PHASE EQUILIBRIUM STUDY OF β-CAROTENE, TOCOPHEROLS AND TRIACYLGLYCEROLS IN SUPERCRITICAL FLUID EXTRACTION PROCESS FROM CRUDE PALM OIL USING CARBON DIOXIDE AS A SOLVENT

by

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LIST OF SYMBOLS

Symbol	Description	Unit
a_{mn}	Interaction parameter between groups m and n	K
$a_{CH3/CO2}$	Interaction parameter between groups CH_3 and CO_2	K
$a_{CO2/CH3}$	Interaction parameter between groups CO_2 and CH_3	K
B_v	Second virial coefficient	cm^3/mole
d_c	Critical hard sphere diameter	nm or Å
d_i	Solubility parameter of component i	
dQ_i/dx_i	Gradient of the molar Gibbs function based on component i	
d^2Q_i/dx_i^2	Second derivative of the molar Gibbs function based on component i	
G_m^{mixing}	Molar Gibbs function of mixing	bar.cm^3/mole
i	Solvent (carbon dioxide)	
j	Solute (heavy component (β-carotene))	
k	Group of component i	
k_i, k_j	k-value (distribution factor) for components i and j	
m^E	weight of the extracted component(s) based on the vapor sample bomb sampling	g
m^S	weight of the extracted component(s) based on the liquid sample bomb sampling	g
m, n	Group in the mixture	
M_w^*	Equivalent molecular weight	g/mole
$(ny)^G$	Number of moles of carbon dioxide in the vapor sample bomb	
$(nx)^G$	Number of moles of heavy component (such as β-carotene) in the vapor sample bomb	

$(ny)^L$	Number of moles of carbon dioxide in the liquid sample bomb	
$(nx)^L$	Number of moles of heavy component (such as β-carotene) in the liquid sample bomb	
$(nx_1)^L$	Number of moles of heavy component (such as β-carotene) in the liquid phase on a carbon dioxide free basis	
$(nx_1)^G$	Number of moles of heavy component (such as β-carotene) in the vapor phase on a carbon dioxide free basis	
$(nx_2)^L$	Number of moles of crude palm oil in the liquid phase on a carbon dioxide free basis	
$(nx_2)^G$	Number of moles of crude palm oil in the vapor phase on a carbon dioxide free basis	
P	System pressure	MPa or bar
P_1	Expansion vessel pressure before expansion	bar
P_2	Expansion vessel pressure after expansion	bar
P_c	Critical pressure	bar
P_r	Reduced pressure	
q_i	Area function for the molecule	
Q_i	$- G_m^{mixing}/RT$	
Q_k	Area function for group k	
r	Number of segments	
T	System temperature	°C
T_c	Critical temperature	°C
T_r	Reduced temperature	
U_{mi}	Molar internal energy of the compressed fluid component i	bar. cm^3/mole
U^0_{mi}	Molar internal energy of the compressed fluid component i at a very low pressure	bar. cm^3/mole

V	System volume	liter
V_{mi}	Molar volume of the pure liquid of i	$cm^3/mole$
w^E	β-carotene weight fraction in the extracted (vapor) phase	
w^S	β-carotene weight fraction in the liquid (solute) phase	
X	Mole fraction of carbon dioxide in the liquid phase	
X_1	Mole fractions of heavy component (such as β-carotene) in the liquid phase on a carbon dioxide free basis	
x_i^E	Component i mole fraction in the vapor phase	
x_i^S	Component i mole fraction in the liquid phase	
$(x_{CO2}^E)^{Theory}$	CO_2 mole fraction in the extracted phase based on the mutual solubility calculations associated with the Gibbs function method	
$(x_{CO2}^S)^{Theory}$	CO_2 mole fraction in the solute phase based on the mutual solubility calculations associated with the Gibbs function method	
Y	Mole fraction of carbon dioxide in the vapor phase	
Y (rate test)	Yield of β-carotene extraction based on DoE model	
Y_1	Mole fractions of heavy component (such as β-carotene) in the liquid phase on a carbon dioxide free basis	
$(yield)^{EXP.}$	Experimental yield of β-carotene	
$(yield)^{DoE.}$	Theoretical yield of β-carotene obtained from DoE	
Z	Interactions with nearest neighbor molecules ~ 10	

Greek Symbols

γ_i	Component i activity coefficient	
$\Gamma_k^{(i)}$	Residual activity coefficient for group k	
$\theta_i, \theta_m, \theta_n$	Area fraction of group i or m or n	
φ_i	Segment fraction	
ξ_i	Non-ideality factor of the vapor phase	
ΔP^E	Pressure increment in the large expansion vessel after and before the expansion process based on the vapor sample bomb sampling	bar
ΔP^S	Pressure increment in the small expansion vessel after and before the expansion process based on the liquid sample bomb sampling	bar
ρ_c	Critical density	g/cm^3
ρ_r	Reduced density	

ABBREVIATIONS

Symbol	Description
ANOVA	Analysis of Variance
CCD	Central Composite Design
CPO	Crude Palm Oil
DAG	Di-acylglycerol
DGDG	Diagalactosyldiacylglycerol
DoE	Design of Experiment (an statistical model)
EOS	Equation of State
FFA	Free Fatty Acid
FFB	Fresh Fruit Bunches
FID	Flame Ionization Detector
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
LLV	Liquid-Liquid Vapor
MAG	Mono-acylglycerol
MGDG	Monogalactosyldiacylglycerol
MMM	Mohsen-Nia-Moddaress-Mansoori
OO	Dioleoylglycerol
PFAD	Palm Fatty Acid Distillate
PO	Palmitoyloleoylglycerol
POO	1, 2-dioleoyl-3-palmitoyl-rac-glycerol
POP	1, 3-dipalmitoyl-2-oleoyl-glycerol
PP	Dipalmitoylglycerol
PR	Peng-Robinson
PSRK	Predictive Soave-Redlich-Kwong

RBD   Refined Bleached Deodorized

RD   Relative Deviation

RK   Redlich-Kwong

RSM   Response Surface Methodology

SCF   Supercritical Fluid

SCE   Supercritical Extraction

SLV   Solid-Liquid Vapor

SRK   Soave-Redlich-Kwong

SSV   Solid-Solid Vapor

SOO   1, 2-dioleoyl-3-stearoyl-rac-glycerol

TAG   Tri-acylglycerol

VLE   Vapor-Liquid Equilibria

WAA   Weeks After Anthesis

KAJIAN KESEIMBANGAN FASA β-KAROTENA, TOKOFEROL DAN TRIASILGLISEROL DALAM PROSES PENYARIAN BENDALIR GENTING LAMPAU DARIPADA MINYAK SAWIT MENTAH DENGAN MENGGUNAKAN PELARUT KARBON DIOKSIDA

ABSTRAK

Keseimbangan fasa bahan-bahan daripada sistem berbilang komponen seperti minyak sawit mentah dalam pelarut karbon dioksida separa lampau dan genting lampau telah menjadi semakin penting khususnya dalam proses-proses unit operasi. Dua radas telah dibina dan ditentukurkan dalam penyelidikan ini untuk mengkaji penyarian bendalir genting lampau β-karotena daripada minyak sawit mentah serta keseimbangan fasa penyarian genting lampau β-karotena, triasilgliserol (TAG) dan tokoferol daripada minyak sawit mentah dengan menggunakan karbon dioksida.

Bagi kajian keseimbangan fasa, kebolehlarutan salingan β-karotena, TAG dan tokoferol dari minyak sawit mentah dengan menggunakan karbon dioksida sebagai pelarut adalah dalam julat suhu 80 ℃ ke 120 ℃. Setiap sampel daripada unit fasa keseimbangan mengandungi dua bahagian. Bahagian cecair telah dianalisa dengan kromatografi dan bahagian wap pula telah dialirkan ke sebuah bekas kembangan untuk diukur kenaikan tekanan semasa proses pengembangan. Spektrofotometer, kromatografi gas (GC) dan kromatografi cecair berprestasi tinggi (HPLC) telah digunakan untuk menganalisa sampel-sampel cecair bagi menentukan komposisi β-karotena, empat komponen tokoferol (α-, β-, γ- dan δ-tokoferol) serta tiga komponen triasilgliserol [1,3-dwipalmitoyl-2-oleoyl-gliserol (POP), 1,2-dwioleoyl-3-palmitoyl-rac-gliserol (POO) dan 1, 2-dwioleoyl-3-stearoyl-rac-gliserol (SOO)] masing-masing. Dua fasa data keseimbangan dua fasa telah dikira dengan menggunakan komposisi fasa cecair dan kenaikan tekanan semasa proses pengembangan. Keputusan menunjukkan bahawa kebolehlarutan maksimum bagi ketiga-tiga

kumpulan komponen adalah sekitar 2.27 % pada suhu 120 ℃ dan pada tekanan 5.44 MPa. Walaupun komposisi komponen-komponen minyak sawit mentah berubah dengan sedikit, namun dari aspek penskalaan menaik, data yang dilaporkan di atas boleh digunakan kerana perkara ini dipercayai berlaku disebabkan oleh pengaruh kenaikan tekanan terhadap data yang dikira. Dengan ertikata lain, kelarutan triagliseral yang tinggi di dalam karbon dioksida mungkin telah bertindak sebagai sepelarut dalam bahan-bahan lain yang mempunyai kelarutan yang tinggi.

Keputusan yang diperoleh daripada ujikaji telah juga dibandingkan dengan data teori yang diperoleh daripada pengiraan kebolehlarutan salingan yang dikaitkan dengan fungsi Gibbs. Pengiraan kebolehlarutan salingan yang dikaitkan dengan fungsi Gibbs telah digunakan untuk setiap fasa melalui penggunaan ungkapan pekali aktiviti. Pendekatan model ini didapati adalah menggalakkan bagi meramal fasa wap sementara beberapa perbezaan antara data ujikaji dan teori untuk fasa cecair telah diperhatikan. Sebahagiannya mungkin disebabkan oleh parameter-parameter ketakpastian dan interaksi yang dikira daripada set data keseimbangan lain yang diperoleh dari pustaka penyelidikan yang lalu. Kajian ini juga menunjukkan bahawa interaksi parameter juga bersandar terhadap tekanan.

Bagi kajian ujian kadar, perisian versi 6.0.6 Rekabentuk Pakar telah digunakan untuk mengoptimakan keadaan operasi. Dua puluh eksperimen telah dijalankan pada julat tekanan 7.5 MPa ke 17.5 MPa, julat suhu 80 ℃ ke 120 ℃ dan julat masa sarian masing-masing pada 1 jam ke 5 jam. Minyak sawit mentah sebagai sampel cecair telah disari dengan radas ujian kadar sarian genting lampau ujian kadar pada pelbagai keadaan dan dianalisa dengan spektrofotometer untuk mengkaji hasil penyarian β-

karotena. Kaedah Sambutan Permukaan dan Rekabentuk Komposit Berpusat telah digandingkan untuk mengkorelasi dan mengoptimumkan keadaan-keadaan operasi. Walau bagaimanapun, data ujikaji menunjukkan bahawa hasil maksimum penyarian β-karotena (sekitar 0.017 %) diperoleh pada keadaan 7.5 MPa, 120 °C dan pada masa anggaran 1 jam. Walau bagaimanapun analisis statistik menunjukkan bahawa keadaan optimum untuk ujikaji ini ialah pada 14.0 MPa, 102 °C dan pada masa sarian 3.14 jam (sebagai titik optimum) bagi memperoleh hasil yang bererti bagi β-karotena daripada proses penyarian. Perselisihan ini dilihat sebagai wajar kerana keluaran daripada suatu model statistik akan berbeza sedikit dengan keputusan ujikaji. Secara kesimpulannya, walaupun masa sarian adalah panjang iaitu 5 jam, ia masih sesuai. Namun begitu, masa sarian yang pendek mungkin digalakkan dimana tekanan dan suhu akan memberi kesan pada hasil penyarian β-karotena.

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ABSTRACT

Phase equilibrium of the substances from a multi-component system such as crude palm oil in sub and supercritical carbon dioxide solvent has become increasingly important particularly in unit operation processes. In this study, two rigs have been fabricated and calibrated to study the supercritical fluid extraction of β-carotene from crude palm oil and also to evaluate the phase equilibrium of the supercritical extraction of β-carotene, triacylglycerols (TAGs) and tocopherols from crude palm oil using carbon dioxide as a solvent.

For the phase equilibrium study, the mutual solubility of β-carotene, TAGs and tocopherols from crude palm oil using carbon dioxide as a solvent were in the temperature range of 80 ℃ to 120 ℃. Each sample from the phase equilibrium unit contained two parts. The liquid part was analyzed by chromatography and the vapor part was conducted to an expansion vessel to measure pressure increment during expansion process. In order to analyze the liquid samples, a spectrophotometer, a gas chromatography (GC) and a high performance liquid chromatography (HPLC) were employed to determine the compositions of β-carotene, four tocopherols components (α-, β-, γ- and δ-tocopherol) and three triacylglycerols components [1, 3-dipalmitoyl-2-oleoyl-glycerol (POP), 1, 2-dioleoyl-3-palmitoyl-rac-glycerol (POO) and 1, 2-dioleoyl-3-stearoyl-rac-glycerol (SOO)] in crude palm oil, respectively. Two phase equilibrium data was calculated using liquid phase composition and pressure increment during expansion process. Results showed that the maximum solubility of three groups of components around 2.27 % at temperature of 120 ℃ and at pressure

5.44 MPa. Although the composition of the components from crude palm oil has slightly changed but, from scale up point of view the reported data as mentioned above would be applicable. This could be due to the pressure increment that considerable influenced on the calculated data. On the other hand, high solubility of triacylglycerols in carbon dioxide may be assisted as a co-solvent in high solubility of other substances.

The results obtained from the experiment were also compared with the theoretical data obtained from the mutual solubility calculations associated with the Gibbs function which were applied as the theoretical model in this research. The mutual solubility calculations associated with the Gibbs function was applied in each phase by applying the activity coefficient expressions. This model approach has been found to be encouraging for the prediction of vapor phase while some differences between the experimental and theoretical data for liquid phase were observed. The reasons of this were partly due to the uncertainty and interaction parameters calculated from another set of equilibrium data obtained from the literature. It also showed that the interaction parameters were dependent on pressure.

For the rate test study, a Design of Expert (DOE) software version 6.0.6 was used to optimize the operating conditions. Twenty runs were carried out at various range of pressure 7.5 MPa to 17.5 MPa with temperature between 80 ℃ to 120 ℃ and extraction time 1 hour to 5 hour, respectively. Crude palm oil as a liquid sample was extracted from the rate test supercritical extraction apparatus at various conditions and was analyzed via spectrophotometer in order to study the yield of β-carotene extraction.

Response Surface Methodology (RSM) coupled with central composite design (CCD) was applied to correlate and optimize the operating conditions. The experimental data showed that the maximum yield of β-carotene extraction (around 0.017%) was obtained at a condition of 7.5 MPa, 120 °C and approximately one hour. However, for analysis, the optimum conditions for this experiment were at 14.0 MPa, 102 °C and an extraction time of 3.14 hours (as optimum point) in order to obtain a significant yield of the β-carotene from the extraction process. This discrepancy seemed to be reasonable as the output from a statistical model would slightly varied with the experimental results. As a conclusion, though the extraction time was longer (5 h), the extraction process was suitable. Nevertheless, a shorter extraction time could be encourageable with pressure and temperature significantly affected the yield of β-carotene extraction.

CHAPTER 1

INTRODUCTION

1.0 Introduction

In the present work, much effort has been made on studying the supercritical fluid extraction based on phase-equilibria under conditions such that one component of the gas is above or very close to its critical temperature and pressure or below the normal boiling point. There are numerous applications of the supercritical fluid extraction technique in various industries. In recent times, this technique has been found to be useful in the petroleum reservoir processes (Pires *et al.*, 2001; Haruki *et al.*, 2003; Johnson and Cornwell, 2007). At normal pressures such systems follow the usual supercritical solubility laws and their behavior is of interest to chemical and bioprocess engineers in gas absorption and striping operations. The solubility of liquids in gas supercritical fluid extraction process is based on the gas extraction.

Experiments have shown that if a liquid is in contact with gas and the pressure is raised sufficiently, the gas will eventually achieve appreciable solvent properties. This effect is of potential interest in the development of supercritical fluid extraction separation processes. In this research, crude palm oil has appreciable solubility in carbon dioxide at an elevated temperature and at pressures which are based on the pressure versus mole fraction plots and this is a major constituent in much natural oil processing (Singh, 2004).

In practice, liquid extraction using solvents such as hexane is used to extract selected components from oil-bearing seeds (Brunner, 1994) while vacuum distillation is used

for separation processes in the food industry. Liquid extraction has many disadvantages, because all traces of harmful impurities must be removed from the solvent. At the same time, the solvent has to be separated from the product. For these reasons and other possible benefits, tests are made in order to examine the feasibility of using the supercritical fluid extraction process for separation and extraction. A supercritical fluid extraction has the following advantages over vacuum distillation and liquid extraction process (Williams *et al.*, 2002):

- low energy consumption.

- easy separation of the solvent from the product.

- it is possible to use a comparatively innocuous solvent such as carbon dioxide.

1.1 What is Supercritical Fluid Extraction?

A supercritical fluid is a substance which is above its critical point. The temperature at the critical point is called the critical temperature and the pressure at that point is called the critical pressure. A supercritical fluid is a unique fluid because it also has the gaseous property of being able to dissolve materials into their desired components. It also exhibits desirable transport properties that enhance its adaptability as a solvent for any liquid extraction processes. A supercritical fluid has high diffusion and low viscosity and low surface tension than a liquid solvent which leads to more favorable mass transfer (Williams *et al.*, 2002).

The supercritical fluid of a pure component and the thermodynamic state of various separation processes are illustrated in Figure 1.1. In this research carbon dioxide was chosen as a solvent in supercritical extraction. The benefits of carbon dioxide as an

ideal solvent were: inert and non-corrosive in dry environment, non-flammable and non-explosive, abundant and inexpensive, non-toxic and accepted as a harmless ingredient in food and beverages and has desirable physical properties such as low critical temperature (T_c=31 ºC) and low critical pressure (P_c=73.8 bar), low viscosity, low surface tension and high diffusivity (Mchugh and Krukonis, 1994).
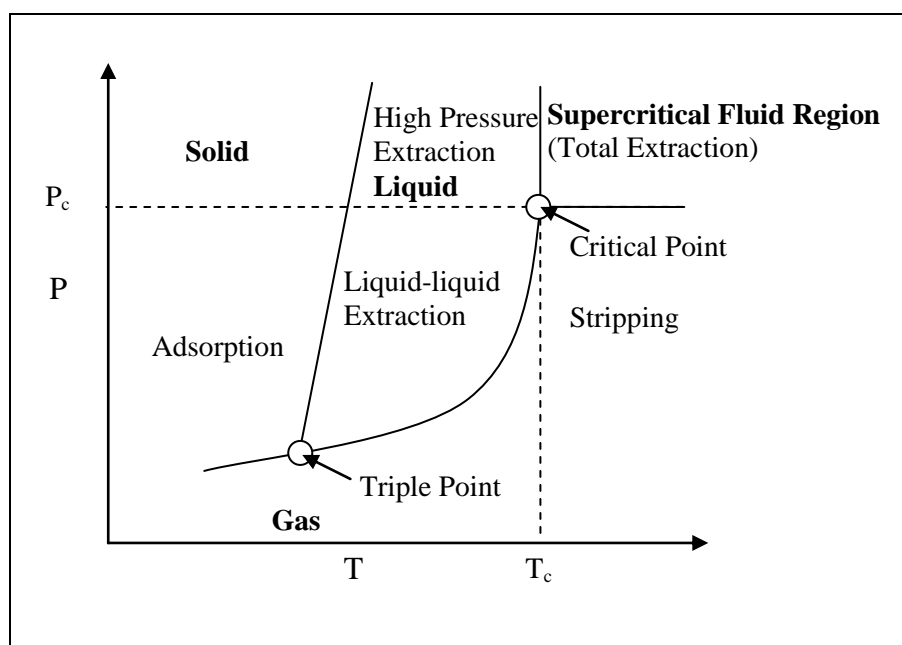
Figure 1.1. Phase diagram of a single substance in supercritical fluid (Mchugh and Krukonis, 1994).

Therefore, the close relationship between fluid density and its dissolving power as well as its favorable mass transfer properties makes supercritical fluids useful processing mediums for the extraction and separation of components. Supercritical fluids exhibit a pressure dissolving power whereby the higher the pressure, the higher the dissolving power it generated. These properties make supercritical fluids as useful solvent or reaction medium for the purification, extraction, fractionation and the re-crystallization of a wide range of materials.

3

1.2 Applications of Supercritical Fluids

Supercritical fluid extraction with carbon dioxide as a solvent offers potential advantages for processing under milder conditions; therefore, the separation and fractionation of crude palm oil related compounds has become a subject of active research due to their potential applications in the food and biochemical industries (Coniglio *et al.*, 1995). Due to their thermal sensitivity, conventional separation techniques such as vacuum distillation, which require high temperatures (more than 200 ℃ for oil systems), are unsuitable. It is possible to use the supercritical extraction technology to separate the heat sensitive compounds such as vitamins in order to avoid toxic solvent residues (Christina and Mollerup, 1999). Further extraction of color, composition, odor and texture is controllable whereby supercritical carbon dioxide retains the aroma of the food sample (Rizvi *et al.*, 1994).

Another area for the use of the supercritical extraction is in the pharmaceutical industry. Pharmaceutical applications of the supercritical fluid extraction are substantially challenging due to the standards for recovery and reproducibility that are more rigorous than those for environment applications. The analytes are usually at trace levels and are highly polar and matrices are exceedingly complex and often possess co-extractives (Mchugh *et al.*, 1994). The extraction of vitamins such as vitamin E from soybean oil and a purification method for its extraction method has been well studied (Johannsen and Brunner, 1997). In addition, the solubilities and the recrystallization of various drugs have been demonstrated in supercritical extractions (Mchugh *et al.*, 1994). One of the most important applications is the extraction of fish oil in which the ω-3 and ω-6 fatty acids have been found to have potential health benefits in various inflammatory diseases; lowers blood pressure and could also

prevent heart attacks (Weaver, 1988). Other applications of the supercritical fluid extraction includes the recovery of organics from oil shale, the separation of biological fluids, bioseparation, petroleum recovery, crude de-asphalting and dewaxing, coal processing (reactive extraction and liquefaction), selective extraction of fragrances, oil and impurities from agriculture, pollution control and combustion (Mansoori *et al.*, 1998).

Currently, supercritical fluid extraction using carbon dioxide as a solvent is the famous technology among researchers for rapid contamination-free extraction in the food and pharmaceutical industries. Large scale supercritical carbon dioxide extraction has been in commercial operation since the late 1970s for the decaffeination of coffee and tea (Singh, 2004), the refining of cooking oil (Hwang *et al.*, 2000), and recovering flavors and pungencies from spices, hops and other plant materials (Moyler, 1993). There have been many supercritical fluid extraction applications in natural products as well as in the food industries such as spice extraction (Udaya and Manohar, 1994), the deodorization of oils and fats (Riha and Brunner, 2000), and the extraction of herbal medicines. Table 1.1 summarizes some of the known applications of the supercritical fluid extraction technique.

Table 1.1. Supercritical fluid extraction applications in natural products and food industries (Mukhopadhyay, 2000).

Supercritical fluid extraction applications	Natural products/ Food industries
Decaffeination	Coffee and tea
Spice extraction	Oil and oleoresin
Deodorization	Oils and fats
Extraction	Vegetable oils, flavors, fragrances, aromas, perfumes and herbal medicines
Hops extraction	Bitter
Stabilization	Fruit juices and lanolin from wool
Deoiling	Fast foods
Decholesterolization	Egg yolk and animal tissues
Antioxidants	Plant materials
Food colors	Botanicals
Denicotinization	Tobacco

Some of the newer applications which are at the developmental stage or are being explored includes the deoiling of crude lecithin and the preparation of ceramic membrane using supercritical carbon dioxide (Sarrade *et al.*, 2003).

The first commercial supercritical plant was built in the 1980s in the United States of America and it was used for the separation of ethanol from water in combination with other preliminary processes such as the prevaporation distillation (Mukhopadhyay, 2000). Several large-scale plants are in operation throughout the United States of America and Europe. Supercritical plants in Germany, France and the United Kingdom were commissioned for tea, hops, spices and flavor extraction, whereas in the USA the most preferred is the coffee decaffeination plant that was built in Houston, Texas, in mid-1985 (Cookson *et al.*, 1987). In mid-1986, Pfizer (USA) began the operations of hops extraction in Sydney which was reported to be the largest carbon dioxide extraction plant in the world (McHugh and Krukonis, 1994).

The advantages of the supercritical techniques in bio-technology and other related areas are it is less pollution in the working and general environment and less solvent disposal costs (Williams *et al.*, 2002). The supercritical fluid extraction uses a supercritical fluid to remove soluble substances from insoluble matrices. Supercritical fluids have attractive properties for extraction because they penetrate a sample faster than liquid solvents; they also have diffusion coefficients which are midway between gases and liquids (Williams *et al.*, 2002). On top of that, usually pressure enhancement has a good effect on supercritical extraction while temperature enhancement does not have a significant effect on supercritical extraction. In addition, co-solvent addition effect and its type depend on polarity of solute and it will assist solvent ability however it does not have an effect on β-catotene extraction using CO_2 solvent (Markom *et al.* 2001). Other applications of supercritical fluids are listed in Table 1.2.

Table 1.2. The other applications of supercritical fluids

Application of supercritical fluid	Example	Reference
Homogenization of reaction mixtures	Production of pure medicine	(Williams, 2002)
Bio-application of chemical reaction	A supercritical medium is the staining of fingerprints on cheques and banknotes using ninhydrin	(Hewlett,1996)
As solvent instead of ozone-depleting	As solvent instead of CFC_{113} in fridge cycle	(Williams, 2002)
As a good inert reactant in interesterification process	In interesterification of edible palm oil by stearic acid in supercritical CO_2	(Liang, 1998)
As a good inert in oxidation process	Oxidation of cyclohexene using catalyzed by halogenated iron porphyrins in CO_2-SF	(Birnbaum, 1999)
As a good inert in hydrogenation	Fixed bed hydrogenation of organic compounds	(Arunajatesan, 2001)
In extraction-reaction process to extract oil from oilseeds and convert it to other valuable products	Lipase-catalyzed hydrolysis of canola oil in supercritical CO_2	(Rezaei, 2001)
Cellulose hydrolyze process	Cellulose is rapidly recovered from glycose, fructose and oligomers in supercritical water.	(Sasaki, 1998)
Thiodiglycol hydrolysis and oxidation	Thiodiglycol degradation to carbon dioxide, water, elemental sulfur and sulfates under sub-and supercritical water	(Lachance, 1999)
Food wastes oxidation	Rapid oxidation of food wastes with hydrogen peroxide in water-SF	(Jin, 2001)
Particles production	Production of pharmaceutical, polymers particles	(Reverchon, 2000)
Supercritical fluids as carriers	Impregnated wood with chemical agent is removed by supercritical fluid	(Acda *et al.*, 1995)
Drying of biological specimen	Drying of wet biological specimen at or above critical temperature of solvent for prevention of tension forces which have harmful effects	(Williams, 2002)

There were also reports of supercritical carbon dioxide applications in citrus oil (Temelli *et al.*, 1988), soybeans (Eldridge *et al.*, 1986), fish muscle (Hardardottir and Kinsella, 1988), meat (Wehling *et al.*, 1992) and egg yolk (Froning *et al.*, 1990).

1.3 Phase Equilibrium in Supercritical Extraction Process

Although the familiar heuristics on solubility found in chemistry textbooks can be employed to assess whether a solute will be soluble in a candidate supercritical fluid, these heuristics do not quantify the pressures and temperatures needed for a given solubility level. Experimentally determined solubilities are ultimately needed in order to make an informed decision of whether a potential supercritical fluid application merits further financial investment. Knowledge of the possible types of phase diagrams which are applicable for the mixtures which are of interest can help to minimize the amount of experimental work needed to determine the solubility levels over wide ranges of temperature and pressure.

Fortunately the wide variety of phase behavior that can occur when operating at high pressures can be cataloged with a small number of schematic phase diagrams (Scott, 1972; Scott and van Konynenburg, 1970; Hicks and Young, 1975). Interpreting as well as extrapolating high-pressure phase behavior data need not be a formidable task even if the mixture is compressed of components that differ in molecular size, shape, structure and attractive potential. Many of the features of the phase diagrams for multi-component mixtures are identical to those found with the phase diagrams of binary mixtures [consisting of a single supercritical fluid solvent and a single solute (Streett, 1983)]. An understanding of the phase behavior of this limiting case

provides a basis for generalizing the phase equilibrium principles that are operative during the supercritical fluid solvent extraction of mixtures.

The degree of freedom equation (Streett, 1983) gave the number of independent, intensive variables that must be set in order to fix all of the intensive properties of the equilibrium system. For example, unique values of the compositions of the equilibrium phases for a two-phase mixture can be obtained either by carrying out experiments or by calculation if the temperature and pressure are fixed. For a two-phase ternary mixture, another variable must be fixed, for example overall mixture composition, in addition to temperature and pressure. The phase rule not only provides the number of independent variables that must be fixed in order to define multi-phase, multi-component mixtures; it also specifies the topology of the phase diagrams used to represent that behavior.

When operating a supercritical fluid process, it is prudent to avoid regions of multiple phases in the pressure-temperature-composition (P-T-x) space such as three-phase liquid-liquid-vapor (LLV), solid-liquid-vapor (SLV) or solid-solid-vapor (SSV) equilibria. When these regions of multiple phases are projected onto a two-dimensional P-T diagram, their geometrical representations are simplified because pressure and temperature are field variables (Streett, 1983); they are the same in each of the equilibrium phases. Pressure, temperature and chemical potential are field variables whereas molar volume is not. Two surfaces which represent the equilibrium between two phases in the P-T-x space are projected as a single surface in the P-T space because pressure and temperature are the same in both phases. Three lines representing the three equilibrium phases in the P-T-x space are projected as a

single line in the P-T space because the pressure and temperature are the same in all three phases. Four points representing four equilibrium phases in P-T-x space project as single point in P-T space. Therefore, complex phase behavior can be more readily interpreted when a P-T diagram (i.e. a field variable diagram) is used. On the other hand, the limitation of a P-T diagram is that the composition and density of the equilibrium phases are not readily apparent. However, many features of the high-pressure phase behavior consistently appear in limited ranges of mixture compositions (McHugh and Krukonis, 1994).

By reviewing and cataloging many examples of the phase behavior of particular classes of mixtures, it is possible to make reasonable estimates of the mixture composition, depending on the operating temperature, pressure and vicinity to a pure component critical point. According to the literature, Scott and van Konynenburg showed that virtually all of the experimentally observed phase behavior can be represented with five schematic P-T diagrams (Scott, 1972; Scott and van Konynenburg, 1970). The phase diagram classification scheme is simplified by using a two-dimensional projection of critical mixture curves and three-phase lines from three-dimensional P-T-x diagrams. The five classes of possible phase diagrams are shown in Figure 1.2. Points C_1 and C_2 are the critical points of components 1 and 2 respectively. The dashed curve in each figure is the critical mixture curve for the binary mixture. Detail explanation can be obtained by Luks and Kohn (1984) and Rowlinson and Swinton (1982).
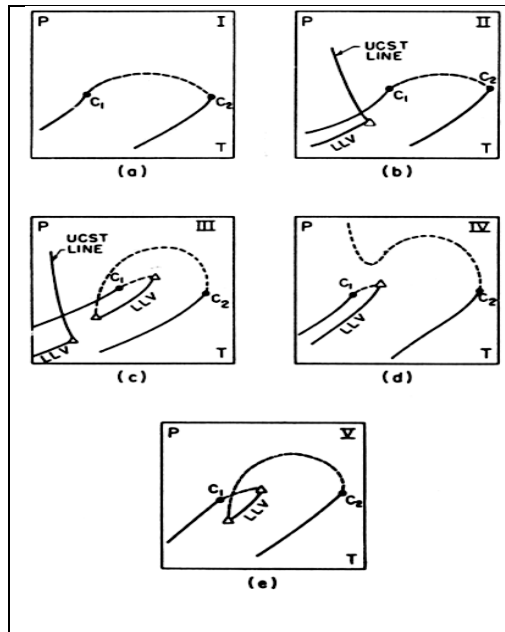
Figure 1.2. Five classes of possible binary phase diagrams as determined from the van der Waals equation of state (McHugh and Krukonis, 1994).

1.4 Best Region for Supercritical Extraction of Some Compounds

Figure 1.3 shows the best region for the supercritical extraction of hop from hop plants using carbon dioxide, supercritical de-caffeinate from its plant using carbon dioxide, supercritical hop extraction from hop plant and also the sub-critical extraction of natural oils from herbs using CO_2. The United Kingdom coal board pilot plant for extracting thermolysis products uses a mixture solvent and the range of conditions in propane de-asphalting column using propane. Researchers have studied lipid extraction by the supercritical carbon dioxide process. Some of these researches are illustrated in Table 1.3.
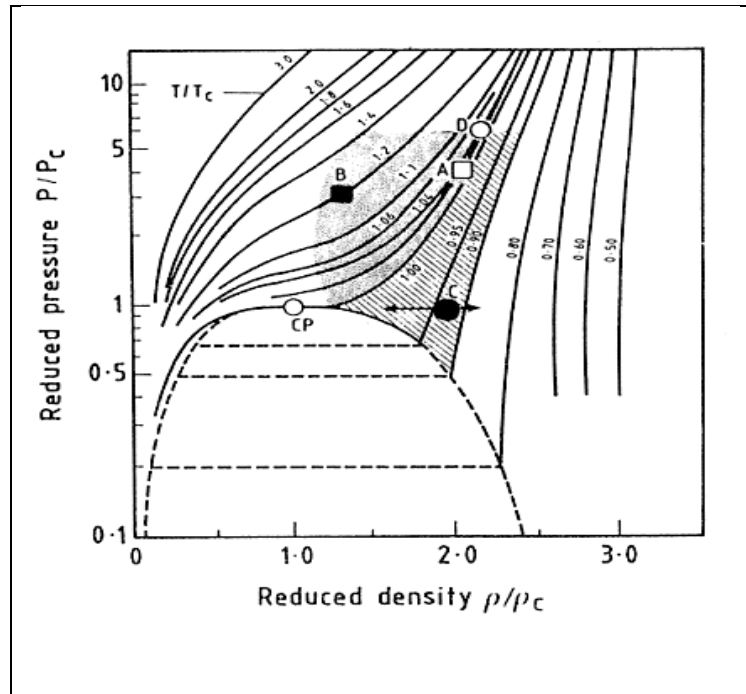
Symbol	Process	Solvent
□	Supercritical hop extraction plant	CO_2
■	Supercritical de-caffeinate plant	CO_2
○	U. K Coal Board pilot plant for extracting products by thermolysis	Mixture
●	Supercritical hop extraction plant and also sub critical extraction of natural oils from herbs	CO_2
↔	Range of conditions in propane de-asphalting column	C_3H_8

Figure 1.3. Range of 'near critical' extraction operations (King and Bott, 1993).

Table 1.3. Research on lipid extraction studies using supercritical CO_2 extraction.

Sample	Analyte(s)	Pressure (MPa)/T °C	Analytical Instrument	Reference
Brown seaweed	Fatty acid composition	24.1-37.9 / 40-50	GC-FID	Akgun *et al.*, 1999
Butter oil	Cholesterol	10-27.6 / 40-70	GC-FID	Akgun *et al.*, 1999
Cotton seed	Fatty acid composition	51.7-52 / 100	GC-FID Gravimetric	Smith *et al.*, 1998
Ground beef	Fatty acid composition	38 / 80	GC-FID	King *et al.*, 1987
Milk fat	Lipid profile Vitamins, solid fat content, PCBs	6.9-17.2 / 40-60	GC-FID HPLC Pulsed NMR DSC	Bharath *et al.*, 1989
Oat bran	Digalactosyl-aclglycerols	40 / 50-70	Supercritical fluid chromatography	Byun *et al.*, 2000
Pistachio	Texture and sensory	20.7-34.5 / 50-70	Instron universal testing machine	Cookson *et al.*, 1987
Pork	Cholesterol / Fat Content and Sensory Attributes	7.3-34 / 50-150	HPLC TLC Hunter Colorimeter	Inomata *et al.*, 1989
Rapeseed	Fatty acid composition Tocopherol Content	51.7 / 100	GC-FID HPLC	Byun *et al.*, 2000
Sunflower	Fatty acid composition	51.7-62 / 100	GC-FID	Smith *et al.*, 1998
Soybean	Fatty acid composition	51.7-62 / 100	GC-FID	Smith *et al.*, 1998

1.5 Problem Statements

Since palm oil has 15 times more retinol (pro-vitamin A) equivalent than carrot and 300 times more than tomato (Choo, 1994) and some of vitamins (such as vitamin E) from crude palm oil were lost during refining process (Sambanthamurthi *et al.*, 2000), therefore crude palm oil as a feed is chosen in this research.

Most of researches in the literature are based on supercritical fluid extraction of β-carotene, TAGs and tocopherols using CO_2. Furthermore, there are few researches on phase equilibrium of these substances in CO_2 which most of them operated in low temperature. According to the literature (Tan *et al.*, 2008), high temperatures have a good effect on TAGs solubility in CO_2 which they can also assist other substances solubility (such as β-carotene and tocopherols) as a co-solvent in CO_2, therefore phase equilibrium of these substances in sub and supercritical CO_2 in high operating temperature is studied.

Phase equilibrium data is a necessary item in order to calculate some unit operation parameters such as mass transfer coefficient. Furthermore, they show mutual solubility data which is a significant data in system scale up from industrial point of view. Since sometimes due to lacking of appropriate apparatus and the other limitations such as safety problems, mutual solubility data is not experimentally measurable. Therefore, an accurate predictable method for multi-component systems (such as crude palm oil) is required by applying activity coefficients while existing methods such as equations of state are applicable for binary systems and they need to develop for multi-component systems. For this purpose they need many parameters which should be extracted from literature and probably some of them are not available in the literature.

Supercritical fluid extraction of natural products is an interesting topic due to having several advantages (such as non-toxic and easy to remove solvent) (Williams *et al.*, 2002). For this purpose, a special apparatus is required and tested which can be applied for a lot of liquid and solid feeds.

1.6 Research Objectives

The general objectives of this research are to study the phase equilibrium and extraction of crude palm oil through CO_2 supercritical approach. The specific objectives of this project are as follows:

1. To fabricate and test the phase equilibrium unit for β-carotene, TAGs and tocopherols from crude palm oil using CO_2 as a solvent in order to obtain mutual solubility data and consider their reliability.

2. To fabricate and test the rate test unit for extraction of β-carotene (pro-vitamin A) from crude palm oil.

3. To obtain theoretical data of β-carotene, TAGs and tocopherols based on the mutual solubility calculations associated with the Gibbs function by applying activity coefficients and compare with the experimental data.

4. To study three operating factors effects including pressure, temperature and extraction time on yield of β-carotene extraction simultaneously. Furthermore, to consider two factors effects from three factors on yield of β-carotene extraction by fixing one of them at the same time.

5. To minimize number of runs and optimize the operating conditions for β-carotene extraction in the rate test study using RSM.

1.7 Scope of This Study

In the first stage, a fabricated apparatus for the phase equilibrium supercritical fluid extraction study and the rate test supercritical fluid extraction unit were applied for the extraction studies using crude palm oil as a feed and carbon dioxide as a solvent. The two apparatus used in this work were explained in Chapter 3. The main focus of this study is a phase equilibrium supercritical fluid extraction using carbon dioxide as a clean, safe and easy to remove solvent to study the mutual solubilities of vitamins (such as vitamin A (β-carotene) and vitamin E (tocopherols)) and some harmful substances such as fatty acids (TAGs) from the CPO in CO_2.

The results obtained from the phase equilibrium supercritical fluid extraction unit were two-phase equilibrium data and they were compared with the results obtained from the mutual solubility calculations associated with the Gibbs function which were calculated by applying the activity coefficients. According to this theoretical method, interaction parameters which were independent of temperature were obtained from the mutual solubility calculations associated with the Gibbs function, specific thermodynamic assumptions and two-phase equilibrium data obtained from the literature. The mutual solubility calculations associated with the Gibbs function is explained in detail in Chapters 3 and 4.

The results obtained from the rate test supercritical fluid extraction unit were used for the yield of β-carotene extraction and the β-carotene weight fractions in both phase studies where the yield of β-carotene extraction in supercritical fluid extraction was studied in detail. Three parameters involving pressure, temperature and time of extraction were studied in this process. The effects of these three process variables

on the yield of β-carotene extraction were also investigated using the response surface methodology (RSM).

1.8 Organization of the Thesis

This thesis is divided into five chapters:

Chapter 1 is brief introduction about the supercritical fluids, the supercritical fluid extraction technique, the properties of supercritical carbon dioxide, applications of supercritical fluids, the phase equilibrium concept, best region for supercritical extraction of some compounds, problem statements, research objectives and scope of this study.

Chapter 2 provided the development of the palm oil industry in Malaysia, the processes in the palm oil mill, crude palm oil substances, solvent properties (especially carbon dioxide) and co-solvents, the solubility concept, the technical aspects of supercritical fluid extraction, crude palm oil fractionation (in particular β-carotene substance) and the phase equilibrium study by supercritical fluid extraction and the yield of carotene extraction. The theoretical prediction of the supercritical process using specific thermodynamic equations such as equations of state, UNIFAC and mutual solubility calculations associated with the Gibbs function are shown. The statistical method is also discussed in detail in this chapter.

Chapter 3 presents more detailed information of the materials and chemicals used in the present study. The overall experimental flowchart is also presented and discussed. Details of the experimental set up are then elaborated in this chapter. This

is followed by the details of the experimental procedure which includes the phase equilibrium study in the supercritical fluid extraction of crude palm oil. In this chapter, the mutual solubility calculations associated with the Gibbs function equations are generated. Furthermore, the rate test study is considered in this section. The details of two supercritical apparatus involving size and type for all sections which followed mathematical design are shown. Finally, applied analytical methods set up and calculations procedures are studied in this chapter.

Chapter 4 is the main part of this thesis. This chapter is divided into two sections. In the first section, the method of experimental data extraction from the phase equilibrium apparatus is explained in detail and followed by the second section that two phase equilibrium data for β-carotene, TAGs and tocopherols are calculated and illustrated. Then, the thermodynamic method which is related to the mutual solubility calculations associated with the Gibbs function is applied in order to predict the mutual solubility of β-carotene substance from crude palm oil in CO_2. In the following section, the yield of the β-carotene extraction using supercritical carbon dioxide solvent in the rate test apparatus is considered. Detailed information on the role of the operating conditions such as pressure, temperature and time of extraction (and their combination) in β-carotene supercritical extraction using carbon dioxide solvent is studied. Then, details of the process optimization related to the statistical model are elucidated.

Chapter 5 concluded the findings from the present study. Furthermore, some useful comments as recommendations in the related field for future studies are suggested.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Industry in Malaysia

Oil palm (*Elaeis guineensis*) is one of the most versatile crops in tropical countries. The palm oil industry is one of the most important contributors to the Malaysia economy. Malaysia is one of the largest producers and exporters of palm oil in the world (Malaysian Palm Oil Board, 2004). The total palm planted area increased by 4.5 percent or 174,000 hectares to 4.0 million hectares in 2005 as compared with that in 2004. The production of crude palm oil continues to increase for seven consecutive years reaching 15.0 million tonnes in 2005 from 14.0 million tonnes in the previous year (Malaysian Palm Oil, 2005).

2.1.1 Palm Oil Production Processes

Figure 2.1 presents a typical process flow diagram for the extraction of crude palm oil. After harvesting, the fresh fruit bunches (FFB) are transported to the mills for processing. Each FFB consists of hundreds of fruits, each of which contains a nut surrounded by a bright orange pericarp which contains the palm oil. These FFBs are sterilized with steam at a pressure of 3 bar and a temperature of 140 °C for 75 to 90 minutes. The objectives of this process are to prevent further formation of free fatty acids due to enzyme action, to facilitate stripping and to prepare the fruit mesocarp for subsequent processing (Zinatizadeh, 2006).
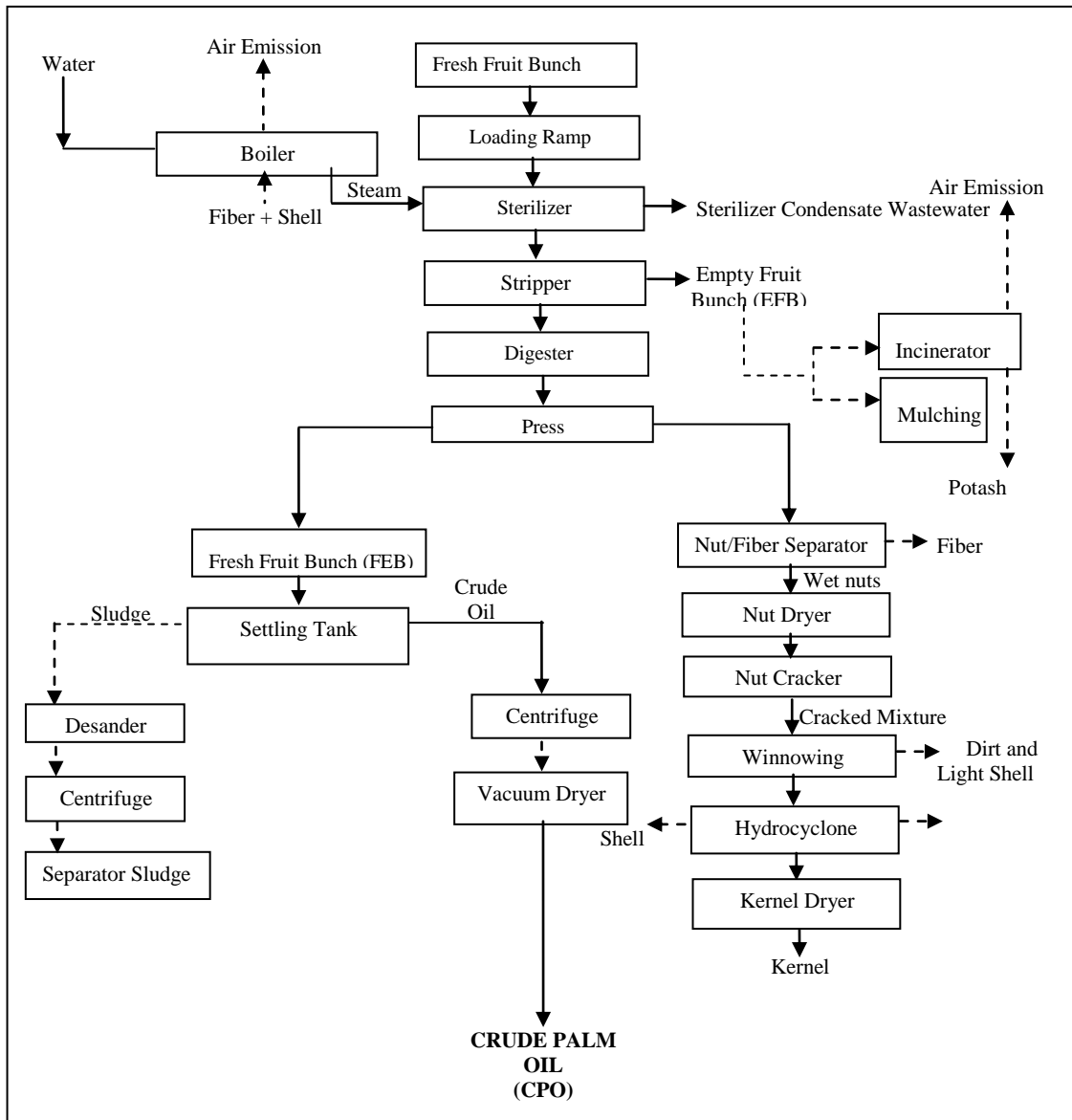
Figure 2.1. Conventional crude palm oil extraction (Zinatizadeh, 2006).

After sterilization, the FFBs are fed to a rotary drum-stripper where the fruits are stripped from the bunches. The detached fruits are passed through the bar screen of the stripper and are collected below by a bucket conveyor and discharged into a digester. In the digester, the fruits are mashed by the rotating arms. In this stage, the mashing of the fruits under heating breaks the oil bearing cells of the mesocarp. Twin screw presses are generally used under high pressure to press out the oil from the digested mash of the fruit. Hot water is added to enhance the flow of the oils. The

crude oil slurry is then fed to a clarification system for oil separation and purification. The fiber and nut (press cake) are conveyed to a depericarper for separation (Zinatizadeh, 2006).

The crude palm oil (CPO) from the screw presses consists of a mixture of palm oil (35 to 45 percent), water (45 to 55 percent) and fibrous materials in varying proportions. The mixture is then pumped into a horizontal or vertical clarification tank for oil separation. In this unit, the clarified oil is continuously skimmed off from the top of the clarification tank. The mixture is then passed through a high speed centrifuge and a vacuum dryer before it is sent to a storage tank. The pressed cakes discharged from the screw press consist of moisture, oily fiber and nuts and they are conveyed to a depericarper for nuts and fibers separation. The fiber is usually sent to the boiler house and is used as boiler fuel. Meanwhile, the nuts are sent to a rotating drum where any remaining fiber is removed before they are sent to a nut cracker. Hydrocyclone is commonly used to separate the kernels and the shells. The discharge from this process constitutes the last source of wastewater steam (Chow and Ho, 2000). A general mass balance of various products generated from a palm oil mill is shown in Figure 2.2.
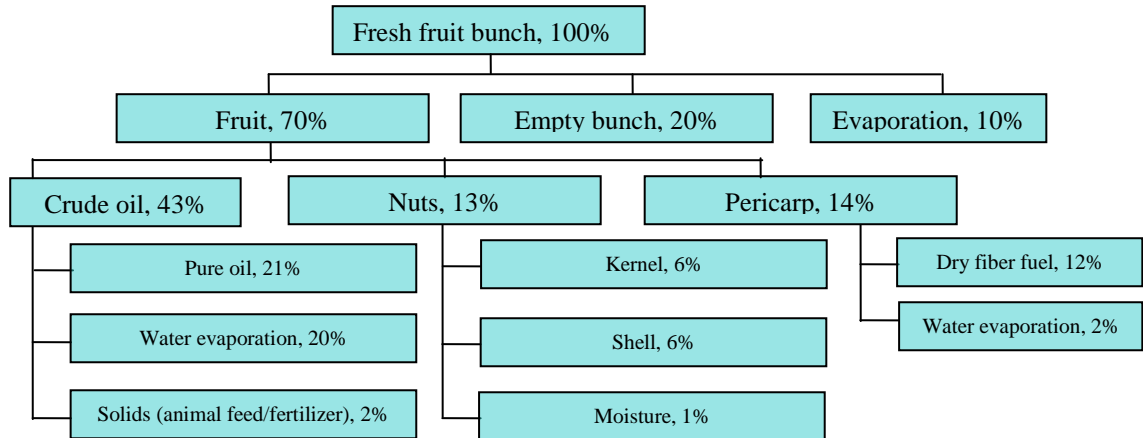
Figure 2.2. Typical fruit and production composition chart of a palm oil (Muttamara *et al.*, 1987).

2.1.2.0 Chemistry of Palm Oil

Like all oils, tri-acylglycerolses (TAGs) are the major constituents of palm oil. Over 95 percent of palm oil mixture TAGs, i.e. glycerol molecules, are estrified with three fatty acids. During the oil extraction from the mesocarp, the hydrophobic TAGs attract other fat- or oil-soluble cellular components. These are the minor components of palm oil: phosphatides sterols, pigments, tocopherols and trace metals. Other components in palm oil are the metabolites from the bio-synthesis of TAGs and the products from lipolytic activity. These also include the mono-acylglycerolses (MAGs), di-acylglycerolses (DAGs) and free fatty acids (FFAs) (Sambanthamurthi *et al.*, 2000).

The fatty acids are any of a class of aliphatic acids, such as palmitic (16:0), stearic (18:0) and oleic (18:1), in animal and vegetable fats and oils. The major fatty acids in palm oil are myristic (14:0), palmitic, stearic, oleic and linoleic (18:2). The typical fatty acid composition of palm oil from Malaysia is presented in Table 2.1 (Tan *et al.*, 2000). The ratio of palmitic/stearic acid in palm oil can vary according to a number of factors. Clegg (1973) reported that the palmitic and stearic acid content in

E. guineensis oil varied because of geographical influences. In addition, palm oil has saturated and unsaturated fatty acids in approximately in equal amounts (Clegg, 1973).

Table 2.1. Typical fatty acid composition (%) of Malaysian palm oil (Tan *et al.*, 2000).

Fatty acid chain length	Mean	Range observed	Standard deviation
12:0	0.3	0-1	0.12
14:0	1.1	0.9-1.5	0.08
16:0	43.5	39.2-45.8	0.95
16:1	0.2	0-0.4	0.05
18:0	4.3	3.7-5.1	0.18
18:1	39.8	37.4-44.1	0.94
18:2	10.2	8.7-12.5	0.56
18:3	0.3	0-0.6	0.07
20:0	0.2	0-0.4	0.16

Most of the fatty acids in palm oil are presented as TAGs. The different placements of fatty acids and fatty acid types on the glycerol molecule produce a number of different TAGs such as 7 to 10 percent of saturated TAGs which are predominantly tripalmitin (Karleskind and Wolff, 1996). The fully unsaturated triglycerides (TGs) constitute 6 to 12 percent (Karleskind and Wolff, 1996; Kifli, 1981). The sn^2 position has a specificity for unsaturated fatty acids. Therefore, more than 85 percent of the unsaturated fatty acids are located in the sn^2 position of the glycerol molecule (Sambanthamurthi *et al.*, 2000). Table 2.2 shows the percentage distribution of individual TAGs of palm oil which is obtained by subjecting the lipolysis data to computer assisted statistical analysis (Kifli, 1981; Sow, 1979).

Table 2.2. Triacylglycerol composition (%) of Malaysian tenera palm oil.

No double bonds			One double bonds			Two double bonds			Three double bonds			Four double bonds		
	A	B		A	B		A	B		A	B		A	B
MPP	0.29	0.5	MOP	0.83	1.4	MLP	0.26	-	MLO	0.14	0.2	PLL	1.08	0.8
PMP	0.22	0.2	MPO	0.15	0.2	MOO	0.43	0.7	PLO	6.59	6.0	OLO	1.71	1.4
PPP	6.91	7.2	POP	20.02	23.7	PLP	6.36	6.3	POL	3.39	3.1	OOL	1.76	1.5
PPS	1.21	1.0	POS	3.50	3.1	PLS	1.11	0.8	SLO	0.60	0.4	OLL	0.56	-
PSS	0.12	0.1	PMO	0.22	-	PPL	1.17	1.0	SOL	0.30	0.2	LOL	0.14	0.1
PSP	-	0.7	PPO	7.16	6.9	OSL	0.11	-	OOO	5.38	5.1			
			PSO	0.68	0.6	SPL	0.10	0.1	OPL	0.61	0.5			
			SOS	0.15	-	POO	20.54	21.5	MOL	-	0.1			
			SPO	0.63	0.5	SOO	1.81	1.4						
						OPO	1.86	1.6						
						OSO	0.18	0.2						
						PSL	-	0.1						
Others	0.16			0.34	0.3		0.19	0.6		0.15	-		0.22	
Total	9.57	9.7		33.68	35.8		34.12	34.6		17.16	15.6		5.47	3.8

A: based on Kan-Ichi Hayakawa's computation (Kifli, 1981).
B: based on van der waal's method (Sow, 1979).
H, myristate; P, palmitate; S, stearate; O, oleate; L, linoleate.