan prestasi yang lebih baik daripada florisil dan aluminium oksida dalam pemisahan karotena daripada minyak yang diekstrak dengan menggunakan proses penjerapan kromatografi. Sistem *n*-heksana:etanol menunjukkan prestasi yang agak konsisten tanpa mengambil kira kesan daripada perbezaan pemuatan minyak permulaan dan suhu yang digunakan pada kromatografi turus terbuka (OCC). Kepekatan karotena dalam *n*-heksana meningkat apabila nisbah minyak:bahan penjerap bertambah. Keputusan eksperimen mendapati bahawa jumlah pelarut dan nisbah minyak:bahan penjerap adalah faktor-faktor penting yang mempengaruhi perolehan semula karotena dan minyak manakala hanya nisbah minyak:bahan penjerap mempengaruhi perolehan semula kepekatan karotena oleh OCC. Suhu adalah merupakan faktor yang tidak penting untuk ketiga-tiga tindakbalas. Elusi profil bagi kromatografi cecair tekanan rendah membuktikan bahawa kepekatan karotena adalah lebih tinggi berbanding dengan kepekatan tokoferol dalam minyak yang diekstrak. Kromatogram menunjukkan puncak yang lebih tajam pada kadar aliran yang lebih tinggi dan pada jumlah muatan yang lebih kecil. Kandungan etanol yang lebih tinggi dalam sistem pelarut menyebabkan pengagihan karotena dan tokoferol yang tidak sekata. Keadaan optimum untuk kromatografi cecair tekanan rendah adalah sama ada beroperasi pada kadar alir rendah dengan muatan minyak yang tinggi atau beroperasi pada kadar alir tinggi dengan muatan minyak yang rendah dengan menggunakan 96:4 (% v/v) n-heksana:etanol. Enapcemar POME kering yang diperolehi dalam kajian ini sesuai digunakan sebagai baja disebabkan oleh paras nitrogen, kalium, kalsium, magnesium, kandungan jirim organik dan nisbah karbon kepada nitrogen yang mencukupi.

RECOVERY OF CAROTENES AND TOCOPHEROLS FROM PALM OIL MILL EFFLUENT VIA EXTRACTION AND CHROMATOGRAPHY

ABSTRACT

Carotenes and tocopherols provide plenty of health benefits and are important in food, cosmetic and pharmaceutical industries. Due to the increasing demand for these natural products, this study aims to investigate the feasibility of recovering carotenes and tocopherols from agricultural wastewater abundantly available in Malaysia, which is the palm oil mill effluent (POME). Solvent extraction was used to retrieve oil from POME whereas adsorption chromatography approach was employed to recover carotenes and tocopherols from the extracted oil. The major components of the extracted oil from POME were found to be similar to crude palm oil, containing mainly α-carotene, β-carotene, α-tocopherol, γ-tocopherol and β-tocopherol. The experimental design results showed that solvent:POME ratio and mixing rate played significant roles in the oil and carotenes recovery from POME by using solvent extraction process. The optimum conditions obtained for extraction of oil and carotenes from POME were 8:10 *n*-hexane:POME ratio; 500 rpm mixing rate and 25 min mixing time. The β-carotene adsorption capacities increased with increasing initial concentration, contact time and temperature. Adsorption of β-carotene on silica gel and florisil were best fitted by the Langmuir isotherm model and pseudo-second-order kinetic model. The adsorption process was endothermic and spontaneous under the conditions studied. Silica gel showed better performance than florisil and aluminium oxide in separation of carotenes from extracted oil of POME by using adsorption chromatography. *n*-Hexane:ethanol system showed rather consistent performance regardless of the different initial oil loading and temperature used on the open column chromatography (OCC). The carotenes concentrations in *n*-hexane fractions increased when the extracted oil:adsorbent ratio increased. The central composite design revealed that solvent amount and oil:adsorbent ratio were significant factors influencing the carotenes and oil recoveries whereas only oil:adsorbent ratio affected the carotenes concentration recovered by OCC. Temperature was an insignificant factor for all the three responses. The elution profiles of low pressure liquid chromatography proved that the carotenes concentration was higher than tocopherols concentration in the extracted oil. The chromatogram showed sharper peaks at higher flow rate and smaller volume loading. Higher percentage of ethanol in the solvent system resulted in uneven distribution of carotenes and tocopherols. The optimum conditions for the low pressure liquid chromatography were obtained either by operating at low flow rate with high oil loading or at high flow rate with low oil loading using 96:4 (% v/v) *n*-hexane:ethanol. The dried POME sludge obtained in this study was feasible to be used as fertilizer due to its notable levels of nitrogen, potassium, calcium, magnesium, organic matter content and appropriate carbon to nitrogen ratio.

CHAPTER 1

INTRODUCTION

1.1 Carotenoids and Tocopherols

Carotenoids as the main groups of coloring substances in nature are responsible for many of the red, oranges and yellow colors of fruits and vegetables (Sabah *et al.*, 2007). Carotenoids are synthesized by all photosynthetic organisms, including phytoplankton, algae, higher plants and phototrophic bacteria. In addition, they are produced by some other bacteria, yeasts and fungi. Carotenoids are selectively absorbed in various food chains, where they may undergo metabolic structural changes (Britton *et al.*, 2004).

In the process of protecting compounds from harmful oxidative reactions via trapping free radicals or quenching singlet oxygen, carotenoids may become the primary oxidizable substrate (Henry *et al.*, 1998). Carotenoids are known to have micronutrients with a large number of functions. Carotenoids are split into two classes which are xanthophylls which contain oxygen and carotenes which are purely hydrocarbons that contain no oxygen. Carotenes possess anti-cancer properties for certain types of cancers, such as oral, throat, lung, stomach and colon cancers (Choo *et al.*, 1997). It may also protect against heart disease and strengthen the body's immune system and protects against toxins, colds, flu and infections. It also helps to prevent night blindness and other eye problems (Lin *et al.*, 2009). In fact, it is being used as an oral sun protectant for the prevention of sunburn and has been shown to be effective either alone or in combination with other carotenoids or antioxidant vitamins (Stahl and Sies, 2005).

Carotenes, in particular β-carotene, are the most important vitamin A precursor (Sambanthamurthi *et al.*, 2000) in human nutrition as it can be transformed into vitamin A *in vivo* (Chuang and Brunner, 2006) and provides the major source of vitamin A for third world populations (Ooi, 1999, Sundram *et al.*, 2003). Currently, vitamin A deficiency is usually treated with commercial vitamin A supplements (Seo *et al.*, 2005). Alternatively, carotenes do not cause hypervitaminosis A, as conversion of carotenoids to vitamin A is regulated and thus, β-carotene has the advantage in humans that it is non-toxic (Chandrasekharan, 1997).

Beside from medicinal uses, the importance of carotenoids has also increased due to the more extensive use of natural compounds in the food, cosmetic and pharmaceutical industries (Sabio *et al.*, 2003, Bhosale and Bernstein, 2004). Carotenoids have an industrial use in food products and cosmetics as vitamin supplements and health food products as well as feed additives for poultry, livestock, fish, and crustaceans (Del Campo *et al.*, 2007). In oil industry, carotenoids are responsible for the long term stability of oils since they have antioxidant properties (Szentmihályi *et al.*, 2002).

On the other hand, vitamin E is the collective name for the eight major naturally occurring molecules, four tocopherols and four tocotrienols. The tocopherols are all pale yellow, viscous oils found in a variety of plants, including almonds, mustard greens, green and red peppers, spinach, sunflower seeds and wheat germ. Vegetables oils, especially the seed oils, are rich sources of tocopherols. Natural tocopherols are recovered from vegetable oil deodorizer distillates, a valuable by product obtained during the deodorization of vegetable oil refining

(Martins *et al.*, 2006). Vitamin E has also traditionally been extracted from the residues of the soybean refining industry (Sambanthamurthi *et al.*, 2000).

Tocopherols which are physiologically active as vitamin E are considered natural antioxidants and find extensive applications in food, cosmetics and pharmaceutical industries (Martins *et al.*, 2006). It is recommended that vitamin E decreases the occurrence of several age-related degenerative diseases. Vitamin E is a potent membrane soluble antioxidant which may prevent colon cancer through several different cellular and molecular mechanisms (Campbell *et al.*, 2003). Besides, α-tocopherol is present in human skin, particularly in the epidermis, which is the outermost skin layer, working as an effective photoprotective agent and functioning as a primary antioxidant in the first line of defence against harmful reactive oxidant species (Fuchs, 1998).

In addition to its use as a vitamin supplement for normal individuals and those at risk for vitamin E deficiency, the tocopherols have a few other uses such as in the curing of meats to block the action of nitrosamines, a group of compound that occurs naturally in meats and may be carcinogenic; as an additive to animal feed to replace vitamins lost during feed processing and as a food additive in vegetable oils and shortening to prevent oxidation or spoilage (Balz *et al.*, 1996, Schlager *et al.*, 2006). Tocopherols can interrupt lipid oxidation by inhibiting hydroperoxide formation in the chain-propagation step, or the decomposition process by inhibiting aldehyde formation. Besides its free radical scavenging activity, α-tocopherol is highly reactive towards singlet oxygen and protects the oil against photosensitized oxidation (Sundram *et al.*, 2003).

Overall, there has been an increasing demand for phytochemical ingredients, particularly in consumer products. This trend is partly due to consumer preference for products containing natural ingredients, which are generally perceived as milder, safer and healthier than their synthetic counterparts (Harjo *et al.*, 2004). The growing worldwide market value of carotenoids is projected to reach over US$1,000 million by the end of the decade (McNally, 2007). Market data contained in the Frost & Sullivan report revealed that the US vitamin E market earned revenues of $209.6 million in 2005 and was estimated to reach $260.8 million by 2012 (Nutraingredients, 2006).

1.2 Palm Oil and Palm Oil Mill Effluent (POME)

Malaysia palm oil industry is growing and the plantation development is accelerating. Malaysia is one of the biggest producer and exporter of palm oil which currently accounts for 41% of world palm oil production and 47% of world exports and also 11% and 25% of the world's total production and exports of oils and fats, respectively (MPOC, 2009). Palm oil production increased from 94,000 tonnes in 1960 to 15 million tonnes in 2005 (Basiron, 2007). The production of crude palm oil (CPO) reached a record of 17.73 million tonnes in 2008 and the total export earnings of oil palm products increased to RM 20.02 billion in year 2008. China, maintained its position as the largest export market for Malaysian palm oil for the seventh consecutive year followed by European Union, Pakistan, USA, India, Japan and Ukraine (Wahid, 2009).

Being an export-oriented industry, the oil palm industry heavily relies on the world market. Therefore it is very vital for the oil palm industry to be sustainable and competitive to increase its long-term profitability and sustainability. The western countries are known for their high environmental standards. Since European Union is the second largest importer of palm oil, it is essential that the oil palm industry is ready to meet the higher expectation of these overseas customers on the environmental performance of the industry. According to Subramaniam *et al.* (2008), there are only two parameters that are causing the potential impacts to the environment from the Malaysian oil palm industry which are the palm oil mill effluent (POME) followed by the boiler ash.

Palm oil mills with wet milling process are accounted for major production of palm oil in the country and a significantly large quantity of water is used during the extraction of CPO from the fresh fruit bunch (FFB). According to Thani *et al.* (1999), about half of the water used in extraction process will result in POME, and the other half being lost as steam, mainly through sterilizer exhaust, piping leakages, as well as wash waters. POME is a high volume liquid waste which is non-toxic, organic in nature but having an unpleasant odour and is highly polluting (Hwang *et al.*, 1978). About 2.5 tonnes of POME is produced for every tonne of oil extracted in an oil mill (Ho *et al.*, 1984, Songip *et al.*, 1996a). Thus 17.73 million tonnes of palm oil production in year 2008 resulted in about 44.33 million tonnes of POME.

POME is a colloidal suspension of 95-96% water, 0.6-0.7% oil and grease and 4-5% total solids including 2-4% suspended solids; originated from the mixture of a sterilizer condensate, separator sludge and hydrocyclone wastewater (Prasertsan and Prasertsan, 1996, Ma, 2000). It is acidic (pH 4-5) and hot (80-90°C). The organic content of POME, as measured by biochemical oxygen demand (BOD, 3 day, 30 °C) averages about 25,000 mg/L with a chemical oxygen demand (COD) of 50,000 mg/L and oil and grease content might exceed 4 000 mg/L which are highly polluting (Ma, 2000). If the POME had been discharged untreated, the amount of BOD produced in year 2008 would be 1.108 million tonnes. By estimating each citizen produces 14.6 kg of BOD every year (Doorn *et al.*, 2006), this pollution load is equivalent to the waste generated by 75 million people which is about thrice the population of Malaysia.

According to the above characteristics, the discharge of untreated POME characterized with high content of degradable organic matters into water streams or rivers definitely cause severe pollution of waterways by oxygen depletion and suffocate the aquatic life. The impact of POME discharge to a relatively small river can be devastating to its eco-system and beneficial uses. The riverine communities and users of rivers and streams are very vulnerable to the adverse pollution impact of indiscriminate discharges of POME. Thus, oil palm industry needs to shift towards more environmental friendly industry as it has to be sustainable and competitive to increase its long-term profitability and sustainability.

In the 1970s, the subject of POME and its impact on the environment had become an issue of much concern to the government and the public. The government acted responsibly in enacting the Environment Quality Act (EQA) in 1974 and specific regulations for POME in 1977 (EQA 1974, 2005). Section 18 of the Act enables the exercise of control through the issuance of a license to the prescribed premises were deemed to provide for the most pragmatic regulatory approach in the case of POME (Maheswaran, 1984). It is mandatory for all palm oil mills to treat their wastewaters on site to an acceptable level before they are allowed to be discharged into water course. Thus, there is a necessity for the palm oil industries to find suitable treatments or processes to ensure that the discharge effluent meets the stringent regulation.

On the other hand, CPO contains a number of important minor components such as carotenoids, tocopherols and tocotrienols, sterols, ubiquinones, triterpenes, phospholipids, glycolipids, terpenic and aliphatic hydrocarbons (Choo, 2000). The percentage of these minor components in CPO is about 1% but their combined economic value is much more (Ooi, 1999). Habib *et al.* (1997) found that POME contained carotenes whereas Chow and Ho (2002) observed that the composition of the major lipids found in the oil droplets separated from the centrifuge sludge is similar to that of commercial palm oil. Therefore, a viable method of recovery of the minor components from the effluent by extraction and chromatography can be explored.

1.3 Extraction and Chromatography

Solvent extraction is frequently used for isolation of antioxidant and extraction yield is dependent on the solvent and method of extraction, due to the different antioxidant potentials of compounds with different polarity. It would be interesting to optimize an extraction process to obtain maximum yield of the antioxidant and in addition removal of undesirable components (Goli *et al.*, 2005). The extraction method must enable to complete extraction of the compounds of interest and must avoid chemical modification (Zuo *et al.*, 2002). The polarities of different compounds present in the samples affected the variation in the yields of various extracts (Hayouni *et al.*, 2007).

The solvents used in the extraction will ultimately be removed or at least reduced by evaporation; therefore solvents with low boiling points should be chosen to avoid prolonged heating of the palm oil. Most hydrocarbons are suitable for the extraction of vitamin A which includes carotenes where light petroleum and n-hexane are almost universally used for this purpose (Davidek, 1975). Moreover, solvents such as hexane, ethanol, methanol, petroleum ether and acetone are commonly used in extraction of oil from vegetables, flowers and oil seeds (Mani *et al.*, 2007). Hexane is often used for vegetable oil extraction mainly due to its efficiency and ease of recovery (Akaranta and Anusiem, 1996). Other solvents such as acetone, iso-propanol and iso-hexane are also often used in oil extraction (Dunford and Zhang, 2003).

In addition, chromatography is one of the most important separation processes in the pharmaceutical and biotechnological industries. It is used both in analytical and preparative applications and separation can be based on different chemical and physical mechanisms. The most common chromatographic processes in preparative applications are based on stationary packed bed columns operated in batch mode (Persson *et al.*, 2004). The first chromatographic experiment was reported in 1905 by Tswett where he created the term ‘chromatography’ inspired in the experiment; elution of a sample of green leaves extract through a column of calcium carbonate which was separated in a yellow fraction (carotenes) and green fraction (chlorophyll). The theory of adsorption chromatography was developed in

1940 by Tiselius and partition chromatography in 1941 by Martin and Synge (Rodrigues and Minceva, 2005).

The selection of various modes in chromatography basically depends on the application. Normal phase liquid chromatography (NPLC) has a polar stationary phase and is usually used to retain polar compounds from a non polar mixture. Hexane soluble compounds are more suitable for separation by NPLC (Harjo *et al.*, 2004). Sample retention in NPLC decreases as the polarity of the mobile phase increases and less polar compounds elute first, while more polar compounds leave the column last. Silica is traditionally applied in NPLC with mobile phases of a low or medium polarity. The strength of the mobile phase increases with increasing solvent polarity (Kazoka, 2002). Using isocratic or gradient elution NPLC can successfully separate the complex biological or industrial materials. The most important advantage of the method is its flexibility where one can change many different parameters to achieve the optimum separation conditions (Borówko and Oscik-Mendyk, 2005).

The major food carotenoids can be reliably determined either by open column chromatography (OCC) or by high performance liquid chromatography (HPLC). OCC has the advantage of using common laboratory equipment and does not require a constant supply of carotenoid standards. Kimura and Rodriguez-Amaya (2002) presented a work on isolating carotenoid standards by OCC. Mortensen (2005) analysed the carotenes from oil palm fruit extract by HPLC, Breithaupt (2004) determined the carotenoids used as food coloring additives by HPLC, Barba *et al.* (2006) used HPLC for determination of lycopene and β-carotene in vegetables, Gimeno *et al.* (2000) developed determination of α-tocopherol and β-carotene in olive oil by reversed phase HPLC and many more. Therefore, combination of solvent extraction and liquid chromatography can be used to recover and isolate the important antioxidants such as carotenes and tocopherols which have increasing demand from different potential sources.

1.4 Problem Statement

World Health Organization (WHO) considers vitamin A deficiency (VAD) as a public health problem in more than half of all countries, especially in Africa and South-East Asia, hitting severely on young children and pregnant women in low-income countries. VAD is the leading cause of preventable blindness in children and increases the risk of disease and death from severe infections whereas in pregnant women, VAD causes night blindness and may increase the risk of maternal mortality. Therefore, supplying adequate vitamin A in high-risk areas is crucial for maternal and child survival which can significantly reduce mortality. Conversely, its absence causes a high risk of disease and death (WHO, 2008). For the time being, most of the vitamin A or vitamin A precursor are attained from food sources which consequently resulting in higher price of the vitamin supplements. Besides, utilization of food sources in order to obtained vitamin A will deplete the raw material itself for other usages. Therefore, a cheaper raw materials or processing methods need to be discovered for easy accessible to the third world country.

Astonishingly, Malaysia palm oil industries can offer the world community towards the prevention of VAD because crude palm oil is the world's richest natural plant source of carotenes in which particularly β-carotene is the most important vitamin A precursor in human nutrition (Choo, 1989). Crude palm oil possesses minor components which amongst them are the carotenoids, vitamin E (tocopherols and tocotrienols) and sterols (Choo *et al.*, 1997). Its concentration normally ranges between 400 ppm to 3500 ppm and it contains about 15 times more retinol equivalents (vitamin A) than carrots and 300 times more than tomatoes (Sundram *et al.*, 2003). However, the process of extraction of CPO from FFB requires large quantity of water which will result in large amount of effluent. Due to the lack of consciousness in environmental issues, treatment of POME has long been labeled as a burden and non-profit activity by most palm oil mill owners.

The standard discharge limit for oil and grease according to Environmental Quality (Prescribed Premises)(Crude Palm Oil) Regulations 1977 is 50 mg/L while the concentration of oil and grease in POME is about 4000 mg/L. Therefore, palm oil mill should seek for an alternative method to remove the oil and grease in the effluent up to 99% before being discharged in order to meet the standard. In response to the government regulations, various treatment and disposal methods have been employed by palm oil mills to treat POME. Conventional biological treatments of anaerobic and aerobic or facultative digestion are used. However, these biological treatments have several disadvantages and the treated effluent may sometimes do not comply with the discharge standard and retrieving oil through these treatments is not viable.

On the other hand, POME has been discovered to contain carotenes and the composition of the major lipids found in the oil droplets separated from the centrifuge sludge was similar to that of commercial palm oil and thus assumption can be made that the carotenes in the oil droplets are also similar to the crude palm oil (Habib *et al.*, 1997, Chow and Ho, 2002). Since POME is abundant, the carotenes contained in POME can therefore be recovered as a valuable source of vitamin A instead of discarding it as waste.

By reclaiming the oil and solid from the POME and further converting it into value added products such as carotenes and fertilizer, this will not only solve the pollution problem but will also create a business opportunity for the industry or venture capitalists. This positive development will result to a paradigm shift in the environmental management of POME. The alternative downstream processing will ensure the treatment of POME to be more efficient, innovative and attractive for the mill owners to apply.

The results obtained from the membrane based POME treatment pilot plant studies by Ismail (2005) and Chong (2007) are promising and favourable to the palm oil mill owners as the treatment system offers water recycling. Therefore, there is a need to develop and design an enhancement system for oil and sludge recovery on top of water recycling treatment system. Recovery of carotenes and tocopherols from POME is important due to its increasing importance and value. This study serves a double purpose, first, the wastewater is converted to value added products and second, the utilization of agricultural waste indirectly solves the environmental problem faced by the palm oil mill.

1.5 Research Objectives

The main aim of this study is to recover oil from POME, separate and concentrate carotenes and tocopherols from the recovered oil and convert its sludge into fertilizer. Foremost, analyses on POME as well as analyses of carotenes and tocopherols in recovered oil are important. The measurable objectives are:

i) To recover oil and grease from POME using solvent extraction and to study the effects of various operating parameters on recovery of oil from POME and its process optimization using response surface methodology.

ii) To investigate the effects of adsorbate initial concentration, contact time and solution temperature on adsorption capacity and equilibrium; to study the isotherms, kinetics and thermodynamics for adsorption of β-carotene on silica based adsorbents using batch adsorption tests.

iii) To evaluate the effects of several parameters on recovery of carotenes from extracted oil by open column chromatography and optimization of the operational parameters on the carotenes recovery process.

iv) To separate carotenes and tocopherols from the extracted oil using low pressure liquid chromatography and to optimize the operating parameters using response surface methodology.

v) To study the feasibility of the POME sludge as fertilizer by determining the nutritive value of POME sludge after oil extraction.

1.6 Research Scope

The main focus of this study is to recover the carotenes and tocopherols contained in the extracted oil of POME which is abundantly available in Malaysia. Solvent extraction method was applied in this study to recover the oil residue from

POME as the oil and grease in the POME was solvent extractable. Non polar solvents were used for easier separation of organic solvents from the aqueous POME. The effects of operating parameters such as solvent:POME ratio, mixing time and mixing rate towards extracted oil and carotenes recovery were determined.

The second part of the study focused on adsorption of β-carotene on silica gel and florisil from *n*-hexane solution. The capacities of silica gel and florisil for the adsorption of β-carotene was investigated. The applicability of common isotherm models to represent the adsorption process was determined. The experimental data were analyzed using pseudo first-order, second-order kinetic and intraparticle diffusion models. Thermodynamics data of the adsorption were analyzed to understand the adsorption process.

Two types of adsorption chromatography were employed to separate and concentrate the carotenes and tocopherols from the extracted oil of POME, which were open column chromatography and low pressure liquid chromatography systems. A comprehensive study of the effects of adsorbents, solvent system, oil:adsorbent ratio and temperature towards the open column chromatography performance in terms of oil recovery, carotenes recovery and carotene concentration were carried out. The temperature which was studied in the experiments was close to the melting point of the extracted oil. An increasing polar solvent system was employed to elute the column where a non polar solvent was first used, and subsequently followed by polar solvent.

A low pressure liquid chromatography was further used in order to examine the separation of carotenes and tocopherols from the extracted oil. Different flowrate, oil loading and solvent mixture were used to examine the elution profiles of carotenes and tocopherols. Fractional elution process was employed. Statistical tool was later applied to determine the significance of experimental variables.

The possibility of converting POME sludge into fertilizer was evaluated by investigating the chemical properties of the extracted sludge. The POME sludge was amended with different ratio of palm oil mill by product to enhance the characteristics of fertilizer.

1.7 Organization of the Thesis

There are five chapters in this thesis. Chapter 1 (Introduction) presents a brief overview on carotenoids and tocopherols, palm oil industry along with the generation of POME and description on extraction and chromatography. The needs to recover carotenes from agricultural by product available in Malaysia are also discussed in this chapter. The research objectives of the present study are elaborated together with the research scope and the overall content of this thesis are summarized in the last section of this chapter.

Chapter 2 (Literature Review) elaborates the characteristic of POME, characteristic of palm oil as well as some information on carotenes and tocopherols. This is followed by discussion on solvent extraction method in oil recovering. Description on adsorption, adsorption isotherms, kinetics and thermodynamics are also provided. Subsequently, explanation on liquid chromatography and adsorption chromatography with chromatographic processing technique is provided. Information on fertilizer is also stated in this chapter. The last part of literature review focuses on the statistical approach used for process optimization.

Chapter 3 (Materials and Methods) covers the methodology for the experimental work done in this research. This chapter presents in detail the materials and chemicals used in the present study. The subsequent sections are the detailed experimental procedures which include details of analysis of samples, solvent extraction, batch adsorption studies, open column chromatography and low pressure liquid chromatography for carotenes and tocopherols recovery as well as fertilizer treatment.

Chapter 4 (Results and Discussion) is the core of this thesis with seven main studies. In the first section, the raw POME characteristics based on the analysis methods followed by oil and carotenes analyses are presented. The second section discusses the solvent extraction of oil from POME and its process optimization. Section three focuses on the batch adsorption studies of β-carotene on silica based adsorbent whereas the following sections cover the adsorption column chromatography. Section four discusses the effects of adsorbents, solvent system, oil:adsorbent ratio and temperature on recovery of oil and carotenes from extracted oil of POME by using open column chromatography and its process optimization. Section five elaborates the effects of flowrate, oil loading and solvent system studied to obtain the optimum condition for recovery of carotenes and tocopherols from extracted oil of POME by using low pressure liquid chromatography. The last section includes the finding on conversion of POME sludge into fertilizer.

Chapter 5 (Conclusions and Recommendations) gives the conclusions and some recommendations for future research. The conclusions reflect the accomplishments of the listed objectives which are obtained throughout the study. Based on the conclusions obtained, recommendations for future research are suggested. These recommendations are presented in view of their implication and importance related to the present research.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Mill Effluent

Palm oil mill effluent (POME) is thick brownish viscous liquid waste and is non-toxic as no chemicals are added during oil extraction but has an unpleasant odor. The typical POME characteristics have been determined and analyzed by several researchers and is shown in Table 2.1 (Chow, 1991, Ma, 2000).

Table 2.1 Characteristics of palm oil mill effluent (Chow, 1991, Ma, 2000).

Parameter	Concentration	Element	Concentration
pH	4.7	Phosphorus	180
Oil and grease	4,000	Potassium	2,270
Biochemical oxygen demand	25,000	Magnesium	615
Chemical oxygen demand	50,000	Calcium	439
Total solids	40,500	Boron	7.6
Suspended solids	18,000	Iron	46.5
Total volatile solids	34,000	Manganese	2.0
Ammoniacal nitrogen	35	Copper	0.89
Total nitrogen	750	Zinc	2.3

* All parameters' units are in mg/L except pH.

The effluent treatment technologies for POME in Malaysia are invariably combinations of physical and biological processes. The physical treatment includes pre-treatment steps such as screening, sedimentation and oil removal in oil traps prior to the secondary treatment in biological treatment systems. The organic content of POME is generally biodegradable and treatment is based on anaerobic, aerobic and facultative processes. The ponding system which consists of combination of the three treatments is the most commonly used where 85% of Malaysia's palm oil mills using this technology. Some of the effluent treatment systems are shown in Table 2.2.

Table 2.2 Current POME treatment processes

Ponding System (Chin *et al.*, 1996)
- Main components of the system are de-oiling tank, acidification ponds, methanogenic ponds, facultative ponds and sand beds
- Extensive land areas are required
- Long treatment period of 45 to 80 days

Anaerobic-cum-Aerated Lagoon System (Thani *et al.*, 1999)
- Similar to the ponding system except the facultative ponds are replaced with mechanically-aerated lagoons
- Operating costs are high due to energy consumption of the mechanical aeration equipment and added maintenance requirements

Conventional Anaerobic Digester (Noor *et al.*, 1990)
- A continuous stirred-tank reactor (CSTR) with no solids recycle
- Requires a longer hydraulic retention time of up to 20 days to prevent washout of microorganisms and to achieve desired treatment efficiency

Up-flow Anaerobic Sludge Blanket Reactor (Zinatizadeh *et al.*, 2006)
- Upward flow of wastewater through a suspended layer or sludge blanket of active biomass
- During contact, organic matter are converted to methane and carbon dioxide
- Biogas is separated, liquor solids settled in the settling zone and treated wastewater is discharges via an overflow weir

Evaporation Process (Songip *et al.*, 1996a, Songip *et al.*, 1996b, Ma, 2000)
- About 85% of the water in POME can be recovered as distillate
- A concentrate of 20-30% solids content was produced
- 89% and 75% reduction in COD and BOD after treatment by adsorption column containing zeolite/activated carbon

Treatment by Tropical Marine Yeast (Oswal *et al.*, 2002)
- Treatment using a marine hydrocarbon-degrading yeast
- A COD reduction of about 95% with a retention time of two days
- Further treatment with flocculant, ferric chloride and consortium developed from garden soil reduced the COD and adjusted the pH to between 6 and 7

Land Application System (Thani *et al.*, 1999)
- The treated effluent with a BOD of less than 5,000 mg/L is applied to cropland as a source of inorganic fertilizer
- Removal of settleable solids and equalization prior to land application by flatbed and long-bed systems

Sludge Recycle by Freezing and Thawing Method (Yee *et al.*, 2002, Yee *et al.*, 2003)
- 70% of the suspended solids were removed by thawing at 5 ml/min
- Retention time for anaerobic treatment could be reduced without affecting organic acids generation

Ultrafiltration (UF) (Nor and Suwandi, 1982, Wah *et al.*, 2002, Ahmad *et al.*, 2003a)
- Protein concentrate, filter cake and clear recycleable water are recovered
- Two stages of treatment consisted of coagulation, sedimentation and adsorption as first stage, ultrafiltration and reverse osmosis membranes as second stage
- Reduction in turbidity, COD and BOD up to 100%, 98.8% and 99.4%, respectively with a final pH of 7

Biological treatment of wastewater relies heavily on a mixed population of active microorganisms which utilize the organic substances polluting the water as nutrients (Ma, 1999). The microorganisms are very sensitive to the changes in the environment and thus great care has to be taken to ensure that a conducive environment is maintained for the microorganisms to grow (Ma, 2000). Besides, the organic matters digestion by the microorganisms requires long treatment period and thus large treatment area is required. In addition, the main air emission from the conventional biological POME treatment ponds during the anaerobic digestion is the biogas which consists of methane, carbon dioxide and traces of hydrogen sulfide (Ma *et al.*, 1999). These biogases are green house gases which harm the quality of the air (Subramaniam *et al.*, 2008).

The Environmental Quality (Prescribed Premises) (Crude Palm Oil) Order 1977, prescribed factories that process oil palm fruit or oil palm fresh fruit bunches into crude palm oil, whether as an intermediate or final products, as "prescribe premises", which shall require a license under Section 18 of the EQA for the occupation of use of their respective premises. Environmental control of crude palm oil mills is exercised through the imposition of appropriate conditions of license which includes ensuring acceptable condition of effluent discharge, proper disposal of scheduled wastes and air emission control throughout the operation (EQA 1974, 2005).

The Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations 1977 are the governing regulations containing the effluent discharge standards. Other regulatory requirements are to be imposed on individual palm oil mills through conditions of license (EQA 1974, 2005). Table 2.3 shows the current effluent discharge standards ordinarily applicable to crude palm oil mills.

Table 2.3 Effluent discharge standards for crude palm oil mills (EQA 1974, 2005)

Parameter	**Unit**	**Parameter Limits (Second Schedule)**
Biochemical Oxygen Demand (BOD; 3 days, 30°C)	mg/L	100
Chemical Oxygen Demand (COD)	mg/L	No discharge standard after 1984
Total Solids	mg/L	No discharge standard after 1984
Suspended Solids	mg/L	400
Oil and Grease	mg/L	50
Ammoniacal Nitrogen	mg/L	150 (Value of filtered sample)
Total Nitrogen	mg/L	200 (Value of filtered sample)
pH	-	5-9
Temperature	°C	45

The BOD_3 concentration limit of 100 mg/L is ordinarily achievable if the treatment systems are well-designed and operated. However, the treatment gets the lowest priority in the operation and maintenance budget due to long treatment duration and large treatment area are required. Therefore, an alternative treatment is needed to substitute the conventional biological treatments.

2.2 Palm Oil

Crude palm oil (CPO) contains mainly glycerides, with 3-5% free fatty acids and about 1% minor components (Choo *et al.*, 1996b). Palm oil contains the highest known concentration of agriculturally derived carotenoids of the vegetable oils that are widely consumed (Ping, 2007). Palm oil has saturated and unsaturated fatty acids in approximately equal amounts (Sambanthamurthi *et al.*, 2000). Most of the fatty acids are present as triacylglycerols (TAG). The different placement of fatty acids and fatty acid types on the glycerol molecule produces a number of different TAGs

(Sambanthamurthi *et al.*, 2000). The palm oil melts over a range of 25-50 °C. The quality of crude palm oil is determined by its free fatty acids content, oxidative parameters such as peroxide values, anisidine value, deterioration of bleachability index (DOBI) and other oxidative measurements (Siew and Cheah, 2007). Some of the physicochemical characteristics of palm oil and sludge palm oil are given in Table 2.4.

The free fatty acid (FFA) content is measured because this is one reliable parameter for food quality and it is used as indication of hydrolysis (Alyas *et al.*, 2006). FFA in vegetable oil result from breaking triglyceride ester bonds (Saleh and Adam, 1994). The crude palm oil contains relatively high amounts of free fatty acids. Processing of fruit without delay or fermentation yields good quality oil with a low free fatty acid content of 2.3%. The oil from freshly harvested fruits contains very little free fatty acids (Salunkhe *et al.*, 1992).

Iodine value (IV) reflects the liquidity and unsaturated fatty acid composition of the oil (Noh *et al.*, 2002). The iodine value of palm oil is lower (44-58) than other vegetable oils because of high proportion of saturated fatty acids. Peroxide value (PV) represents a quality assessment measuring the extent of primary oxidation deterioration in fats and oils (Low *et al.*, 1998). Oxidation (primary and secondary) is described in terms of PV and anisidine value (AV). The total oxidation value (TOTOX) is equal to 2PV + AV (Baharin *et al.*, 2001).

Table 2.4 Characteristics of crude palm oil and sludge palm oil

Parameters	**Palm oil (DSM, 2007)**	**Special quality CPO (DSM, 2007)**	**Standard quality CPO (DSM, 2007)**	**Crude palm oil (Liew *et al.*, 1993)**	**Crude palm oil (Low *et al.*, 1998)**	**Sludge palm oil (Ainie *et al.*, 1995)**		**Sludge palm oil (Ibrahim and Kuntom, 2000)**	
						Range	**Average**	**Range**	**Average**
Moisture content, %	-	< 0.2	< 0.2	0.1	-	below 2.0	0.99	0.08 – 14.79	3.3
Free fatty acid (FFA), %	-	< 2.5	< 5	2.2	4.19	5 – 45	44.43	1.18 - 86.66	36.4
Peroxide value (PV), meq/kg	-	< 1.0	< 2.0	2	1.7	1.36 – 21.81	9.98	1.77 – 41.36	9.4
Iodine value (IV)	52.0	-	-	55	56.1	40.2 – 55.9	49.8	5.4 – 55.3	42.8
Saponification value (mg KOH/g oil)	199	-		-	-	173.82 – 197.86	197.47	143.9 – 284.7	239.8
Unsaponifiable matter, %	0.32	-	-	-	-	0.082 – 0.90	0.348		
Impurities, %	-	< 0.05	< 0.05	-	-	-	-	0.05 – 12.89	0.39
Anisidine value (AV)	-	< 4.0	< 5.0	-	-	-	-	0.70 – 39.22	14.1
DOBI	-	2.8	2.3	-	-	-	-	0.02 – 3.16	0.92
Carotene, ppm	581	-	-	-	587	-	-	7.7 – 678.2	241.4

The extent of deterioration of palm oil can be determined by DOBI method (Baharin *et al.*, 2001). Based on Ibrahim and Kuntom (2000), it was observed that sludge palm oil (SPO) samples had high FFA, moisture, impurities, PV, AV and saponification value while generally low values were observed for carotene content, DOBI and IV. The low FFA values of the SPO samples indicated that they had been collected soon after the oil palm fruits were processed. SPO collected fresh from the sludge pit can have a free fatty acid content of 3-5% while samples collected at the pond contain at least 30% FFA (Ainie *et al.*, 1995).

The unsaponifiable fraction of palm oil is rich in carotenoids and tocopherols. The oil also contains some sterols, waxes and hydrocarbon. The palm fruit pericarp contains a relatively high proportion of carotenoids, an orange pigment precursor of vitamin A. The carotenoid content varies with the degree of ripeness and the genotype of the fruit.

2.3 Carotenoids and Tocopherols

Carotenoids are divided into two main classes: carotenes and xanthopylls. β-carotene, α-carotene and lycopene are prominent members of the carotene group which includes carotenoids composed only of carbon and hydrogen atoms. Xanthopylls, however carry at least one oxygen atom (Sundram *et al.*, 2003, Stahl and Sies, 2005). Table 2.5 shows the average composition of carotenes in crude palm oil (Ng and Tan, 1988, Ooi, 1999). Its concentration can range from 400 to 3500 ppm, depending on the species of oil palm (Ooi, 1999).

Table 2.5 Composition of different carotene fractions as a percentage of total carotenes derived from crude palm oil (Ng and Tan, 1988, Ooi, 1999)

Carotene	**Composition (%)**
Phytoene	1.27
Cis-β-carotene	0.68
Phytofluene	0.06
β-Carotene	56.02
α-Carotene	35.16
Cis-α-Carotene	2.49
ζ-Carotene	0.69
γ-Carotene	0.33
δ-Carotene	0.83
Neurosporene	0.29
β-Zeacarotene	0.74
α- Zeacarotene	0.23
Lycopene	1.30

According to Choo (1994), the different species and hybrids of palm oil tree are comparable with regards to the major components, α- and β-carotenes which constitute about 80-90% of the total carotenoid content with lesser amount of γ-carotene, lycopene and xanthophylls. Yap *et al.* (1991) found 13 different types of carotenoids with the major isomers, α-carotene and β-carotene, accounting for 54% to 60% and 24% to 60% of the total carotenoids, respectively. The α- and β-carotenes were predominant and were present in a ratio of 1:2.

Crude palm oil contains 600 to 1000 ppm of vitamin E and is a mixture of tocopherols (18-22%) and tocotrienols (78-82%) (Sambanthamurthi *et al.*, 2000). Tocopherols and tocotrienols are fat-soluble vitamin E isomers and the major antioxidants of vegetable oils. The major vitamin E isomers in palm oil are α-tocotrienol (29%), α-tocopherol (28%) and δ-tocotrienol (14%) (Sundram *et al.*, 2003). Refined palm oil contains about 350-450 ppm vitamin E, present as the RRR-α-tocopherol (30%) and tocotrienol (70%) isomers. The concentration of vitamin E found in palm oil is shown in Table 2.6 (Ooi, 1999).