UNIVERSITY OF
BIRMINGHAM

School of Chemical Engineering

Supercritical Fluid Technology Research Group

Extraction and Enrichment of Minor Lipid Components of Palm Fatty Acid Distillate Using Supercritical Carbon Dioxide

By

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Synopsis

Currently the extraction of valuable components from oils is of growing interest. Palm fatty acid distillate is a by-product from palm oil refining process which contains valuable minor components. The scope of the present work was to investigate the enrichment of high value low concentration components of palm fatty acid distillate namely squalene (1.8-2.3 wt.% squalene) using supercritical carbon dioxide as solvent with counter-current packed column kept under isothermal and longitudinal thermal gradient.

The overall objective of this work was to explore the effect of extraction process parameters such as pressure, temperature, and solvent to feed ratio to optimize the conditions that lead to high separation efficiency. This work has been centred on the study of the solubility of the main lipid components in supercritical carbon dioxide, isothermal counter-current extraction, longitudinal thermal gradient fractionation and the effect of feed concentration.

Solubility studies have been conducted for binary, ternary and quaternary systems as function of state of conditions through the application of a dynamic method. Binary systems of CO2/squalene, CO2/oleic acid, CO2/α-tocopherol, and CO2/pseudo-component palm olein were measured at temperatures of 313, 333 and 353 K, and at a pressure range of 10 to 30 MPa. A ternary system of CO2/squalene/palm olein and a quaternary system of CO2/squalene/ palm olein/ oleic acid were also investigated at 313 K and pressures of 10 to 25 MPa. Comparison of the ternary system with the binary system showed a decrease in the solubility of squalene, with a corresponding rise in the solubility of palm olein. In the quaternary system, the presence of oleic acid decreased the selectivity of squalene.

Extraction of squalene has been carried out on a counter-current glass beads packed column with the dimensions of 11.45 mm internal diameter and 1.5 m of effective height. The pressure and temperature were the operating conditions investigated and they varied from 10 – 20 MPa and 313 – 353K, respectively. Experimentation has demonstrated that squalene high fraction is achievable, however, squalene recovery has been found to be highly dependent on the extraction pressure and temperature. Squalene and free fatty acids content in the extract increased, and triglycerides content decreased during most of the fractionation runs. Longitudinal thermal gradient profiles along the column were investigated for further recovery of squalene, results showed the highest squalene recovery of more than 95% was reached and concentration of squalene was increased from 2wt% in the feed to 16wt% in the top product.

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

وَقُل رَّبِّ زِدْنِي عِلْمًا

“In the name of Allah, the Entirely Merciful, the Especially Merciful”

“Say, My Lord, increase me in knowledge.”

Chapter 20: Surat Taha
Holly Quran

أهدي هذا العمل المتواضع إلى أجمل و أغلى ما عندي ، إلى والديَّ العزيزين

أمينة عزيز و ابراهيم كرم
(ماما أمينة و بابا كرم)

This Work is dedicated to my beloved Parents

Mama Amina & Baba Karam

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Chapter 1

Introduction and Objectives

1.1 Introduction

One of the great ancient philosophers, Aristotle, had a statement on solvents and solutions. The statement in English is "...it is chiefly the liquid substances which 'react' "or "...for instance, liquids are the type of bodies most liable to mixing". Christian Reichardt mentioned that solid state chemists were not happy with Aristotle statement (Reichardt, 2006). Adding to that, supercritical fluids chemists neither! However, supercritical fluids have properties of liquids, but it can't be identified as liquid yet. Chemistry of solvents is moving toward new era where basic definitions and statements are narrow compared to the wide modern research.

The regulations towards using hazardous solvents in food and pharmaceutical industries, promote research to find neoteric alternative solvents. Extraction has been carried out using a variety of conventional solvents such as dichloromethane for the decaffeination of coffee beans (McHugh and Krukonis, 1994) and hexane for the extraction of olive husk oil (Esquivel et.al, 1999). Cost, accessibility, hazard and post treatment of the extract to remove solvent residue are main drawbacks of these conventional solvents especially in the food industry.

Functional foods and cosmetics are in revolution of innovation in food and cosmetic market due to the growing health awareness among people. This leads to alter and improve the existing products to be enriched with bioactive compounds such as vitamin E, squalene, caritenoids, etc. These bioactive valuable compounds can be found in various resources, mainly in vegetable oils and their by-products, e.g., olive oil, palm oil, sunflower oil, etc. The extraction of these compounds can be obtained using various extraction methods and it is vital to locate a suitable method to avoid degradation of these valuable compounds.

The supercritical extraction technology using supercritical carbon dioxide (Sc-CO2) as solvent makes processing and manufacturing bioactive material at ambient temperature possible in view of the fact that carbon dioxide (CO2) has near ambient critical temperature. CO2, which is non-toxic solvent, non-flammable and cheap gas compared to other solvents, has been investigated intensively in the past decades to design environmental friendly processes alternative to the tradition separation processes of bioactive materials.

1.2 Background

Vegetable Oils and fats naturally exist in plant seeds and fruits. The highly demand of these oils lead to its higher production rate. Extraction of these oils from plants such as palm oil, soybean oil and sunflower oil are produced widely all over the world. The annual production of the world's most important vegetable oils is shown in Table 1-1. Palm oil is one of highest plant based oil produced along with soybean oil.

Table 1-1: Annual production of the world's main vegetable oils (USDA, 2010).

	Production (Million Metric Tons)			
	2006/07	**2007/08**	**2008/09**	**2009/10**
Oil Coconut	3.22	3.53	3.53	3.62
Oil Cottonseed	5.13	5.22	4.82	4.67
Oil Olive	2.91	2.84	2.95	2.91
Oil Palm	37.33	41.02	43.94	44.82
Oil Palm Kernel	4.43	4.86	5.15	5.3
Oil Peanut	4.53	4.91	5	4.67
Oil Rapeseed	17.13	18.43	20.49	22.32
Oil Soybean	36.45	37.71	35.74	38.73
Oil Sunflower	10.6	9.92	11.87	11.47

Palm oil contributed about 32% to the total production of vegetable oil in 2009/10, as shown in Figure 1.1. Indonesia and Malaysia are the first and second largest producers of palm oil in the world, see Figure 1.2.

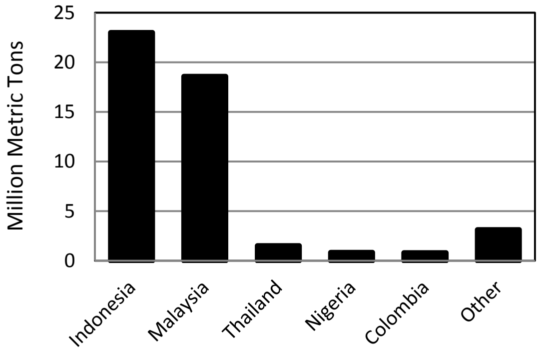


Figure 1.1: Main palm oil producers in the world 2009/10 (USDA, 2010).

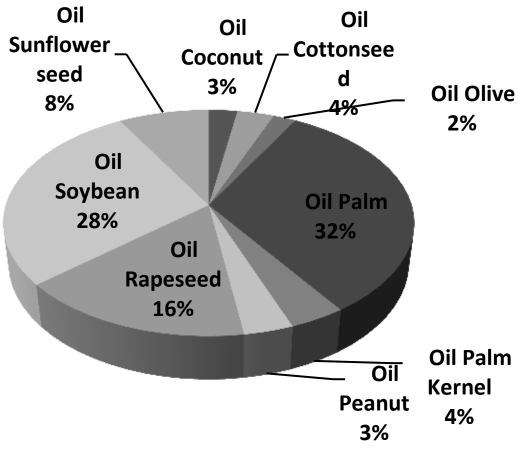


Figure 1.2: Distribution of main vegetable oils production 2009/10 (USDA, 2010).

Prior to export of the oil product, the crude palm oil is passed through chemical and physical refinery processes in order to produce an edible form of oil by removing undesired compounds and removing the odour of the oil. Palm fatty acid distillate (PFAD) is a by-product of deacidification and deodorization processes of the crude palm oil refinery. PFAD is mainly used in laundry soap industry as well as animal feed industry (Ab Gapor Md, 2010). It is composed mainly of free fatty acids (FFA) and glycerides as well as valuable components squalene, vitamin E and phytosterols. These components have potential value mainly for nutraceutical and cosmetic industries. Beside the applications of PFAD in many industries, it can be a valuable source for the extraction of these added-value components, especially with the high production rate of PFAD.

Supercritical fluid technology received high interest in many applications and it has high demand in the area of extraction and fractionation as an interesting alternative to the conventional separation processes. The fractionation of free fatty acids and tocopherols from PFAD are commonly carried out in distillation systems and/or two-step condensation systems. . Moving towards less environmental impact processes is one of the main industrial policies in this century particularly in the food and pharmaceutical industries.

Supercritical fluid extraction process has several advantages including that the process operating temperature is near-ambient which can enhance product quality by avoiding thermal degradation of thermal-sensitive compounds. Supercritical fluid process can be operated continuously in multistage extraction column and it can be applied in many potential applications in chemical and food industries.

1.3 Aims and objectives

PFAD contains squalene, FFA, tocopherols and sterols which are of special interests in the cosmetic, pharmaceutical and food industries. Based on the high production rate of palm oil, therefore high production of PFAD, it can be great source for the production of commercial amounts of these valuable components. Therefore, the main objective of this research is to separate and enrich the minor lipid components namely squalene, from PFAD via the application of supercritical extraction technology with Sc-CO2 as solvent, by evaluating the effect of extracting pressure, temperature and flow rate of carbon dioxide on the separation of lipid components presented in PFAD. The present work is devoted mainly to the recovery of squalene from PFAD via Sc-CO2 fractionation in counter-current mode. To our knowledge, there are no studies devoted on the recovery of squalene from PFAD. This raw material is particularly significant due to its high production rate with the content of valuable components. The previous investigations of Bondioli and Ruivo were conducted on counter-current extraction process with squalene feed concentration of 40 to 60% by weight from olive oil deodorizer distillate (Bondioli et.al, 1993, Ruivo et.al, 2007). The raw material employed in the present study contains only 2 to 4 wt. % of squalene. There is more challenge for the recovery of squalene at low concentration than that at higher concentration found in the olive oil distillate. In this work, the supercritical fluid fractionation and extractions experiments were designed and carried out in a bench-scale packed column and operated under isothermal and thermal gradient modes.

1.4 Research Plan

The research plan was developed and conducted based on the following phases to achieve the main aims and objectives of the project:

Phase I: Project Initiation

1. *Literature Review*: a literature survey was carried out to identify the main investigations up to date about supercritical fluids (mainly CO2) and its applications to isolate minor lipid components.
2. *Planning, Design and Construction of equipment*: the rigs for the solubility studies and counter-current fractionation column were previously built, but modifications were

necessary to prior to experimentation. Moreover it was very necessary to learn how to operate the rig and be familiar with the lab and safety regulation.

3. *Identification of the analytical techniques*: the analytical methods were determined from the literature review and training was conducted to learn how to use the analytical equipment, mainly gas and liquid chromatography.

Phase II: Solubility Studies

1. *Solubility Data anthology*: previous data were collected from literature, homogenized and plotted for all minor components and based on that, experiments will be carried out in a binary system (single component + CO2) in order to have a complete spectrum of the solubility data required. In order to develop a level of understanding of the solubility of components in CO2, solubility data were correlated using Chrastil equation (J. Chrastil, 1982).
2. *Binary, ternary and quaternary solubility studies*: Information on equilibrium solubility behaviour is essential to obtain basic knowledge regarding the feasibility of supercritical fluid separation and fractionation processes. Therefore, solubility studies were designed to be carried out for binary systems and multi-component systems.

Phase III: Fractionation and Enrichment Studies

1. *Fractionation rig components*: it was necessary to modify the rig and the fractionation column to meet the main objectives of the study prior to experimentation.
2. *Isothermal and thermal gradient Experiments*: fractionation experiments were designed based on various operating conditions namely temperature and pressure, feed flow rate, CO_2 flow rate. The experiments were designed to be conducted in isothermal mode and temperature gradient along the extraction column.
3. *Study various feed materials*: Following the fractionation experiment (above) the study of the effect of different PFAD feed materials was conducted at optimum conditions.

1.5 Thesis Structure

The structure of this research work is presented in eight distinct chapters as following:

Chapter 1: Introduction and Objectives

Chapter 2: State of the Art

Chapter 3: Materials and Analytical Techniques

Chapter 4: Solubility Studies of Lipid Components

Chapter 5: Counter-current Fractionation of PFAD

Chapter 6: Thermal Gradient Fractionation

Chapter 7: Effect of Different Feed Concentrations

Chapter 8: Conclusions and Future Work.

In this thesis, the main objectives of this research work are defined and summarized in Chapter 1. An overview of the supercritical fluids, minor lipid components and supercritical fluid extraction, solubility in supercritical fluids, and enrichment of natural matters state of the art are discussed in Chapter 2. Followed by a full description of materials and analytical techniques used throughout the research in Chapter 3. Afterwards, concise review of the results of solubility measurements is discussed in chapter 4. Chapter 5 and chapter 6 are mainly about the fractionation and enrichment of PFAD components conducted in isothermal mode and thermal gradient mode along the column, respectively. The feed compositions effect is discussed in chapter 7. Finally, the thesis is concluded with main concluding marks and brief statement of future work plans in Chapter 8.

1.6 Publications

The main results of this research work have been published in peer-reviewed journals and presented in conferences. Full versions of the publications are presented in the Appendix.

1.6.1 Publications

- N. Al-Darmaki, T. Lu, B. Al-Duri, J.B. Harris, T.L.F Favre, K. Bhaggan and R.C. D. Santos, "Isothermal and Temperature Gradient Supercritical Fluid Extraction and Fractionation of Squalene from Palm Fatty Acid Distillate Using Compressed Carbon Dioxide" **The Journal of Supercritical fluids** , 61 (2012) 108-114.

- N. Al-Darmaki, T. Lu, B. Al-Duri, J.B. Harris, T.L.F Favre, K. Bhaggan and R.C. D. Santos, "Solubility Measurements and Analysis of Binary, Ternary and Quaternary Systems of Palm Olein, Squalene and Oleic Acid in Supercritical Carbon Dioxide" **Separation & Purification Technology**, 83 (2011) 189-195.

1.6.2 Conferences

- N. Al-Darmaki, B. Al-Duri, T. Lu, J.B. Harris, T.L.F Favre, K. Bhaggan and R.C. D. Santos, "Phase Equilibria of Squalene, Oleic Acid and Palm Olein in Supercritical Carbon Dioxide ", **Chemical Engineering Congress**, Montreal Canada, August 2009.

- N. Al-Darmaki, B. Al-Duri, T. Lu, J.B. Harris, T.L.F Favre, K. Bhaggan and R.C. D. Santos, "Counter-Current Packed Column Supercritical CO2 Recovery of Squalene", **UAE Annual Research Conference**, Al-Ain, April 2008.

Chapter 2:

Literature Review,

State of the Art

2.1 Introduction

The use of supercritical fluids in the extraction and the purification of natural valuable components have been investigated in the past decades. It is one of the most studied applications in the field of separation in the last twenty years. This chapter is focusing on three dominant themes; fats and oils, supercritical fluids, and intensive literature survey on the extraction and separation of lipid components from liquid raw materials using supercritical fluids extraction. This particular research project is to examine the extraction and fractionation of particular lipid components of PFAD; however the scope of this review is expanded to include studies that examine various systems of feed material.

The first theme of this chapter is on the subject of fats and oils, section 2.3. Sources of main vegetable fats and oils are illustrated, followed by a brief description of the chemical and physical palm oil refinery and the main by-products including PFAD are described. Description and importance of minor lipid components present in PFAD were illustrated. Summary of conventional extraction methods and its limitations is presented at section 2.4 of this chapter.

Within the second theme of the chapter, definitions of solvent and supercritical fluids are illustrated. Available alternative green solvents are presented in section 2.5, followed by a brief discussion of phase behaviour of pure component. Sc-CO2 and its polarity attributes are discussed in section 2.6. Section 2.6.4 of this chapter is focused on the solubility of lipid components in Sc-CO2, followed by a presentation of main applications of supercritical carbon dioxide in chemical and food industries, with more attention on extraction and fractionation processes.

Finally, on the last theme of the review state of the art of supercritical fluid extraction and separation using Sc-CO2 is intensively studied in section 2.7. A review on the selection of parameter for the optimization of supercritical fluid extraction is illuminated. The effect of the solvent flow rate and the effect of additional of co-solvent are briefly discussed in section 2.7.2 and section 2.7.3, respectively. Finally the choice of the packing material and its effect on the overall supercritical fluid extraction efficiency is reviewed. This chapter is ended with main concluding marks found throughout this survey.

2.2 Objectives

The literature review is the initiation of this research project and the main objective of this review is to present and analyse the directions in the research on using supercritical fluids for the extraction of natural matters. To spot the perspective in which the research project is being carried out, thus identifying the implications this research might have on this field. Also, to highlight basic definitions of solvents, supercritical fluids and solubility.

2.3 Fats and Oils

Oils and fats are an essential commodity in a number of wide applications. In our daily life, there is hardly a product that we use does not contain fats as one of its ingredients. Oils and fats are used in various industries such as food products, cosmetics, medicines, paints and lubricants. Oil and fats are chemically similar however they differ in their physical properties. At room temperature, oil is in liquid phase while fat is in solid phase. Fats and oils are known for their hydrophobic property, and they are soluble in non-polar solvents. They are mainly consists of triglycerides and minor components such as FFA, tocopherols and phospholipids. They are derived from animal, fish and vegetable matter. This review is focused on the fats and oils produced from vegetable sources.

2.3.1 Sources of Vegetable Fats and Oils

There are unlimited sources for the derivation of vegetable fats and oils. Crude vegetable oil is obtained from oil milling units. The oil is further refined before distribution for edible use. Palm oil, soy bean oil and sunflower are examples of vegetable oil; they are vastly produced for commercial uses in various industries. Annual production rate of various vegetable oil are illustrated in Chapter 1 of this work. Palm oil showed higher production rate among other vegetable oils.

2.3.2 Palm Oil Refinery Process

Crude palm oil (CPO) can be refined either physically or chemically in order to eliminate impurities such as FFA, soaps and colouring pigments. Refined, bleached and deodorizer palm oil (RBDPO) is produced after physical refining, and neutralized, bleached and deodorizer palm oil (NBDPO) is produced chemically. Figure 2.1 illustrates main steps involved in both physical and chemical refinery of CPO.

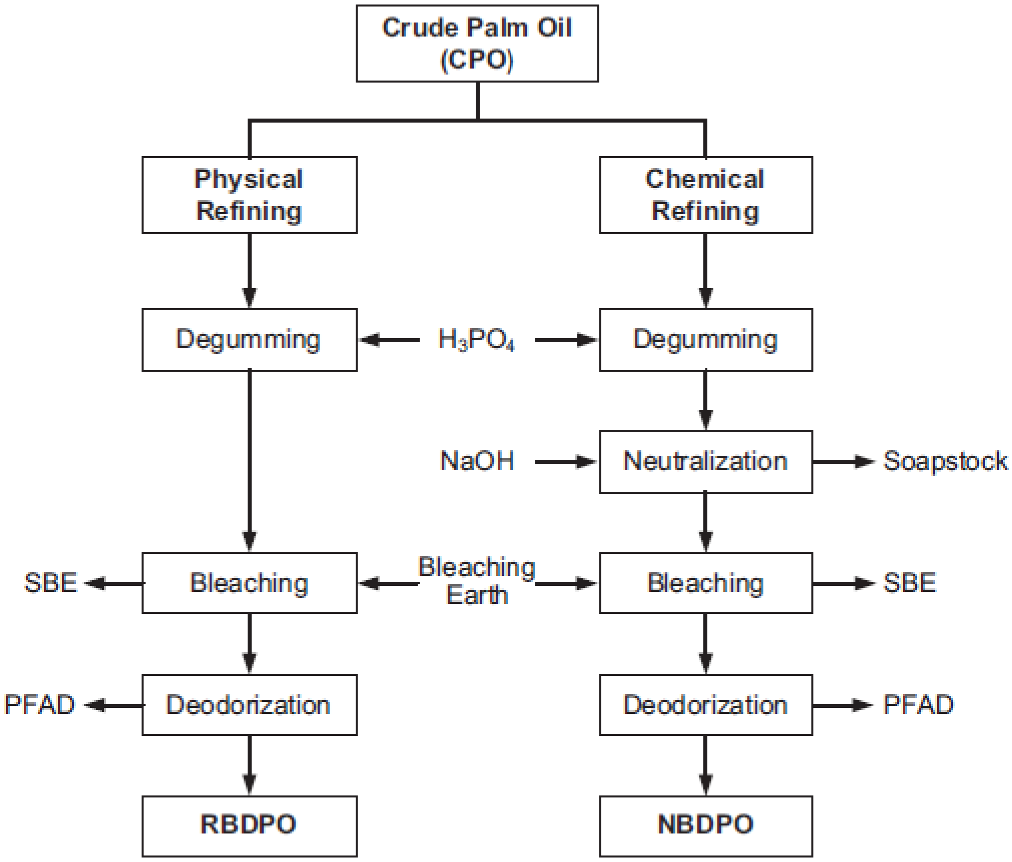


Figure 2.1: Refinery process of crude palm oil (Haslenda and Jamaludin 2011).

Degumming using phosphoric acid H_3PO_4 is an important step to remove phospholipids from CPO. A vacuum bleaching process is carried out to remove colouring pigments, trace metals and oxidative substances by applying slurry bleaching earth. Large amount of spent bleaching earth (SPE) is generated during this process. Steam distillation is applied for deodorization of CPO under vacuum pressure to ensure complete removal of FFA volatile compounds and other contaminants. The principal difference in between physical and chemical refining is the step of neutralization process where phospholipids and FFA are removed by caustic soda NaOH solution reaction with CPO. In general, physical refining is preferred where chemical usage and water consumption is minimized (Haslenda and Jamaludin 2011).

Various by-products are produced from CPO production. Fibre residue is formed from the mechanical extraction of palm fruits to produce palm oil; the residue is a yellow-reddish grass shape waste. This waste is mainly burned. The residue contains mainly carotenes; 36.4% α-carotene and 54.4% ß-carotene. Palm tree leaves are another by-product (waste) that is produced from harvesting palm fruits. They contain α-tocopherol, ß-tocopherol and y-tocopherol (Birtigh. et al, 1995). During the refining process of CPO several by-products are generated mainly soap-stock, SBE and PFAD. PFAD has the potential to be sold and processed to produce value-added products. PFAD is used in two main industries; animal feed and laundry soap industries. PFAD also can be a good potential source of valuable components such as squalene, tocopherol and FFA. Composition of PFAD varies and squalene content is in the range of 0.8 – 4 wt%.

2.3.3 Palm Fatty Acid Distillate Main Components

Recognition of the health benefits of bioactive lipid components has led to growing interest in understanding the separation and isolation of these components from vegetable source such PFAD. The properties and application of some of these components present in PFAD are summarized in Table 2.1. An overview of these components are presented in the following section.

2.3.3.1 Triglycerides

Triglycerides are mainly esters of fatty acids and glycerol, and they can vary with variation of fatty acids. Chemical structure of triglycerides is presented in Figure 2.2. The pseudo-component palm olein mainly consists of triglycerides. It is widely used as a food oil due to the fact that monoacylglycerol production from palm olein increases advantages and applications in pharmaceutical, cosmetic and food industries (Kaewthong and Kittikun 2004; Cheirslip et al. 2007).

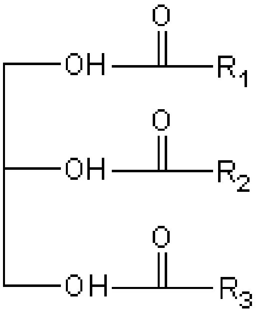


Figure 2.2: Chemical structure of triglycerides where R1, R2 and R3 are free fatty acids chains.

2.3.3.2 Free Fatty Acids

FFAs in chemistry are carboxylic acids with a long unbranched aliphatic chain that can be either saturated or unsaturated. Most naturally occurring fatty acids have a chain of 4 to 28 carbons and are used in many pharmaceutical and nutritional applications, such as soap production and cosmetics (Reinish 1956; Pinto and Lancas 2006). The length of alkane chain contributes to non-polarity of the acid. Thus the acid functional group of acids with short chains dominates and gives the whole compound a polar character (Sahena et al. 2009). Fatty acids in PFAD are mainly composed by palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and stearic acid (C18:0). Chemical structure of each fatty acid is presented in Figure 2.3, Figure 2.4, Figure 2.5 and Figure 2.6, respectively.

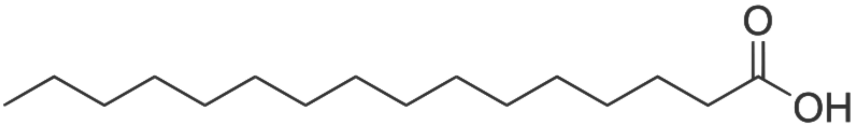


Figure 2.3: Chemical structure of saturated palmitic acid.

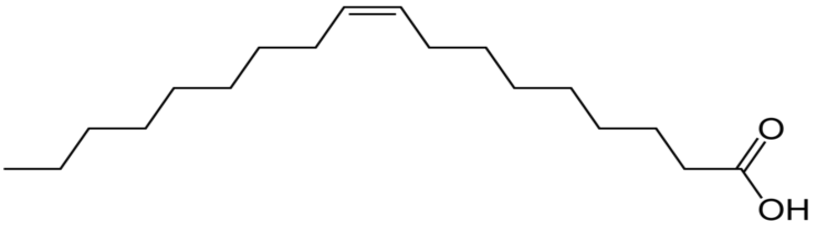


Figure 2.4: Chemical structure of unsaturated oleic acid.

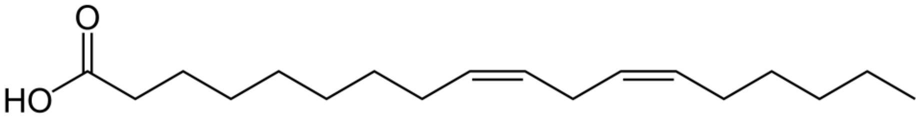


Figure 2.5: Chemical structure of unsaturated linoleic acid.

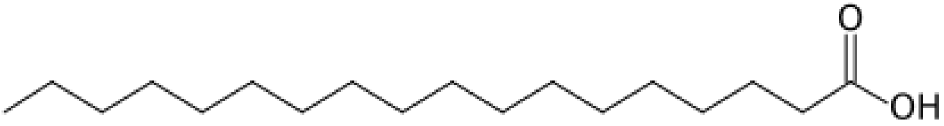
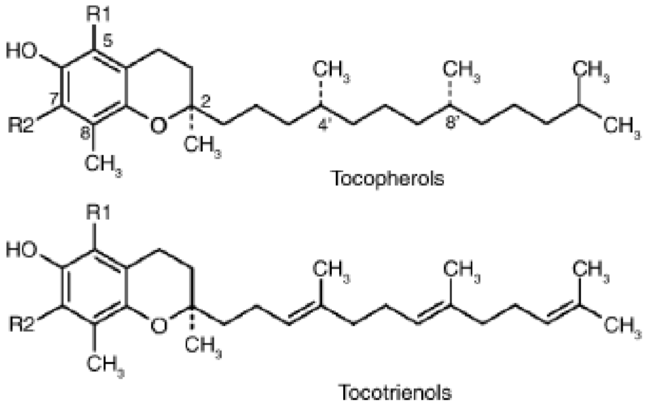


Figure 2.6: Chemical structure of saturated stearic acid.

2.3.3.3 Tocopherols and Tocotrienols

Vitamin E, as presented by Gast et al. 2005, is a total of eight substances with varying biological activity. These substances are called tocochromanols which are also divided into two groups: tocopherols and tocotrienols. Each group is a mixture of four isomers α, ß, y, δtocos (Mendes et al., 2005). Figure 2.7 shows the chemical structure of tocopherols and tocotrienols where the double bonds appear in the side chain of the tocotrienols. α–tocopherol has the highest biological activity and antioxidant characteristic among other substances of tocochromanols. Tocopherols are antioxidants that eliminate free radicals for primary defence in our body (Zacchi et al. 2006).



	R1	R2
α–	CH_3	CH_3
β–	CH_3	H
γ–	H	CH_3
δ–	H	H

Figure 2.7: Chemical structure of tocochromanols.

2.3.3.4 Phytosterols

Phytosterols are presented in PFAD in minor concentrations. The main Phytosterols that are presented in vegetable deodorizer distillate are cholesterol, ß.sitosterol, campasterol, stigmasterol and isofucosterol. Chemical structures of these components are illustrated in Figure 2.8 and Figure 2.9. These compounds cover a major portion of the un-saponifiable portion of the oil. Phytosterols are potential substances for utilization of functional foods (Dunford and King, 2000).

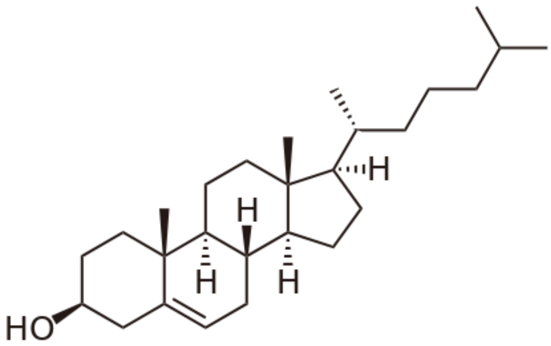


Figure 2.8: Chemical structure of cholesterol.

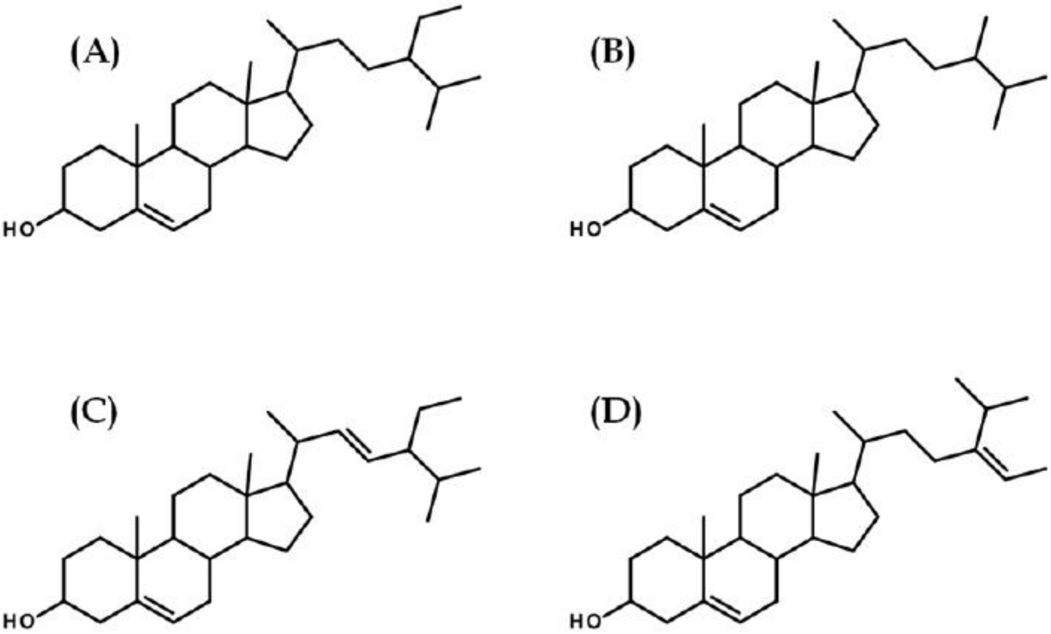


Figure2.9: Chemical structures of examples of phytosterols present in oil deodorizer distillate; (A) ß.sitosterol, (B) campasterol, (C) stigmasterol and (D) isofucosterol (Sugihara et al.2010).

2.3.3.5 Squalene

Squalene $C_{30}H_{50}$ (2, 6, 10, 15, 19, 23-hexamethyl tetracosaheaxaene) is an important non-polar hydrocarbon component in the chemical and pharmaceutical industries. The main natural source of squalene is fish liver oil which contains up to 70% by weight (Bakes and Nichols. 1995). Also it occurs naturally in olive oil, palm oil, wheat germ oil and other vegetable oils in lower concentrations. Squalene is also synthesised chemically under enzymatic reactions (Spanova and Daum, 2011).

Squalene has several advantageous properties. It is responsible for cholesterol biosynthesis (Mendes et al. 2000) and it is a natural antioxidant. It has been reported that squalene also enhances blood oxygenation as well as it is considered as anticancer agent. Squalene is being used as a moisturizing or emollient agent in cosmetic preparation (Bondioli et al. 1993; Moreda et al, 2001). As Squalene has a very low freezing point (-72 °C), a high boiling point (430 °C at 760 mmHg), and good thermal and frictional resistance, it has also been used to produce high-grade oil and lubricant for certain scientific instruments (Kayama 1999). The unique physical properties and physiological functions of squalene construct its importance as resource for functional food and supplement (Bhilwade et al. 2010). Chemical structure of squalene is presented in Figure 2.10.

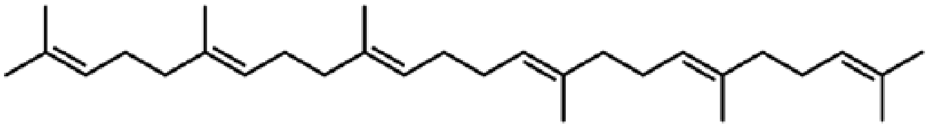


Figure 2.10: Chemical structure of squalene.

Squalene is non-polar hydrocarbon with strong hydrophobic nature. High degree of un-saturation of squalene causes instability and oxidation of this molecule. Squalene is one of the important lipids in human skin cells thus it is considered to be one of promising constituent in cosmetics and skin products.

Shark skins and livers are the main source of natural squalene and squalene present abundantly in whales with total concentration up to 70 wt. % (Catchpole et al.1997; Catchpole et al. 2000). Although, shark liver oil can be considered a main source of squalene, environmental and marine protection has placed regulations on the hunting of shark (Spanova and Daum 2011). Therefore, motivation on researches towards finding alternative sources aroused. There is great potential on the extraction and enrichment of squalene from PFAD.

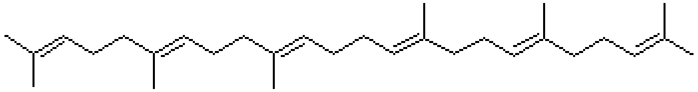
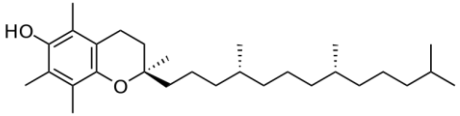
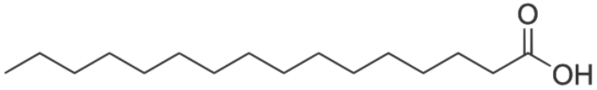
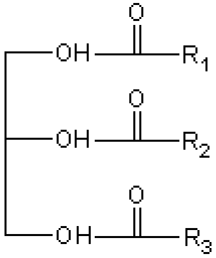
2.4 Conventional Separation Methods

There are several conventional methods for the extraction of lipids available in the separation and purification industry. Organic solvent extraction and vacuum distillation are common separation techniques existing in the industry.

2.4.1 Organic Solvent Extraction (Soxhelt Extraction)

Soxhelt extraction is a traditional extraction technique based on the affinity of the solute to the solvent. There are many disadvantages of this extraction method; hazardous liquid solvents are applied, low selectivity extraction, time consuming and costly (Sahena et al. 2009). Additional thermal procedure is required for the removal of solvent from final product and this aspect limits the applicability of this method on thermal labile compounds. There is threat on the environment because of the massive wide scale of the use of organic solvent extraction in global industries.

Table 2-1: Summary of properties and applications of squalene, α-tocopherol, oleic acid, palmitic acid and palm olein.

Component	Properties			
	Formula	Mw (g/mole)	Chemical structure	Typical Applications
Squalene	$C_{30}H_{50}$	410.73		Cholesterol biosynthesis Cosmetics as natural moisturizer Drug Carrier
α-tocopherol	$C_{29}H_{50}O_2$	430.17		Food and pharmaceutical antioxidants
FFA				
Oleic Acid	$C_{18}H_{34}O_2$	282.46		Soap productions Cosmetics
Palmitic acid	$C_{16}H_{32}O_2$	256.42		
Palm Olein		830[a]		Food oil/ Frying oil Monoacylglycerol production Cosmetic and food industries

[a] Pseudo-component palm olein molecular weight is estimated using its saponification value.

2.4.2 Vacuum Distillation

In vacuum distillation, the operating pressure is reduced to be less than the vapour pressure of lipid mixture. Thus, components with high volatility evaporate without the need of high operating temperature to avoid degradation of thermal sensitive components. Boiling point of a liquid decreases with pressure causing higher relative volatility (Liu et al. 2008). This extraction process is more advantageous compared to organic solvent extraction. However, these advantages are offset by the higher capital and operating cost of vacuum process. Difficulty of extract separation in the condenser is another problem associated with this technique (Westerman, 2004).

Extraction procedures ideally should have low environmental impact, low capital and operating cost, and low final product impact. Organic solvent extraction and distillation do not meet these criteria. Large volumes of contaminated and hazardous solvents are associated with organic solvent extraction and large cost coupled with vacuum distillation. Furthermore, there is pressure on the industry from United Nation Environmental Program to adopt sustainable low environmental impact processes (Herrero et al. 2010). Limitations of conventional methods and environmental regulations motivated scientists to discover and utilize alternative green solvents for the extraction of lipid components from natural matters.

2.5 Towards Green Solvents

In most chemical processes, a solvent is often involved and its importance is well recognized by the scientists of chemical technology. A chemical process without the solvent utilization scarcely exists. Therefore, the choice of solvents is of enormous importance. There is a huge range of solvents available and the number is increasing, indicating there are infinite numbers of the mixtures of these available solvents. There are 250 to 300 solvents available for industry and research use (Marcus, 1998; Marcus, 2002; Wypych, 2001). The vast majority of the solvents used in industry are organic toxic solvents which account high environmental impact. The hazards characteristics of conventional solvents on the environment and on the natural products, led to the development of more friendly alternative solvents. Consequently, moving towards green solvents is of great interest in this century (Sheldon, 2005).

A group of neoteric solvents are considered to be one of alternatives to green solvents. They are, as shown in Figure 2.11, *supercritical solvents*, perfluorinated (fluorous) solvents, and ionic liquids also called nanoaqueous at room temperature liquid ionic solvents (NARTLIS) (Reichardt, 2006). These are environmentally benign solvents of growing interest in recent scientific researches. Amongst green alternative solvents, particularly supercritical fluids have established many industrial applications; especially extraction and separation processes (Reverchon and Marco, 2006).

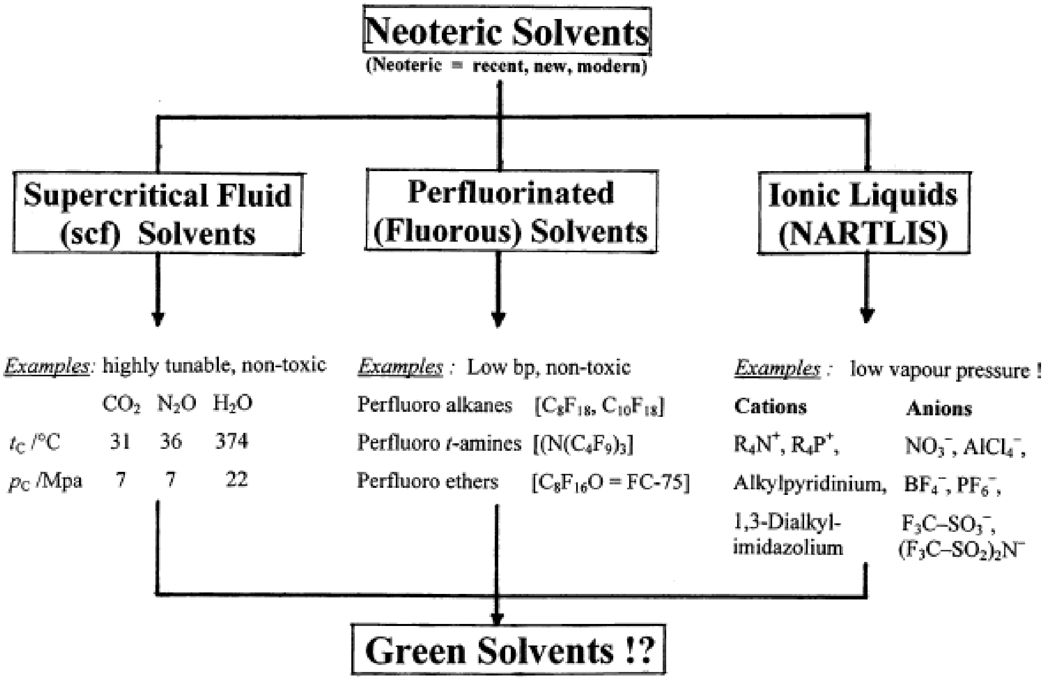


Figure 2.11: Potential green solvents alternative to common organic solvents (Reichardt, 2007).

2.6 Supercritical Fluids

Solvent power of compressed gas has been discovered over 100 years ago by Hannay and Hogarth (1879). It was the beginning of new era in the field of solvents, as supercritical fluids exhibit liquid-like solvent power. Supercritical fluids became one of the most attractive fields of research due to their revolutionary properties, which lead to their applications in many fields in chemical and pharmaceutical industries. Extraction and fractionation of high value minor (in concentration) lipid components of edible oil using supercritical fluids have been of particular interest for food and pharmaceutical industries.

2.6.1 Phase Behavior of Pure Component

Pure components present in one of the classical state of aggregation (solid, liquid or gas) at certain conditions. On the pressure-temperature (PT) diagram of a pure component demonstrated in Figure 2.12, the regions of the three phases are separated by the saturation curves, where two phases coexist in thermodynamic equilibrium.

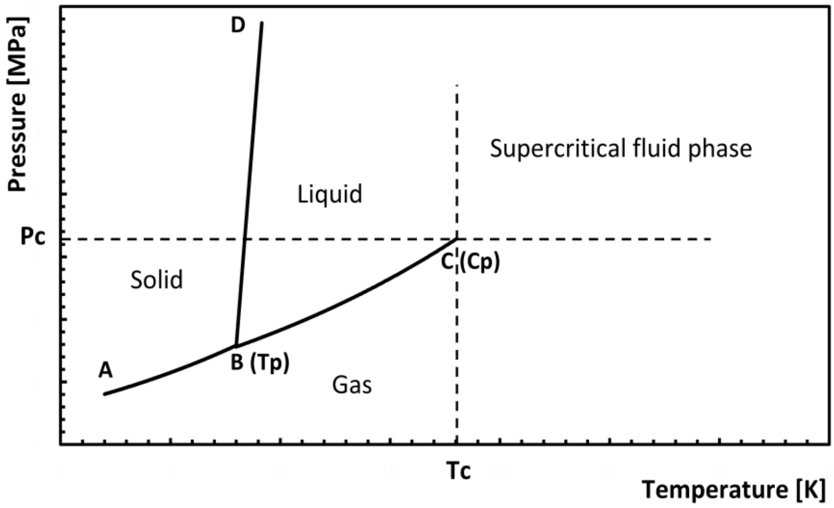


Figure 2.12: PT diagram of a pure component demonstrating the state of aggregation.

A-B curve is solid-gas or sublimation curve, B-D line is solid-liquid or melting point curve and B-C line is liquid-gas or boiling point curve. The sublimation curve ends at the triple point T_p, where all the three phases of a substance coexist in thermodynamic equilibrium. The boiling point curve attains to the critical point C_p, at which the properties of gas and liquid are equivalent and the phases are un-discriminated. Ahead of the critical point, the state of aggregation is described as supercritical fluid phase.

Supercritical fluid phase merges the properties of both liquid and gaseous phases of the matter forming unique characteristics (McGlashan, 1977). They are Liquid-gas like solvents which combine dissolution and diffusivity passing up the need of phase change. Supercritical fluids are 'tuning sensitive', as a small adjustment of the temperature and/or pressure (near/above critical point), results in large change on its density. This is advantageous because the solvent can be completely removed from the solutes of interest. Characteristic values (density, diffusivity and viscosity) of different sate of matter are shown in Table 2.2.

Table 2-2: Characteristic values of different state of aggregation, adopted from (Brunner, 1987).

State of the matter	**Density** g/cm^3	**Diffusivity** cm^2/s	**Viscosity** g/cm.s
Gas			
P = 1 atm, T = 15-30°C	$(0.6\text{-}2.0) \times 10^{-3}$	0.1-0.4	$(0.6\text{-}2.0) \times 10^{-4}$
Liquid			
P = 1 atm, T = 15-30°C	0.6-1.6	$(0.2\text{-}2.0) \times 10^{-5}$	$(0.2\text{-}3.0) \times 10^{-2}$
Supercritical Fluid			
$P = P_c$, $T \sim T_c$	0.2-0.5	0.7×10^{-2}	$(1\text{-}3) \times 10^{-4}$
$P = 4P_c$, $T \sim T_c$	0.4-0.9	0.2×10^{-3}	$(3\text{-}9) \times 10^{-4}$

2.6.2 Supercritical Carbon Dioxide

The distinctive properties of supercritical fluids promote scientists to explore and investigate the utilization of these fluids in various chemical applications. Critical properties of some substances used as supercritical solvents are shown in Table 2.3 (Arai et al, 2002). CO2 is well investigated as the main supercritical solvent used in extraction processes of natural compounds and production of pharmaceuticals due to its superior properties. It is non-toxic, non-flammable and has mild critical conditions, 304 K and 7.3 MPa for temperature and pressure respectively (Gast, et al. *2005*). Also it has advantages of low cost, and most importantly, its density (and therefore solvating power) is very sensitive to pressure changes around the critical region, typical phase diagram of CO2 is illustrated in Figure 2.13 revealing density's lines. This facilitates fractionation of components to a high degree of purity, according to their solubility in Sc-CO2 (Hurtado-Benavides et al, 2004).

Table 2-3: Critical point of substances which are used as supercritical fluid solvents.

Molecule	Formula	Critical Properties		Toxicity and flammability
		T_c(K)	P_c(MPa)	
Argon	Ar	151	49	-
Methane	CH_4	191	45	Highly flammable
Carbon Dioxide	CO_2	304	73	-
Ethane	C_2H_6	305	48	Highly flammable
Propane	C_3H_8	370	42	Extremely flammable
Ammonia	NH_3	406	111	Toxic
Water	H_2O	647	218	-

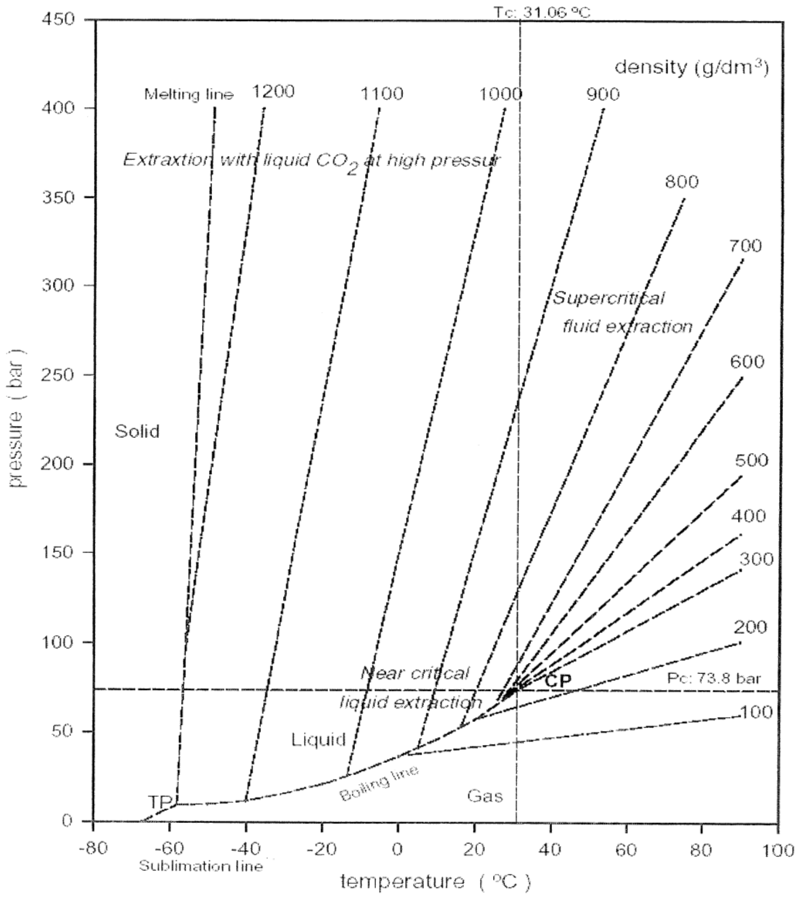


Figure 2.13: Typical phase diagram of CO2 (McHugh and Krukonis, 1994).

Liquid CO2 was studied in the mid of fifties as solvent to be applied in the extraction of hydrocarbon mixtures at room temperature (Francis, 1955) and it did receive high attention as an alternative solvent. However, there are noteworthy differences between liquid CO2 and Sc-CO2. Sc-CO2 offers more advantages compared to liquid carbon dioxide. It has lower viscosity, lower surface tension and higher diffusivity than the liquid CO2. Also, wider range of operating pressures and temperature offers the achievements of specific separation or dissolution (Hubert and Vitzthum, 1978). Figure 2.14 illustrate the density isotherm of CO2 as function of pressure at temperatures of 25 °C (liquid CO2) and 50°C (Sc-CO2), data is from NIST web-book. Great changes occur at pressures between 70 and 200 bar at supercritical conditions compared to that for liquid CO2. Both isotherms show insignificant changes above pressure of 300 bar.

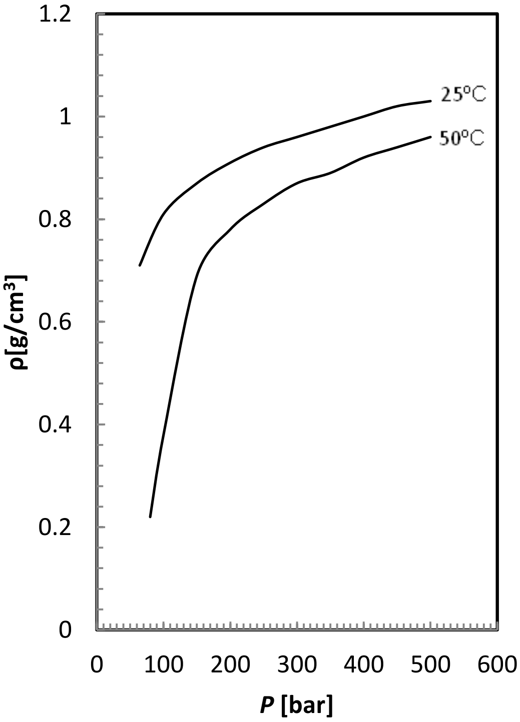


Figure 2.14: Density isotherm of CO2 as function of pressure (liquid CO2 at 25°C, and Sc-CO2 at 50°C)

2.6.3 Polarity Attributes of Carbon Dioxide

Solvent effects or solvation power are usually understood in terms of solvent polarity. Understanding a solvent and its influence in the solution is of high importance especially with a highly tuneable solvent like Sc-CO2. Near the critical region of CO2, the solvent strength is sensitive to any small variation of both pressure and temperature. Therefore, it is vital to know the solvent strength of Sc-CO2 as function of variables of state. The physical characteristics such as density and diffusivity of the solvent are not sufficient to understand its effects, but also the molecular level characteristics are in the same level of importance.

The combination of macroscopic and microscopic approaches (solvent polarity) is therefore important to describe the solvent-solute interactions (Katritzky, 2004).

Historically, Sc-CO2 was considered as a nonpolar solvent with low dielectric constant and zero dipole moment comparable to that of alkanes, and with the ability of microscopic measurements of solvent polarity at microscopic level, this view is changing slowly. Hyatt (1984) and Ikushima et al. (1991) measured Sc-CO2 solvent polarity parameter via Infrared Spectroscopy. Their findings concluded that the Sc-CO2 behaves very much like a hydrocarbon solvent with very low molecular polarizability.

Carbon dioxide is also described as a quadrupolar solvent (Kauffman, 2001) because of it significant quadrupole moment that is caused by the charge-separated molecule with significant nonzero bond dipole moments. These quadrupole characteristics are considered to be responsible of the Sc-CO2 polar attributes, as the overall electronic structure of CO2 allows it to act as either a Lewis acid or a Lewis base (Raveendran, 2005).

Molecular level understanding of the nature of CO2 and its polarity characteristics lead to a further perceptive of the sort of molecular systems that can dissolve in CO2. Classes of compounds that are soluble in liquid and Sc-CO2 are illustrated in table 2.4.

Although there is no significant difference in polarity between liquid and SC-CO2 (Hyatt, 1984), but the unusual properties of Sc-CO2 (infinite compressibility, low surface tension, and low viscosity) lead to its utility as solvent for the extraction and separation of natural matters.

Table 2-4: Classes of compounds soluble in liquid and Sc-CO2 (Westerman, 2004)

Liquid CO2		
Very soluble	Sparingly Soluble	Insoluble
Non-Polar and slightly polar molecules <250 RMM* eg. Terpenes, thiols, esters, short chain hydrocarbons, alcohols ketones	Organic compounds <500 RMM or moderate polarity eg. Oleic acid, decanol, lipids <C18	Sugars, Proteins, long chain waxes, chlorophyll, amino acids

Supercritical CO2		
Very soluble	Sparingly Soluble	Insoluble
Non-Polar and moderately polar molecules <500 RMM Eg. As liquid CO2 + triterpenoids, alkaloids, lipids < C22	Chlorophyll, waxes, carotenoids	Sugars, proteins, amino acids

* Relative molecular mass

2.6.4 Solubility of Natural Matters in Supercritical Carbon Dioxide

"Like dissolves like" is a well-known aphorism in the area of solvents and solubility. As stated in the previous section Sc-CO2 can dissolve solutes with similar polarity. In order to examine the solvent power of Sc-CO2 it is essential to determine the solubility of the material to be dissolved. Solubility is one of the main properties of supercritical fluid and it should be properly studied to provide a full understanding of the extraction process. Knowledge of solubility of pure and multi-component is the basis for the design of a supercritical fluid extraction process.

The ability of Sc-CO2 to dissolve low volatility substances enhances the concentration of solute in the supercritical fluid phase far beyond their vapour pressure. This enhancement in the volatility provides the basis for extraction processes using supercritical fluid (Brunner, 2010).

Solubility curves of low volatility compound naphthalene are given in Figure 2.15 as a general example of solubility trend in SCF. It is clear that supercritical fluid exhibits pressure dependant salvation power, the higher the pressure (higher density) the higher the solubility. The influence of temperature follows different trend, it varies depending on pressure range. At lower pressure range, increasing temperature results in a decrease of the solubility, while at higher pressure similar increase in the temperature enhances the solubility.

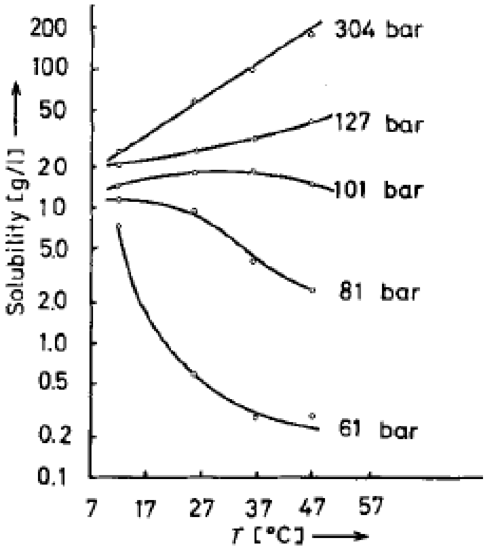


Figure 2.15: Solubility curves of low volatility naphthalene in supercritical fluid solvent (Hurbert and Vitzthum, 1978).

The binary phase equilibrium data of lipid components available in the literature are numerous and the solubility data varies based on studied components and experimental methods of solubility. Chrastil (1982) has published one of the first solubility studies of lipid components such as α-tocopherol, triglycerides and fatty acids in supercritical fluids. He performed solubility measurements in temperature range of 313-353 K and pressure of 8-25 MPa. Triglycerides and fatty acids are the main components present in vegetable and animal fats. King et al. (1997), Nilsson et al. (1991), and Bharath et al. (1992) have measured solubility of binary systems of mono-, di- and triglycerides as triolein in temperature range of 308.2-353.2 at pressures of 0.1-31 MPa. Recently, Fernandez-Roco et al. (2010) have presented experimental data of the solubility of triglycerides in CO2. The solute was triolein and measurements were performed at one temperature of 315.2 K in pressure range of 9.8-30.2 MPa.

FFAs studied by Chrastil (1982) were stearic acid and oleic acid. Maheshwari et al. (1992) and Skerget et al. (1995) have also studied the solubility of oleic acid in Sc-CO2 at temperatures of 313K, 333K and 353K at pressure range of 5-30 MPa. Many researchers are keen to produce new solubility data of lipid components in Sc-CO2 in the last decade because of the discrepancy in the data developed. Comparison of solubility data are discussed later in the text (Chapter 4). These differences between authors could be attributed to many factors such as components purity and the difficulty of sampling of solutes. Recent solubility measurements of oleic acid were performed by Fernandez-Roco et al. (2010), Hong et al. (2010) and Penedo et al. (2009).

Components present in trace amounts in many vegetable oils and their by-products have gained substantial attention in the study if their solubility in supercritical fluids. Particularly the solubility of α-tocopherol has been measured by several researchers. Johannsen and Brunner (1997) have measured the solubility of α-tocopherol at temperatures of 313 K, 333 K and 353 K at pressure range of 19.9-34.9 MPa. They found the highest solubility at temperature and pressure of 333 K and 35 MPa (higher density), respectively. Fang et al. (2004) performed α-tocopherol solubility measurements at the same temperature range but at pressure not more than 29 MPa. Fang found the maximum solubility to be at temperature of 313 K at 29 MPa. Comparison of the solubility data found in the literature has been discussed later in the text.

Compared to the other lipid components, few studies have been conducted for the solubility of squalene. Catchpole et al. (1997) have conducted the first solubility measurements for the binary system squalene-CO2. The study was performed via continuous packed column method over pressure range of 10-25 MPa and temperature range of 313-333 K. Another study was performed by Brunner et al. (2009) over a pressure range of 12-30 MPa and temperature range of 333-373 K. Recently new squalene solubility data were published by Hernandez et al. (2010) and Martinez-Correa et al. (2010). Martinez performed the measurements at pressures above 30 MPa.

Solubility data of binary systems on its own does not provide sufficient information for estimating if certain substance is extractable from its original matrix. Thus, solubility data of multi-component systems are essential. The solute-solute interactions are raised in these systems and adequate understanding of these interactions is important for the design of supercritical fluids separation processes. Data available on such systems is scarce and a small number of studies have been found in the literature.

Zou, Yu and Rizvi have developed a series of publications on phase equilibrium studies on multi-component systems; ternary systems of fatty acids and fatty acid esters (1992), data of anhydrous milk fat in Sc-CO2 at wide range of pressure and temperature (1992) and phase equilibrium data of mixture of methyl oleate and oleic acid (1993).

Catchpole et al. (1997) predicted the ternary phase equilibrium of model mixtures of squalene and triglyceride using Peng. Robinson equation. The interaction between squalene and triglycerides was assumed to be zero.

Stoldt and Brunner (1998) have investigated the phase equilibrium of real systems of fats such as crude palm oil and soybean oil deodorizer distillate. All measurements were conducted at pressures above 20 MPa. A further study was found by Stoldt and Brunner (1999) focused on the phase equilibrium of complex model systems of palm oil deodorizer condensates in Sc-CO2. They performed the measurements at pressure range between 20 to 29.5 MPa at temperatures of 343, 353 363 and 373 K. Three mixtures of palm oil deodorizer condensate where studied with various concentrations of fatty acids and tocopherols.

Squalene has shown great potential in the food and cosmetic industries and the extraction of it in organic-solvent-free processes is vital according to regulations surrounding these industries. Few researchers have addressed the solubility of squalene in multi-component culture. Stoldt and Brunner (1998) have investigated phase equilibrium of soybean oil deodorizer condensates with different squalene concentrations. However, soybean oil deodorizer condensates used in their study varies in the content of other components such as sterols, tocopherols and glycerides. Thus, reliable conclusions on the effect of squalene content on the solubility are relatively difficult to achieve. Also, investigations were conducted at pressure above 20 MPa. Two studies on phase equilibrium of ternary system methyl oleate/squalene/CO_2 (Ruivo et al. 2004) and ternary system oleic acid/squalene/CO_2 (Ruivo et al. 2007) have been conducted. The purpose of Ruivo study was to verify the ability of Sc-CO2 to separate methyl oleate from squalene at pressure and temperature range of 11-21 MPa and 313-343 K, respectively. The investigations on the ternary system of oleic acid and squalene were performed at temperatures of 313-333 K at pressures of 14-22 MPa with various concentrations of squalene content between 25-75 wt%. In both studies, the highest solubility of the mixture was obtained for the oil feed with the highest content of the most soluble component in CO2.

2.6.5 Applications of Supercritical Fluids

The unique and environmental friendly nature of supercritical fluids has led scientists to explore the utility of supercritical fluids in various applications especially that for Sc-CO2. The applications of Sc-CO2 are in continuous grow and are spread worldwide to replace hazardous solvents. Some of the applications are in the development stage while others moved forward to the industrial stage. In this context few examples of these applications are discussed briefly as following:

2.6.5.1 Supercritical Fluid Chromatography

Chromatography is an analytical technique which intends to separate a mixture of components into individual components. Supercritical fluids chromatography combines features of both gas chromatography and liquid chromatography therefore it offers many interesting possibilities under suitable choices of operating parameters, see Figure 2.16; unification of chromatography (Taylor, 2009). The use of supercritical fluid as carrier solvent overcome the limitation of high working temperature to reach the required volatility, especially for the thermal sensitive component systems. In SCF chromatography, the use of the elevated column pressures can offer new dimensions of flexibility in controlling the chromatographic retention process (Roth, 1992).

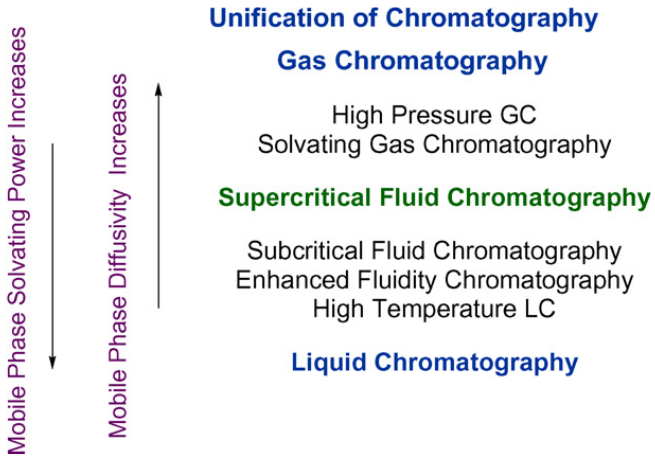


Figure 2.16: Unification of chromatography illustrating the supercritical fluid chromatography position compared to liquid and gas chromatography (Taylor, 2009).

2.6.5.2 Particle Formation

The outstanding features of supercritical fluid transformed it to a great solvent with various (on-going) applications in many fields. Particle formation is one of the important applications in numerous industries especially in the area of pharmaceutical. Conventional methods showed poor control on particle size and harmful solvents residue in the formed particles (Palakodaty and York, 1999). The advantages of the properties of supercritical fluid capable of overcoming the limitations of the conventional powder formation processes. There are four main categories on the application of particle formation using Sc-CO2 based on the role of CO2 and the application of second solvent (Hakuta et.al., 2003). Sc-CO2 particle formation categories are discussed briefly as following;

- Rapid expansion of supercritical solutions (RESS)

  In RESS process, the target (active) compound is first dissolved into Sc-CO2 at certain conditions of state to form supercritical solution. Supercritical solution then sprayed at atmospheric pressure via capillary nozzle. Rapid drop in pressure leads to formation of particles with micron size. Schematic diagram of RESS process is illustrated in Figure 2.17 (a).

- Supercritical anti-solvent (SAS)

  Contrary to RESS process, Sc-CO2 acts as anti-solvent in SAS particle formation method. The compound first dissolved in an organic solvent such as dichloromethane. Subsequently Sc-CO2 is fed into the system which leads to solution expansion forcing the dissolved compound to drop in micro-size particles. SAS diagram is illustrated in Figure 2.17 (b).

- Particle formation from gas saturated solutions (PGSS)

  In the PGSS process, fine particles of polymers can be formed. As Sc-CO2 can dissolve into melted polymers, polymers first melted at certain temperature then Sc-CO2 is fed to the process. The saturated solution then sprayed and expanded into a collection chamber (as shown in Figure 2.17 (c) causing super-cooling, thus fine particles polymer is produced.

- Depressurization of an expanded liquid organic solution (DELOS)

  Instead of melting polymers at certain temperature described previously in PGSS method, in DELOS process polymers are dissolved in organic solvents in order to avoid thermal degradation. Polymer-organic solvent solution is then mixed with Sc-CO2 in high pressure mixing vessel as shown in Figure 2.17 (d). Mixed solution is sprayed in a precipitation vessel where fine particles are formed and collected.

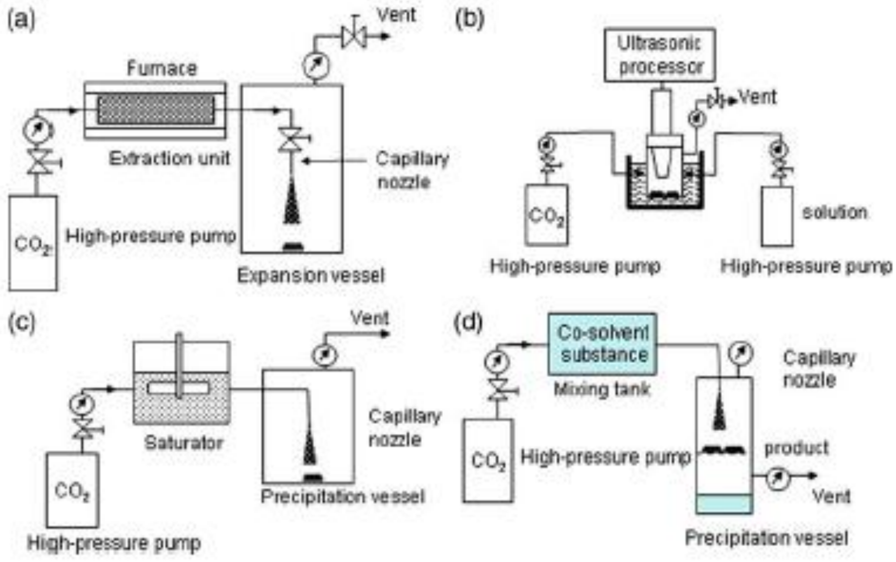


Figure 2.17: Schematic diagrams of the main categories of supercritical CO2 particle formation: (a) RESS process, (b) SAS process, (c) PGSS process, and (d) DELOS process (Hakuta et.al. 2003).

2.6.5.3 Supercritical Drying

The application of Sc-CO2 drying in the production of aerogels have been reported and studied by various researchers (Shimoyama et.al. 2010, Kawakami et.al. 2000) because of the great advantage of surface tension fading in SCFs. In this process, Sc-CO2 is used to remove an organic solvent, e.g., ethanol or acetone, from the gel. A schematic diagram of the drying process is given in Figure 2.18 for the production of titania aerogels. Aerogels production via Sc-CO2 have been investigated for varies functions mainly to be used as catalysts supports and as pharmaceuticals carriers (Brunner, 2010).

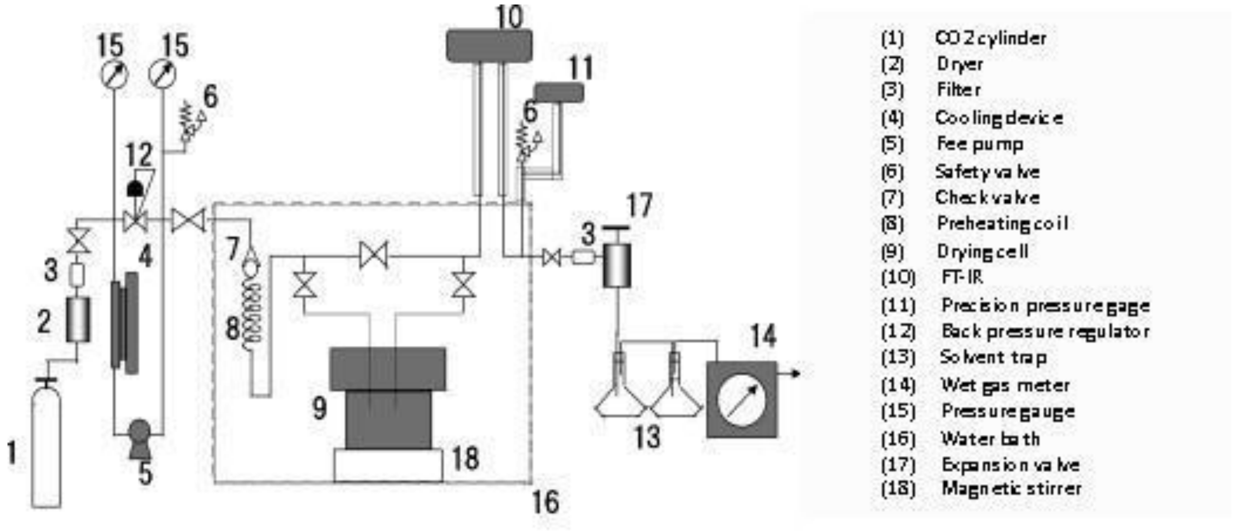


Figure 2.18: Schematic diagram of Sc-CO2 drying for the preparation of titania aerogels (Shimoyama et.al. 2010).

2.6.5.4 Supercritical Fluid Extraction

Extraction of natural products is one of the earliest and widest applications of supercritical fluids and there are many large extraction units operated worldwide. The unique characteristics of supercritical fluids enable it to compete with the conventional extraction methods, e.g., solvent extraction and distillation. Sc-CO2 has been thoroughly investigated as the main supercritical solvent in the extraction of the bioactive natural materials, largely due to it being non-toxic, non-flammable and having relatively low critical conditions suitable for thermally sensitive materials. The natural vegetable materials used for the supercritical fluid extraction can be either solids such as seeds or liquids such as vegetable oils.

- Extraction from solid materials

  Most of the natural materials used for the extraction of important components are normally solids. They are processes in batch or semi-batch processing which is commonly employed. Figure 2.19 shows a typical semi-batch solid processing under supercritical conditions. Raw solid materials are fed into the high pressure extraction vessel to form a fixed bed of solid particles. Sc-CO2 is pumped continuously into the vessel at certain flow rate. Solute is collected in one or more separation units (Reverchon, 1997).

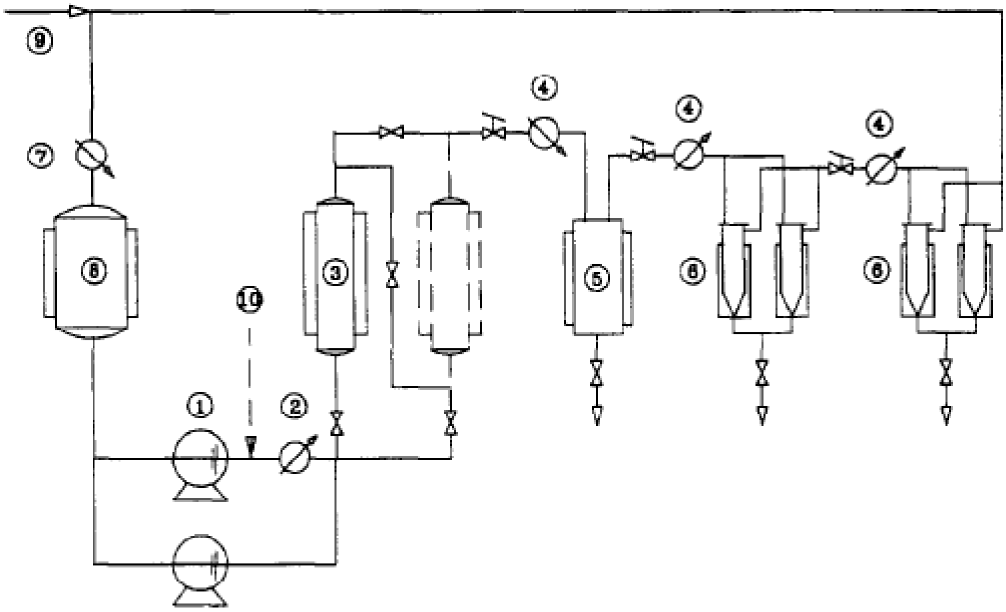


Figure 2.19: Schematic representation of SCF extraction pilot plant (Reverchon, 1997).

- Extraction from liquid materials

SCF extraction has been applied for various liquid mixtures for example; extraction of sterols from vegetable oil (Sovova et. al., 2010), separation to tocopherols from soybean oil by-product (Fang et. al. 2007), and fractionation of model liquid mixtures of squalene and oleic acid (Ruivo et. al., 2008). The extraction and fractionation of liquid mixtures via supercritical fluids is based on the different solubilities of the liquids. The fractionation process can be effectively carried out in a contentious counter-current high pressure column. Schematic representation of the process is shown in Figure 2.20. The process mainly consists of two main cascades; stripping section and enriching section where reflux is applied at the top of the extraction column. Sc-CO2 is fed contentiously at the bottom of the column (Brunner, 2009). Two main fractions are collected: the extract (top product) where the light components are collected and the raffinate (bottom product) where the heavy components are collected.

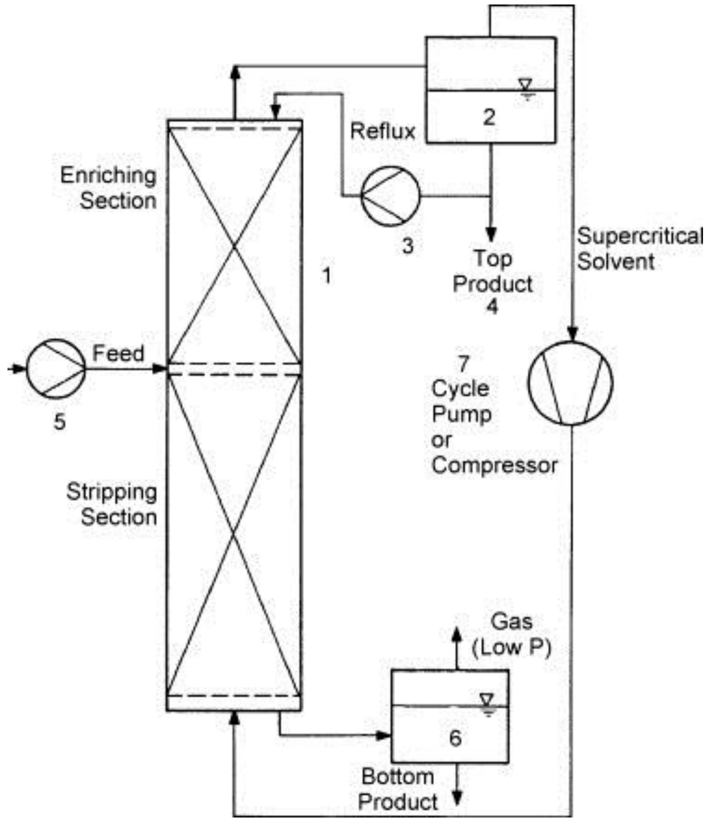


Figure 2.20: Schematic representation of continuous counter-currents high pressure column for the fractionation of liquids by supercritical fluid (Brunner, 2009).

2.7 State of the Art of Supercritical Fluids Extraction and Separation

The fundamentals of supercritical fluids and its principles in the application of separation had been known for many years. However, techniques for such processes were only developed and studied in the fifties and sixties. One of the first studies applying supercritical fluids for the purpose of separation in the middle of the 1960s was reported by Sie and Rijnders (1967). Their study was mainly for analytical purposes by applying supercritical fluids in chromatography. During the period of 1970s till the beginning of the 1980s, many studies were devoted on the application of supercritical carbon dioxide in chromatography (Jentoft and Gouw,1972; VanWasen and Schneider, 1975; Springston and Novotny, 1981; Wilsch and Schneider, 1983; Wilsch et al., 1983).

The first study on the extraction of lipid materials via Sc-CO_2 was conducted by Stahl et al. (1982) with batch process approach. Afterwards the researchers were interested on the application of supercritical fluids in a continuous mode due to the importance and enormous advantages of continuous approaches over batch processes. The early supercritical column fractionation studies were concentrated on the separation of alcohol from alcohol/water mixtures in a counter-current continuous mode as (Rathkamp et.al, 1987; Lahiere and Fair, 1987; Seibert and Moosberg, 1988). These studies investigated three different modes of continuous counter-current extraction of alcohol: spray column, sieve tray column and packed column, under supercritical conditions. Rathkamp investigated the mass transfer performance of two systems: Sc-CO2/ethanol/water system and Sc-CO2/iso-propanol/water system in spray and packed column modes. Higher mass transfer efficiencies were found in packed column compared to those of spray columns. The packing had the effect of increasing the availability of mass transfer area by decreasing the drop rise velocity of the dispersed phase. Lahiere and Fair determined the mass transfer efficiencies of extraction column operated as a spray, a sieve tray, and a packed column for systems similar to that used by Rathkamp. They concluded that the efficiency of packed column is greater than that of tray and spray column. Analogous conclusions were found by Seiberst and Moosberg for the iso-propanol/water system.

By the end of 1980s and the beginning of the 1990s, the interest towards the separation of real systems received attention such fish oils, vegetable oils and deodorizer distillates. Rizvi et al. (1988) and Nilsson et al. (1989) have studied the separation of esters from fish oil using Sc-CO2. Processing of lampante olive oil (Bondioli et al. 1992) and olive oil deodorizer distillate (Bondioli et al. 1993) in supercritical counter-current extraction were also studied. Work and investigations conducted on the separation processes via supercritical fluids in the period of 1980s and 1990s are summarised in Table 2.5 and Table 2.6, respectively.

In the recent years, the number of publications on the extraction and recovery of various components from real natural matters has grown and the interest towards Sc-CO2 as an alternative solvent motivated researchers and scientist to put in further investigations on this area from bench scale to pilot plant scale. Fractionation of crude palm oil was studied (Markom et al. 2001; Chuang and Brunner 2006). Similar investigations were performed for shark liver oil and fish oils by several authors (Catchpole et al. 2000; Riha and Brunner 2000; Vazquez et al. 2008). Table 2.7 summarise the investigations on supercritical fluid separation process of liquid materials for the period 2000 to 2010.

The interest in the isolation and separation of minor valuable lipid components is in continuous growth. Particular emphasis has been paid on the separation of these components from model mixtures and real systems. Separation and extraction of tocopherols from liquid feed materials has been studied by various researchers: tocopherols from wheat germ oil (Zacchi et al. 2006), extraction of α-tocopherol from model mixture of tocopherol/squalene (Mendes et al. 2000) and from crude palm oil and soy oil deodorizer distillate (Gast et al. 2005) with extraction pressure above 20 MPa. Tocopherols were also studied to be isolated from soybean oil by-product (Fang et al. 2007) at pressures lower than 20 MPa.

Limited studies were donated for the enrichment of sterols. Sterol extraction and enrichment from olive oil have been studied intensively by Ibanez et al. (2002) and Hurtando-Benavides et al. (2004). Rice bran oil found and its deodorization distillate have found to be good source of phytosterols (Dunford and King 2001; Sugihara et al. 2010). Investigations concluded that sterols are more likely to be enriched in the raffinate because of its low solubility in Sc-CO2.

Few works has been carried out on the separation of squalene with the application of Sc-CO2. Bondioli et al. (1993) carried out counter-current continuous separation of squalene from olive oil deodorizer distillate. High purity and yields were achieved because of the conversion of FFA and ester to their corresponding triglycerides. Vazquez et al. (2008) has also suggested a chemical esterification step of olive oil deodorizer condensate prior to SCF extraction in order to convert FFA to esters. Thus, raw material composition has great influence on the extraction of squalene, in supercritical fluid extraction column it can be either in top product or in the raffinate based on the feed system composition. In the case of olive oil deodorisation distillate squalene was recovered in the extract (Bondioli et al. 1993), while squalene from esterified deodorization distillate was recovered in the raffinate (Vazquez et al. 2008). In the latter, different conditions were carried out for squalene isolation compared to those used when olive oil deodorizer distillate and shark liver oil are the raw material.

The squalene content in the raw material is more than 20 wt% in most of the publications found. Limited research donated on the separation of squalene with relatively low concentration <5 wt%. Chuang and Brunner (2006) studied the enrichment of minor constituents from crude palm oil using supercritical fluid mixer-settler. Squalene was concentrated in the raffinate from 400 ppm to 33000 ppm.

Research studies on processing PFAD are scarce compared to other vegetable oils condensates. Stoldt and Brunner (1999) have determined experimental phase equilibrium for Sc-CO2 and complex mixtures of PFAD. Since PFAD concentration can vary, the mixtures they have analysed were mainly consisting of FFA and tocopherols. To our best knowledge there is no study found on the extraction of squalene from PFAD using Sc-CO2.

Table 2-5: Literature review of Sc-CO2 extraction and separation of compounds of various liquid systems in 1980s.

System	Target Compounds	Pressure (MPa)	Temperature (K)	Mode	Co-solvent	Packing material	Reference
Soya, Castor seed oil	TG Phospholipids glycolipids	10-30	313 - 353	Semi-continuous	Hexane Water	n.a	Stahl (1982)
Milk Fat	TG and FFA	10-35	323 and 343	Semi-continuous	n.a.	n.a	Arul (1987), Buningpfaue (1989)
Fish oil ethyl esters	Ethyl esters	11- 15 incremental	313 – 373 gradient	Semi-continuous	n.a.	n.a.	Nilsson (1989), Rizvi (1988)
Alcohol water mixture	Ethanol Iso--propanol	10	308 and 313	Counter-current continuous	n.a.	Raschig rings packed column and spray column	Rathkamp (1987)
Alcohol water mixture	Ethanol Iso--propanol	10	308 and 313	Counter-current continuous	n.a	Sieve Tray column and spray column	Lahiere (1987)
Alcohol water mixture	Iso--propanol	8 to 15	297 - 318	Counter-current continuous	n.a	Sieve tray, packed (Raschig rings, metal Intalox saddle) and spray column	Seibert (1988)
Fish oil	Ethyl Esters	12 and 15	308 - 333 gradient	Semi-continuous	n.a.	Dixon and stainless ball	Suzuki (1989)

n.a. not applicable

Table 2-6: Literature review of Sc-CO2 extraction and separation of compounds of various liquid systems in 1990s.

System	Target Compounds	Pressure (MPa)	Temperature (K)	Mode	Co-solvent	Packing material	Reference
Lampante olive oil	TG Squalene FFA	8-15	313-343 Thermal gradient	Counter-current continuous	n.a	Sulzer EX rings	Bondioli et al. (1992)
Olive oil deodorizer distillate	Squalene	11-17	40-60 Temperature gradient	Counter-current continuous	n.a	Sulzer EX rings	Bondioli et al. (1993)
Butter Oil	TG	10-27	40	Semi-continuous	n.a	n.a	Chen et al. (1992)
Peanut oil	FFA	10-20	300-330	Counter-current continuous	n.a	Stainless steel mesh packing	Zaiegler and Liaw (1993)
Soybean deodorizer condensate	Tocopherols	20	343 and 353	Counter-current continuous	n.a	Teflon rings	Brunner et al. (1991)
Soybean deodorizer condensate	Tocopherols	13-17.5	323-363	Semi-continuous	n.a	Teflon rings	Brunner et al. (1991)
Model Mixture of glycerides	TG, DG MG	20.7-34.5	340-380 Thermal gradient	Semi-continuous	n.a	Propack stainless steel	King (1997)

n.a. not applicable

Table 2-6 (Continued): Literature review of Sc-CO2 extraction and separation of compounds of various liquid systems in 1990s.

System	Target Compounds	Pressure (MPa)	Temperature (K)	Mode	Co-solvent	Packing material	Reference
Glycerides Mixtures	TG, DG MG	17.5-35	338-368 Thermal gradient	Semi-continuous	n.a	Propack stainless steel	King et. al (1997)
Anhydrous Milk Fat	FFA	24	313-348	Continuous	n.a	n.a	Bhaskar et al. (1993)
Anhydrous Milk Fat	FFA	n.a	n.a	Continuous Packed and spray columns	n.a	n.a	Lim and Rizvi (1995)
Anhydrous Milk Fat	cholesterol	8-40	313-343	Semi-contentious	n.a	n.a	Huber et al. (1996)
Shark Liver Oil	squalene	12.5-25	313-333	Counter-current continuous	n.a	Glass Raschig rings-glass Fenske helices-stainless steel wire	Catchpole et al. (1997)

n.a. not applicable

Table 2-7: Literature review of Sc-CO2 extraction and separation of compounds of various liquid systems in 2000-2010.

System	Target Compounds	Pressure (MPa)	Temperature (K)	Mode	Co-solvent	Packing material	Reference
Rice bran oil	Tocopherols Phytosterol	20.5 - 32	318-353 Thermal gradient	Semi-continuous	n.a	Propak internals	Dunford and King (2000), Dunford and King (2001)
Soy deodorizer distillate	tocopherols	18 -30	313-333	Semi-continuous	n.a	n.a	Nagesha et al. (2003)
Model mixture of squalene and methyl oleate	squalene	11.5-18.5	313-333	Counter-current continues	n.a	Sulzer EX	Ruivo et al. (2004)
Canola oil deodorizer distillate	Sterols FFA	20 -25	343 -373 Thermal gradient	Semi-continuous	n.a	Stainless steel Propack	Guclu-Ustundag and Temelli (2007)
Deodorization distillate of rice bran oil	Squalene Phytosterols	8-22	303-353	Semi-contentious	n.a	n.a	Sugihara et al. (2010)
Shark Liver oil	Alkoxyglycerols	14-18	318-338 Thermal gradient	Counter-current continues	n.a	Fenske rings	Vazquez et al. (2008)

n.a. not applicable

Table 2-7 (continued): Literature review of Sc-CO2 extraction and separation of compounds of various liquid systems in 2000-2010.

System	Target Compounds	Pressure (MPa)	Temperature (K)	Mode	Co-solvent	Packing material	Reference
Soybean oil deodoriser distillate	Tocopherols	14-18	318-343 Thermal gradient	Semi-continuous Counter-current	n.a	Stainless steel Dixon packing	Fang et al. (2007)
Olive oil deodoriser distillate/esterified	Squalene	15-23	343	Semi-continuous Counter-current	n.a	Fenske rings	Vazquez et al. (2008)
Wheat germ oil	Tocopherols FFA	26.5-35	338 and 341	Counter-current continuous	n.a	Sulzer BX	Zacchi et al. (2006)
Crude Palm oil and Soy oil deodoriser distillate	tocopherols	20-30	340-370	Counter-current continuous	n.a	Sulzer Ex	Gast et al. (2005)
Olive oil	Tocopherols and sterols	20	313	Counter-current continuous	n.a	Raschig rings, Dixon rings, Fenske rings and glass beads	Hurtado-Benavides eta al. (2004)
Crude palm oil	FFA ß-carotene	11-20	313-333	Semi-continuous	n.a	n.a	Markom et al. (2001)

n.a. not applicable

Table 2-7 (continued): Literature review of Sc-CO2 extraction and separation of compounds of various liquid systems in 2000-2010.

System	Target Compounds	Pressure (MPa)	Temperature (K)	Mode	Co-solvent	Packing material	Reference
Fish oil	Ethyle esters	6.5-19.5	313-353	Counter-current continuous	n.a	Sulzer CY wire mesh	Riha and Brunner (2000)
Fish oil	Vitamin A squalene	20-30	333	Counter-current continuous	ethanol	Sulzer EX	Catchpole et al. (2000)
Orange Roughy oil	FFA peroxides	20-26	333	Counter-current continuous	ethanol	Sulzer EX	Catchpole et al. (2000)
Synthetic mixture of tocopherol /squalene/FFA	Squalene	13-25	308-363	continuous	n.a	n.a	Mendes et al. (2000)
Soy oil deodoriser distillate	Squalene FFA sterols	24.1and 31	323 - 363	Semi-continuous	n.a	Mesh stainless steel filling	Chang et al. (2000)
Shark liver oil and olive oil deodoriser distillate	squalene	Mixer:10-25 Column: 16-25	313-333	Semi-continuous	n.a	Static mixer (helicalstages) Packed column (Sulzer EX)	Catchpole et al. (2000)

n.a. not applicable

2.7.1 Selection of Conditions of State; Pressure and Temperature.

These parameters; pressure and temperature, have the scope of achieving the desired extraction and fractionation. The correct selection of these parameters leads to successful extraction process of the desired components. The degree of the success of the fractionation of certain mixtures is based on the selectivity of the Sc-CO2, which is directly related to the state of conditions.

2.7.1.1 Effect of Pressure

In most studies found in the area of separation and extraction at supercritical conditions, pressures were in the range of 10 – 35 MPa in order to attain adequate densities. Further raise in extraction pressure > 35 MPa is not recommended economically. Also, further enhancement of the extraction is limited. With increasing pressure from 5 to 10 MPa, a linear sharp increase of CO2 density occurs. Density only slightly increases at pressure above 35 MPa as shown in Figure 2.21.

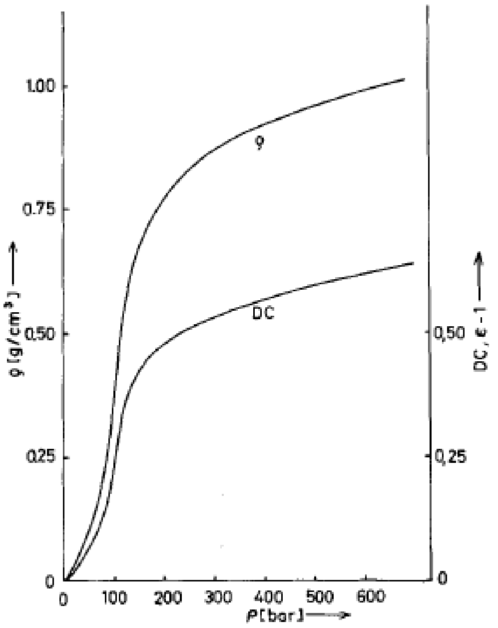


Figure 2.21: Density and dielectric constant of CO2 as function of pressure at temperature of 323 K (Hubert and Vitzthum, 1987).

2.7.1.2 Effect of Temperature

The effect of temperature is of special interest in supercritical solvent extraction. Temperature range studied is virtually similar in most of the research studies on the extraction of lipid components. Maximum extraction temperature limit was chosen to avoid heat degradation of thermal sensitive compounds. Minimum temperature was set to ensure two important parameters: sufficient temperature for CO2 to reach supercritical region and to ensure that the feed matrix in liquid phase to avoid plugging of the column (Guclu-Ustundag and Temelli 2007).

The influence of thermal gradients in a counter-current column was investigated: Dunford and King (2001) have investigated deacidification of rice bran oil under thermal gradients. In recent years, Fang et al. (2007) and Vazquez et al. (2008) induce internal reflux by employing thermal gradient in the study of separation of tocopherols from soybean oil by-product and nonesterified alkoxyglycerols from shark liver, respectively. The effect of internal reflux was remarkable and improved tocopherols and nonesterified alkoxyglycerols recovery.

2.7.2 Effect of Solvent Flow Rate

Solvent flow rate is another crucial parameter in the supercritical extraction and fractionation processes. Usually it is related or referred as solvent to feed (S/F) ratio. As a role of thumb, supercritical CO2 flows from the bottom to the top of the extraction column, while the liquid feed material/solution is pumped to the column from the top to assure counter-current attainment. The conditions taken to account in the traditional packed column operations, should be also respected when supercritical CO2 used as the medium. For instant, complete wetting of the packing material with the liquid feed material should be assured by pumping a required liquid flow. Also, a proper selection of S/F ratio is vital to avoid flooding in the extraction column (Reverchon and Marco, 2006). An access of CO_2 occurs with high S/F ratio, results in unsaturated CO2 and high axial velocity. Consequently, undesirable components content in the final product will increase (Fang et al. (2007).

2.7.3 The Use of Co-solvents

In the view of the literature, solvent mixtures are often more appropriate media compared to pure solvents. However, it is important to note that there are limitations with the use of co-solvent. These include:

- The problem of preferential salvation; this phenomena is particularly related to the composition of the solvent mixture as the composition of the bulk mixed solvent can differ from the composition in solute immediate vicinity. This can lead to local inhomogeneities in multi-component solvent systems (Ben-Naim, 1990, Egorov, 2000).
- An additional separation step is required for the separation of the co-solvent from the product, thus higher capital cost.

Catchpole et al. (2000) have investigated the effect of ethanol mixtures on the solubility and fractionation of fish oils using Sc-CO2. Solubility increased exponentially with linear increase of ethanol concentration, and it increased rapidly of polar components than that of non-polar components. Degree of separation of squalene from shark liver was not superior

2.7.4 The Effect of Packing Materials

The scope of the packing material is to allow adequate contact area between the feed material and solvent. Usually the packing material is inert material packed in the column either randomly or discriminately. Hurtado-Benavides determined the efficiency of supercritical fractionation of olive oil in a counter-current column for different types of random packing materials (Hurtado-Benavides et.al, 2004). The study was based on the determination of the number of transfer units (NTU) and height of transfer unit (HTU) of each packing, and the performance of extraction was analysed by the measurements of vitamin E and sterols in the extract and the raffinate. Hurtado-Benavides found that each packing material favoured the separation of certain solute; for instant Dixon rings for the separation of sterols and glass beads for the separation of vitamin E. In terms of efficiency and performance of counter-current supercritical system, Fenske rings showed the best results, Figure 2.22 is adopted from Hurtado-Benavides study. On the other hand, Catchpole et al (1997) found no reliable results were achieved with Fenske packing in the study of extraction of squalene from shark liver oil. Excessive holdup of liquid in the column was caused with Fenske packing.

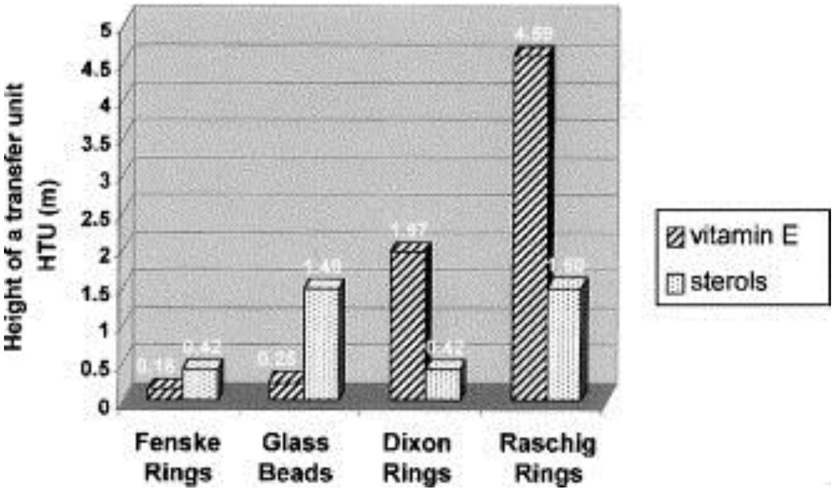


Figure 2.22: HTV values (m) vs. packing materials at a CO2 flow rate of 2000 m/h and S/F ratio of 23.14 kg CO2/Kg olive oil (Hurtado-Benavides et.al, 2004).

2.8 Concluding Remarks

The main objective of this chapter was to identify and illustrate the context in which this research project is carried out. After reviewing the main background behind extraction of lipids via supercritical fluids, the following points might be concluded:

i. Crude vegetable oils, their refined products and deodorizer distillate by-products provide alternative sources for minor voluble constituent such as squalene.
ii. Squalene gained from natural sources has great potential in the food and cosmetic industries.
iii. There is an increase on public awareness of health, safety and environment impact coupled with the use of hazardous organic solvents and there are numerous food regulations toward the use of these solvents for extraction purposes.
iv. Supercritical fluids extraction offers an alternative method for the extraction of lipid components, with Sc-CO2 as extraction medium.

Based on the review of this chapter, the separation of lipid component from liquid raw material is possible and reliable. Therefore, this project is directed towards the investigation of SCF extraction and separation of squalene from PFAD.

Chapter 3

Materials and Analytical Techniques

3.1 Introduction

PFAD main components have considerable differences in their physical and chemical properties. Accurate and precise analytical methods are important for the identification and quantifications of the chemical components of PFAD. Description of main material used and analytical methods applied is discussed throughout this chapter. Material used for solubility measurements were described in section 3.2.1, followed by characterization of material used in extraction experiments in section 3.2.2. Full description of the procedures involved in the analytical technique use for the quantification of squalene and FFA is illustrated in section 3.3. Also, description of the titration method for FFA quantification is given in section 3.3.3. Finally this chapter ended with notes on the storage of sample materials throughout the research.

3.2 Materials Used in Solubility and Extraction Experiments

3.2.1 Solubility Experiments

Throughout this research work, solubility measurements were aimed to be conducted for binary systems of pure lipid solutes and solubility of ternary and quaternary systems of solute mixtures in Sc-CO2.

3.2.1.1 Binary System Pure Materials

Four main components were studied; squalene, oleic acid, α-tocopherol and palm olein. Squalene 98% purity and –tocopherol 96% purity were purchased from Sigma Aldrich. Oleic acid 98% and pseudo-component palm olein were supplied by IOI Group-Loders Croklaan. The composition of palm olein is given in Table 3.1, the data provided by IOI Group.

Palm olein is mainly consists of triglycerides 89%. In this work, palm olein was considered to be pseudo-component for the simplifications of solubility measurements. It should be emphasized that there is no general rule in solubility measurements exists that can be applied to simplify a mixture into pseudo-component. In this research project, it was vital to understand and measure palm olein solubility in Sc-CO2 as it is one of main components in PFAD.

3.2.1.3 Ternary and Quaternary Systems Solute Mixtures

For solubility measurements of ternary system of squalene and palm olein, a mixture of 10-wt% squalene were prepared. Two stocks of 100g were prepared by accurately weighing 10g of squalene and 90g of palm olein. The mixture was prepared at room temperature .The lipid components mixture then mixed appropriately via mixer.

In the case of quaternary system of squalene, oleic acid and palm olein, the concentration of squalene was kept similar to that of ternary system 10-wt% in order to compare between the two systems. Two stocks each of 100 g were prepared by accurately weighing 10 g of squalene, 25 g oleic acid and 65 g of palm olein. The mixture of the quaternary system was prepared under the same conditions of that in ternary system.

Table 3-1: Palm Olein composition in wt%.

Component	**Composition %**
Glycerides	**99.3**
Triglycerides	89.2
Diglycerides	9.6
Monoglycerides	0.5
FFA	**0.03**
Others*	**0.67**

* Mainly tocols, squalene and sterols.

3.2.2 Extraction Experiments

Extraction and fractionation experiments were conducted at various conditions of state. Continuous extraction experiments were performed for the extraction of minor components mainly squalene from PFAD with various compositions.

3.2.2.1 Palm Fatty Acid Distillate

Palm fatty acid distillate PFAD is a by-product from the physical refinery of palm oil. PFAD was supplied by IOI Loders Croklaan, Netherlands. The composition of PFAD may vary from sample to another and thus it melting point (Chua et al. 2007). Throughout this work, main separation experiments were performed for one main PFAD with squalene composition of ~ 2 wt% (PFAD 1). At room temperature, the sample is yellow brownish in colour with butter-like structure. It's found to be liquefied at temperature of ~ 40 °C.

3.2.2.1 High FFA and Low FFA Palm Fatty Acid Distillate

The study of the effect of the feed material composition was also investigated in this work at optimum conditions. Three additional PFAD stock with various compositions were provided by IOI Loders Croklaan, Netherlands. Composition of PFAD samples are given in Table 3.2 below. The samples were requested to have different FFA content to study its presence on the separation and selectivity of squalene. High FFA content PFAD (PFAD 4) is consists mainly of FFA and small concentration of squalene. Low FFA content PFAD (PFAD 3) is consists of only ~5 wt% FFA. For all PFAD samples, stock was melted in warm water bath of ~40°C to assure sample homogeneous prior to extraction experiments.

Table 3-2: Composition in wt%. for various PFAD studied in this work.

Component	**Composition %** [a]			
	PFAD 1	**PFAD 2**	**PFAD 3**	**PFAD 4**
Glycerides	**66.0**	**51.9**	**n.a**	**n.d**
Triglycerides	33	34.5	n.a	n.d
Diglycerides	22	11.8	n.a	n.d
Monoglycerides	11	5.6	n.a	n.d
FFA as Oleic	**25**	**42**	**5.0**	**96**
Squalene	**2.2**	**3.7**	**1.7**	**3.6**
α-tocopherol	**0.4**	**0.51**	**0.67**	**0.1**
Sterols	**1.1**	**0.8**	**1.5**	**0**
Others [b]	**5.3**	**1.0**	**n.a**	**n.d**

[a] Slight variation can be found for various sample stocks. [b] Mainly tocols, polymers and non-identified components. nd; not detectible. n.a.; not available

3.3 Analytical Methods

Modern analytical methods are powerful tools for the identification and quantification of chemicals. These methods including Gas Chromatography (GC), Mass Spectroscopy Gas Chromatography (MsGC) and High Performance Liquid Chromatography (HPLC) have great potential on the identification of lipid components.

3.3.1 Analysis of Squalene Content

Squalene content of model mixtures and PFAD samples were measured by direct carbon number GC with 5-α-cholestane added as internal standard. Carbon number peak separation was optimized to assure two main things: squalene response peak to be as close as possible to that for the internal standard (5-α-cholestane), and sufficient separation of squalene from any FFAs. In order to ensure total removal of high molecule weight of triglycerides from the capillary column, total analysis run interval was extended. Complete elution of these compounds was necessary to avoid analytical error.

3.3.1.1 Carbon Number GC Conditions

6850 series GC with FID detector from Agilent technologies was used coupled with capillary column DB-5 (length 30 m x diameter 0.25 mm x film thickness 0.1μm). The GC was used with split mode, and helium as carrier gas. Oven temperature set to 160°C followed by temperature ramp, from 160°C to 350°C at 5°C/min and hold for 2 minutes. Detector temperature was kept at 350°C.

3.3.1.2 Internal Standard Preparation and Correction Factor Calculation

A known concentration solution of squalene and internal standard was prepared. Mass of 11 mg of each component was accurately measured and dissolved in 15 ml toluene. Prior to GC injection, the solution was diluted: 200 μl solution with the addition of 800 μl toluene. This procedure is required to measure the recovery of squalene against the internal standard. The corrected value of squalene concentration calculated was defined as correction factors CF. This solution was analysed in different time intervals during the research project along with sample analysis to assure accuracy of the method (Westerman, 2004). Typical squalene chromatogram is given in Figure 3.1.

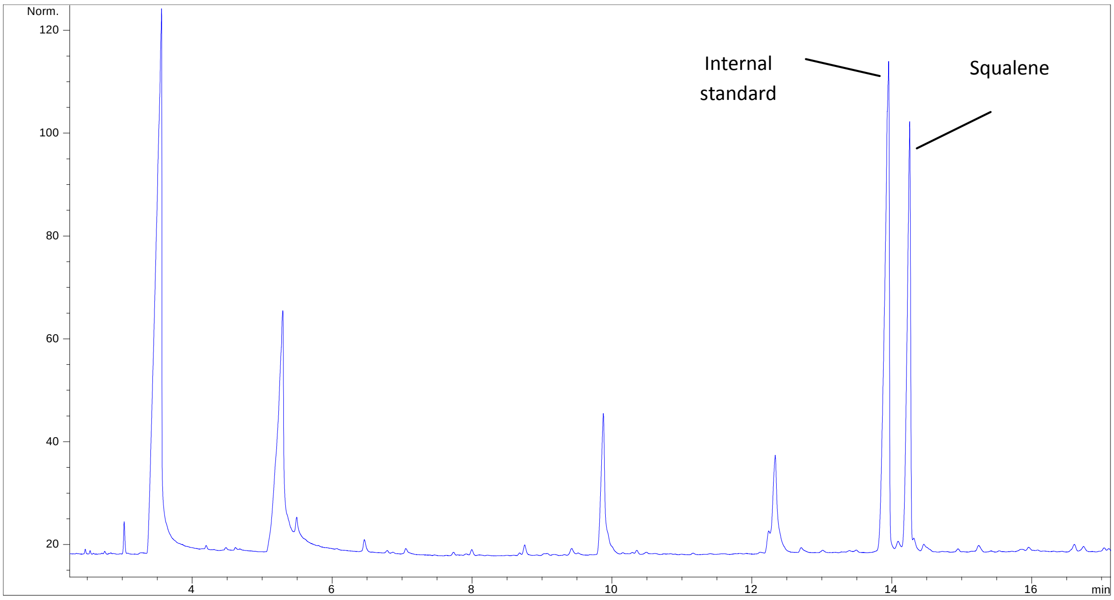


Figure 3.1: Typical GC chromatogram showing peaks of internal standard and squalene.

Internal standard solution stock was prepared with concentration of ~ 50 mg 5-α-cholestane /5 ml toluene. Typically 200 µl of the solution was added to ~25mg sample for squalene expected to be at 5 wt% to 20 wt%. In few samples, mainly raffinate sample of the extraction experiments, this value was decreased to be 100 µl due to the low squalene content. Internal standard solution stock was kept to a maximum period of one month in the fridge with temperature of 2 – 4 °C to avoid change in solution concentration due to solvent evaporation.

3.3.1.3 Sample Preparation and Squalene Content Calculation

Approximately 20 to 25 mg of oil sample (samples from solubility studies and extraction experiments) was accurately weighed and recorded. PFAD samples were heated at temperature of 40 °C before measurements in order to liquefy the butter-like PFAD samples. Then, 200 µl of internal standard solution was added followed by 3 ml toluene. The GC used was equipped with split mode of sample injection and no further dilution is required. Squalene content in the sample was calculated based on the following equation Eq. 3.1:

$$Squalene\ wt\% = \frac{Peak\ Area\ of\ Squalene}{Peak\ Area\ of\ IS} x \frac{IS\ Mass}{Sample\ Mass} x \frac{100\%}{CF} \ldots\ldots\ldots\ldots\ldots Eq. 3.1$$

Where Peak Area is the area under the curve of components determined from the GC, IS is stands for the internal standard and CF is the correction factor.

3.3.2 Analysis of FFA Content via GC

FFA content of model mixtures and PFAD samples were measured by direct carbon number GC with Heptadecanic acid as internal standard. FFAs cannot be directly injected to the GC system due to their high melting point. Thus, Silylation of FFA was essential to determine their content. In order to ensure total removal of high molecule weight of triglycerides from the capillary column, total analysis run interval was extended. Complete elution of these compounds was necessary to avoid analytical error. The total run time was 53 min.

3.3.2.1 Carbon Number GC Conditions

The FFA concentrations were measured using the same GC and column mentioned above for squalene analysis but using different conditions. The oven temperature program was 100 °C for 3 min, 10 °C/min to 150 °C, 5 °C/min to 250 °C, 10 °C/min to 350 °C, with a 15 min holding time. The GC was used with split mode, and Helium was used as the carrier gas.

3.3.2.2 Internal Standard Preparation

A known concentration sample of FFA mixture was used to determine the correction factors CF of each FFA component. In this work, this sample was mainly PFAD because the concentration of FFA was known. This sample was prepared and analyzed along with other sample analysis with unknown FFA to assure accuracy of the method.

Internal standard solution stock was prepared with concentration of ~ 50 mg Heptadecanic acid /5 ml toluene. 200 µl (2 mg internal standard) of the solution was added to ~25mg sample. Internal standard solution stock was kept to a maximum period of one month in the fridge with temperature of 2 – 4 °C.

3.3.2.3 Sample Preparation and FFA Content Calculation

Approximately a drop of oil samples (approximately equal to 20 to 25 mg) was accurately weighed and recorded. Then, 200 µl of internal standard solution was added followed by 1 ml Chloroform as solvent. For Silylation of FFA, 1ml of Hexamethyldisilazane (HMDS) was added to the sample solution. Solution then was heated at temperature of 50 to 60 °C for 50 minutes (Alenezi et al. 2009). Sample solution was then left to cool down to room temperature prior to GC injection. Each of FFA component content in the sample was calculated based on the following equation Eq. 3.2:

$$FFA\,wt\% = \frac{Peak\ Area\ of\ FFA}{Peak\ Area\ of\ IS}\,x\,\frac{IS\ Mass}{Sample\ Mass}\,x\,\frac{100\%}{CF} \ldots\ldots\ldots\ldots..Eq.3.2$$

Where Peak Area is the area under the curve of components determined from the GC, IS is stands for the internal standard and CF is the correction factor. Typical FFA chromatogram showing FFA main components present in PFAD is given in Figure 3.2. FFA are palmitic acid C16:0, linoleic acid C18:2, oleic acid C18:1 and stearic acid C18:0.

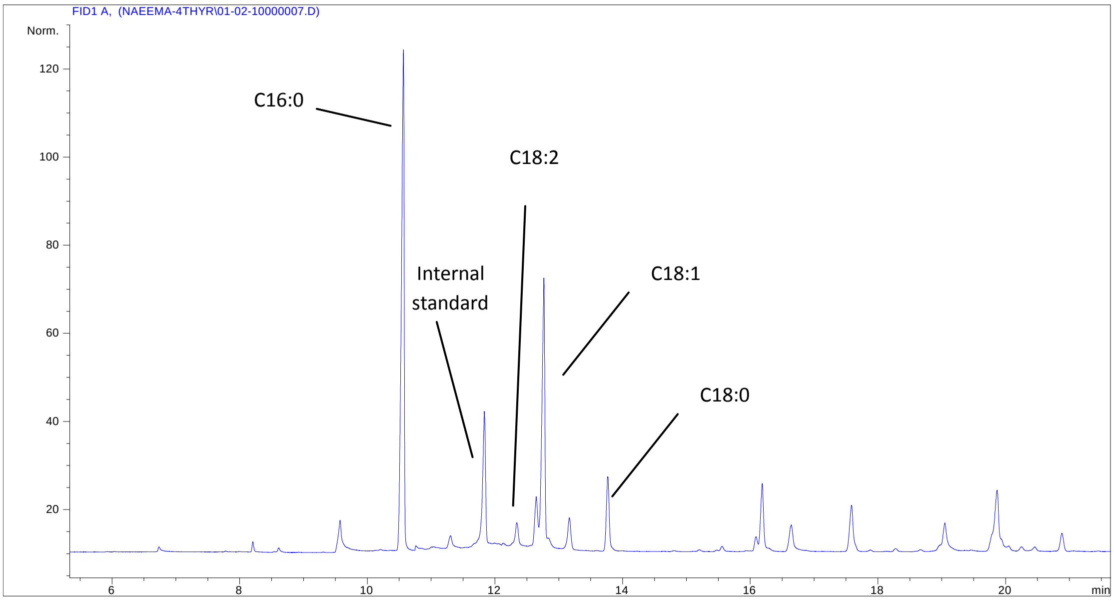


Figure3.2: Typical GC chromatogram showing peaks of internal standard and main components of PFAD; palmitic acid C16:0, linoleic acid C18:2, oleic acid C18:1 and stearic acid C18:0.

3.3.3 Analysis of FFA Content via Titration

Titration is a well know method used for the measurement and quantification of total organic acids. In this method weight percentage of FFA was calculated on oleic acid basis. Sample oil was weighed in a flask and recorded, 50 ml of ethanol was added to the sample and sample was allowed to completely dissolve in the solvent. Phenolphthalein indicator was added to the solution. Titration of sample solution was performed using sodium hydroxide solution until a permanent pink colour was observed (Wan et al. 2007). Each sample was titrated in duplicate. Calculation of FFA content by titration is based on the following equation Eq. 3.3:

$$FFA\% = \frac{V\ x\ N\ x\ 28.2}{m} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..Eq.3.3$$

Where *V* is sodium hydroxide volume, *N* is normality of the titration solution, and *m* is the mass of the sample. FFA concentration measurements via titration were compared to FFA concentration measured by GC, see Figure 3.3 below. Both methods give strong correlation of $R^2=0.98$, therefore both methods are acceptable for the measurements of FFA concentration in PFAD samples. The main advantage of GC is the capability of the equipment to identify the quantification of individual FFA presented in the oil sample compared to titration. Titration analytical method can identify only the total FFA presented in the sample. Few samples were analyzed for FFA content via titration and GC analysis was skipped in order to measure total FFA.

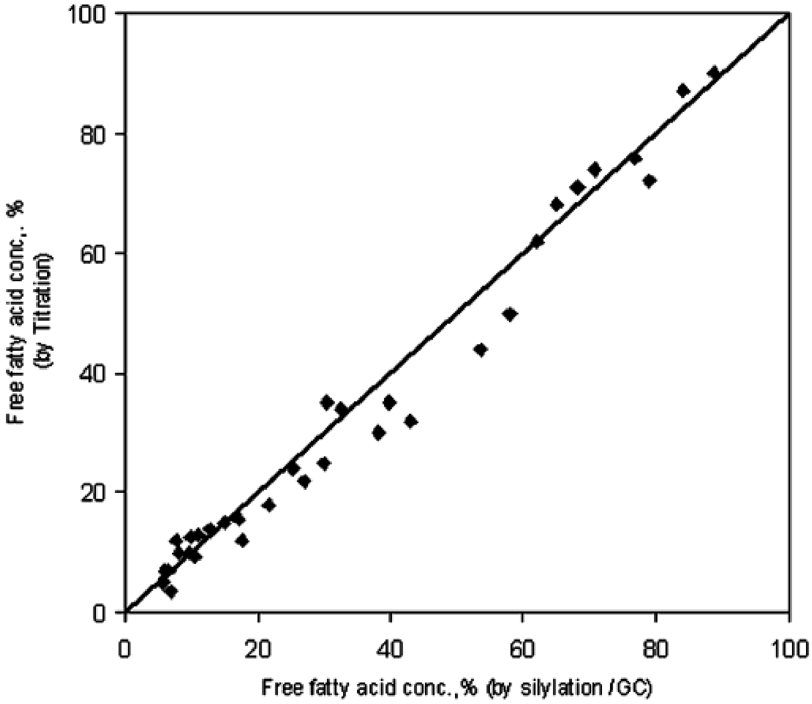


Figure 3.3: FFA analysis comparison between GC method and titration (Alenezi, 2010).

3.4 Samples Storage

Each chemical were stored based on Material Safety Data Sheet MSDS provided by the manufacturer. Stocks of 2 and 2.5 litres of PFAD samples were provided from IOI Group-Loders Croklaan as requested. Samples in use were stored in fridges available in the laboratory at temperature between 2-4 °C. The rest of PFAD samples were stored in cold room provided by the School of Chemical Engineering. Stock solutions prepared for analytical purposes were not stored for long period, solution kept for maximum one month. All samples (Chemicals and/or PFAD) stored at low temperature environment were kept in the laboratory until they reached room temperature before start working.

Chapter 4

Solubility Measurements of Liquid Lipid Based Components in Sc-CO2

4.1 Introduction

Solubility measurements are essential for the development of supercritical fluid extraction and fractionation processes. In order to find the optimum conditions of a separation process it is necessary to know the solubility of the components of interest in supercritical fluid. It provides information about the capacity of the solvent, the selectivity of the solvent, and the dependence of solvent properties on conditions of state, pressure and temperature. If this information is known, a good prediction can be made about whether the separation is possible (Brunner, 2005). Solubility of a substance at given solvent strength (given temperature and pressure) is determined by finding the mass of solute saturated in 1 kg solvent.

This chapter explores the flow type method used in this work for the determination of PFAD main lipid components solubilities at several solvation strength of Sc-CO2. Followed by a full presentation of the results in section 4.4 of this chapter, binary solubility studies, ternary solubility studies and quaternary solubility studies are discussed in section 4.4.1and 4.4.2, respectively.

4.2 Objectives

This study has three main objectives. The first objective is to determine the solubility of individual component; squalene, α-tocopherol, oleic acid and palm olein in Sc-CO_2 under certain conditions of pressure and temperature. The second objective is to determine the solubility of the multi-component system of squalene and palm olein as ternary system. The third objective is to study the effect of the presence of FFA (as oleic acid) on the solubility of squalene in Sc-CO_2, and to provide an analogue to assess the likeliness of these components to dissolve in Sc-CO_2.

4.3 Methodology

4.3.1 Solubility Measurement Rig

Solubility measurements were carried out using a dynamic flow-type apparatus, this method was adopted by various researchers (Li et al. 2009, Jin et al. 2005, Markum et al. 2001, Sovova et al. 1997). It is capable of operating at a maximum pressure and temperature of 30 MPa and 343 K, respectively. The dynamic flow-type methods consists of four main stages; sample preparation and loading, system pressurization, solvent-sample contact and depressurization/solute collection. Each stage will be discussed in details as following. Solubility flow diagram is shown in Figure 4.1. Solubility apparatus illustrated schematically in Figure 4.2.

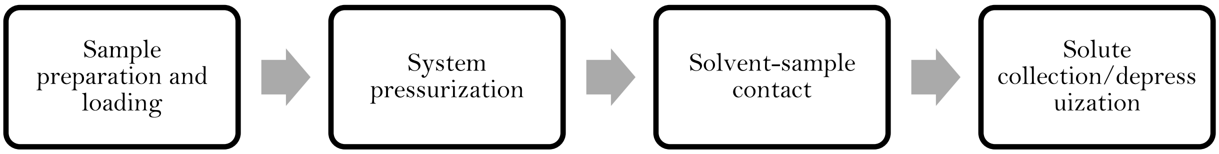


Figure 4.1: The 'four S' flow diagram of the solubility measurements.

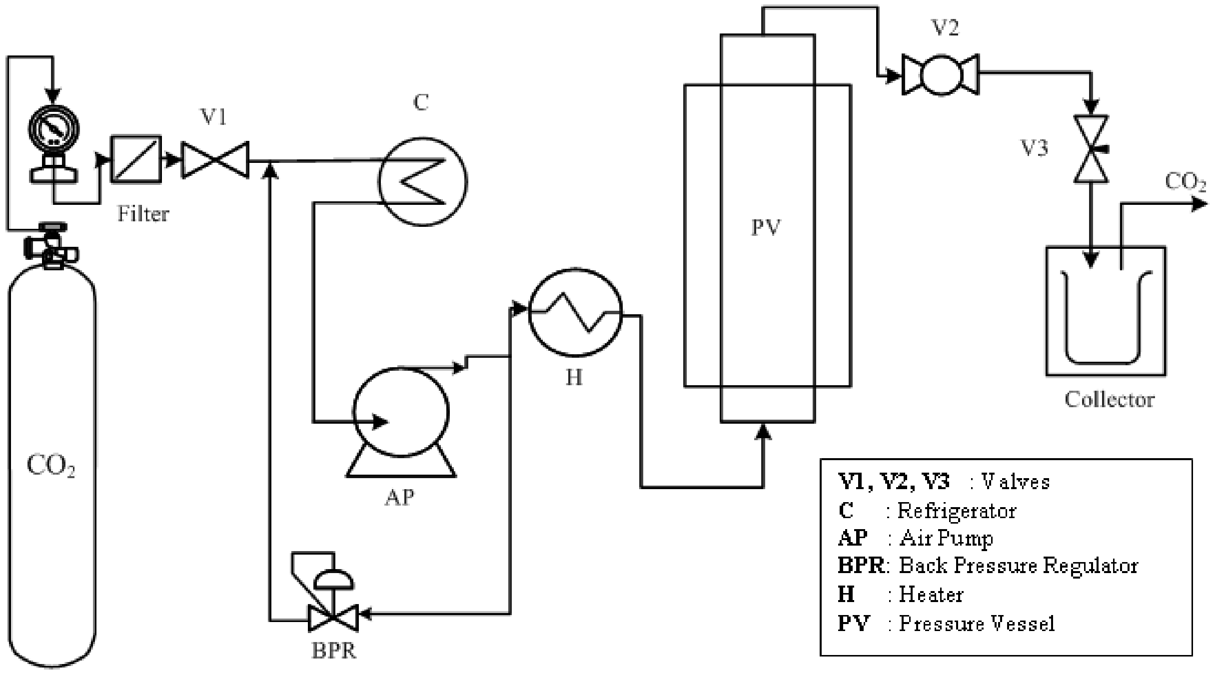


Figure 4.2: Schematic diagram of dynamic flow type solubility apparatus.

4.3.1.1 Sample Preparation and Loading

All of the lipid samples (either pure component or mixture) were at liquid phase at room temperature. More information about the lipid components and the preparations of the lipid mixtures were discussed in section 3.2.1 of Chapter 3; Materials and Analytical techniques. The sample is loaded in a special liquid sample holder designed to insure solvent-sample contact. Sample holder consists of three pieces made of glass material connected to each other as shown in Figure 4.3.

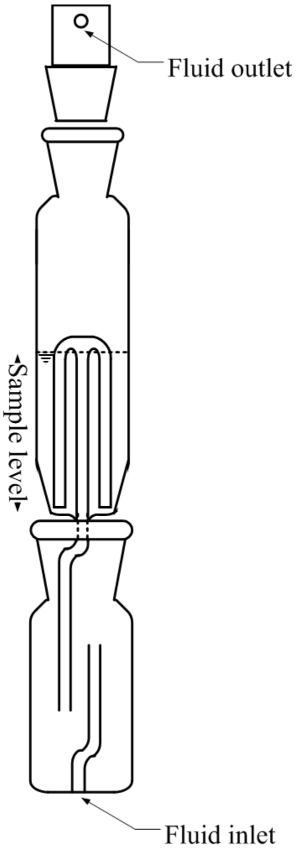


Figure 4.3: Sample holder

The middle section with a capacity of 15ml is where the liquid sample is held. During the run, carbon dioxide passes through the small diameter m-shape tubes (~3mm diameter) in the middle section and develops small bubbles for better contact area with liquid sample. The bottom section is designed to prevent any liquid leakage to the system solvent streams during the run with easy solvent access. The top part is filled with glass wool to trap carried liquid sample (not-dissolved in CO2). The glass sample holder is placed in to another stainless steel holder, illustrated in Figure 4.4, which is finally fitted in the pressure vessel. The system then is ready for solvent delivery.

Figure 4.4: Sample holder

4.3.1.2 System Pressurization and Solvent Delivery

Liquid carbon dioxide CO2 from a cylinder was fed at pressure of 5 MPa to a refrigerated heat exchanger (C) maintained at approximately 0 to 5 °C, through a non-return valve (Autoclave Engineers, TWO4400) and an inline 50 μm filter (Nupro, SS-4TF Filter), which was fitted before the pump to maintain the CO2 in the liquid phase to avoid cavitations. CO2 was pumped via air driven pump AP (Teledyne Fluid Systems, model, 4F64) to the desired process pressure above its critical pressure. The distance between the refrigerator and the pump was kept to the minimum with the feed pipe covered with insulating material to avoid vaporization of liquid CO2 . Compressed Air was regulated and adjusted to control the stroke rate of the pump via air regulator valve (Wilkerson CB6-C4-F00). The system pressure was maintained by adjusting a back pressure regulator BPR (GO-66, 0-6000 psig) and excess CO2 was recycled through a recycle loop back to the low pressure feed point of the pump. To avoid compressed CO2 from returning to gas supply, a non return valve was fitted in the line. The compressed liquid CO2 was preheated through a thermostatically controlled shell and tube heat exchanger (H) with hot water via water bath (Grant Technical Specification, LtD 6G), thus attaining the desired process temperature (above critical temperature). At this point Sc-CO2 is attained prior to the pressure vessel.

4.3.1.3 Pressure Vessel and Solvent-Sample Contact

Sc-CO2 then entered the 500 cm^3 pressure vessel PV contained Liquid sample holder, where phase equilibrium was achieved. The vessel was heated using water jacket thermostatically controlled via water bath (Grant Technical Specification, LtD 6G). A closure gate valve and bursting disk are connected to the stream prior to the vessel inlet. The temperature in the PV was measured using J type thermocouple at several points. One thermocouple probe was fitted in the inlet solvent stream to the PV, and a further two were mounted in the top of PV and the existing stream of the PV. All thermocouples were connected to a digital temperature display. While the system pressure was monitored using a pressure transducer with a digital display. Accuracy of pressure measurement is +/-0.05 MPa and that for temperature is +/- 0.1 K.

4.3.1.4 Depressurization and Solute Collection

Sc-CO2 was contacted with the sample in PV by bubbling through the liquid phase and was, thus saturated by the sample. The solute laden CO2 stream was then depressurised across a closure gate ball valve (V2) into a first collection vessel (Baskervilles Ltd.) fitted with heated micro-metering needle valve (V3) to maintain the pressure at 0.8 – 1 MPa (8 – 10 bars). Solute was separated from CO2 and was collected in a glass beaker placed in this vessel, as shown in Figure 4.2. The exiting stream of PV was insulated and the collection vessel was heated to prevent formation of dry ice during expansion, thus avoid needle valve blockage. For protection of collection vessel, a valve was connected to it for pressure relief. The glass beaker fitted in the collection vessel was filled with glass wool to prevent solute lost within the collection vessel. In order to avoid dry ice formation, the collection vessel was maintained at conditions above triple point of CO2 where the phase changes are from supercritical phase to liquid and gas phases. The exiting solvent stream was passing through another solute trap at atmospheric pressure through another micro-metering needle valve. The second trap is a U-shape trap placed to capture mainly the volatile components; it was filled with glass wool and immersed in dry ice. The solute free CO2 left the solute trap was measured by passing through a flow-meter (U.G.I METERS LTD, model, 804741) and finally vented off from the laboratory. Subsequently, the extract was weighed and analysed. Measurements were made at flow rate range of 0.6 to 0.9 kg.hr-1 where there was no effect on the observed solubilities found. In order to ensure CO2 saturation, loading measurements were carried out at various solvent flow rates ranged between 0.3 and 1.3 kg.hr-1. The system is deemed to have reached equilibrium when no further change was recorded in the solute concentration with the CO2 flow rate, see Figure 4.5 below.

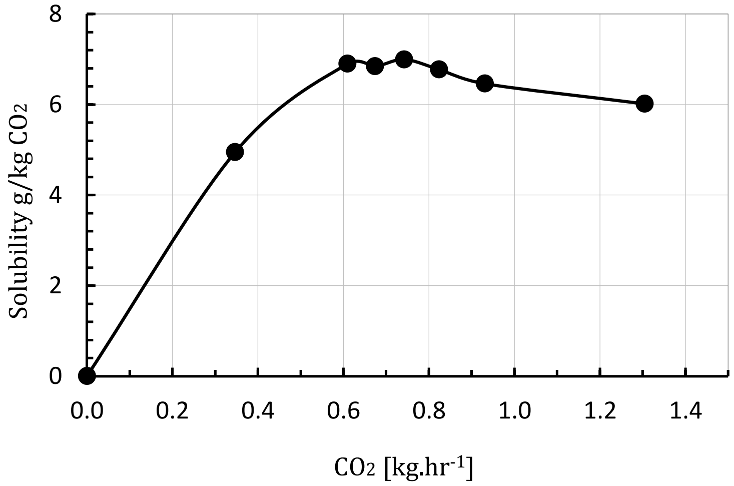


Figure 4.5: Loading of vegetable oil at different solvent flow rate at pressure of 15 MPa and temperature of 313 K.

Equilibrium was achieved at solvent flow rate of 0.6-0.9 kg.hr^{-1} at which the solvent was saturated with the solute and considered as solubility measurements. As the solvent flow rate was increased (above 0.9 kg.hr-1), the solubility was decreased due to the insufficient contact time to reach saturation.

4.3.2 Calculations

The following are the equations used for solubility calculation and they are applied to all solubility measurements. While conducting a solubility experiment, certain data were recorded which are used later to carry out the main calculations.

4.3.2.1 Gas consumption calculation

The CO2 flow rate used during extraction experiments was between 0.6 to 0.9 kg.hr-1 and was calculated by the following calculation procedure (equation 4.1). Atmospheric pressure and laboratory temperature were taken to account; the ideal gas equation was used:

$$\dot{m}CO_2 = \frac{\dot{V}\,P}{R\,T} \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq\ (4.1)$$

Where $\dot{m}$ stands for mass flow rate of CO2 in g.h^{-1}, $\dot{V}$ is CO2 volumetric flow rate measured during the experiment expressed in Litres per hour, P is the atmospheric pressure in atm, T is room temperature in Kelvin, and R is gas constant 0.08206 L.atm. K^{-1}.mol^{-1}.

4.5.2.2 Solubility Calculations

Solubility S (g.kg^{-1}) of a certain component i in Sc-CO2 was presented in two forms, first as mass (g) of component i dissolved in one kg of solvent (Sc-CO2), as stated in equation 4.2. Also, it was calculated as mole fraction of component i, y_i, expressed in equation 4.3.

$$S_i = \frac{m_i\,(g)}{m_{CO_2}(kg)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.Eq\ (4.2)$$

$$y_i = \frac{moles\ of\ component\ i\ (n_i)}{total\ moles\ of\ solution\ (n_{(i+CO_2)})} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..Eq(4.3)$$

4.5.2.2 Standard Deviation Calculations

Each run of solubility measurements at certain conditions of state, was reproduced to and N number of times and variation of the results were calculated via standard deviation StDEV, see equation 4.4. The results were represented in the figures as error bars.

$$\text{StDEV} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots..Eq(4.4)$$

Where, (x_1, x_2,..., x_N) are the observed values of solubility at each run of N, and $\bar{x}$ is the mean value of these solubility observations.

4.4 Results and Discussions

4.4.1 Solubility of Binary Systems of Pure Solutes

In order to understand the solubility behaviour in multi-component systems, it is first essential to investigate the solubility of the binary systems of the component. The experimental solubility of the binary systems of α-tocopherol (αT/Sc-CO2), oleic acid (OA/Sc-CO2), squalene (SQ/Sc-CO2), and pseudo-component palm olein (PO/Sc-CO2) were measured in the temperature range of 313-353 K and pressure range of 10-30 MPa. To our knowledge, the solubility of the binary system of pseudo-component palm olein in Sc-CO2 has not been reported in the literature. The solubility results in mole fraction for each component in the binary systems are presented in Table 4.1, Table 4.2, Table 4.3 and Table

4.4 respectively, with each point being the average of a minimum of three measurements. Density data of Sc-CO2 is obtained from NIST web-book.

Solubility isotherms for the four main components are illustrated in Figure 4.6 – Figure 4.9. Solubility isotherms for lipid components follow similar trends of increasing with rising pressure at constant temperature. Solubilities of oleic acid and tocopherol at temperature of 313 K is higher than that at higher temperatures at pressure lower than 25 MPa, while at pressure higher than 25 MPa the effect was reversed. At pressure higher than 25 MPa, the increase in vapour pressure of oleic acid and α-tocopherol predominates over the decreasing of solvent power of Sc-CO2. Fang et al. (2004) observed this effect in his investigations on the solubility of α-tocopherol in Sc-CO2, his results shown that solubility isotherms at 353 K is higher than 313 k and 333 K isotherms at pressure above 25 MPa.

Table 4-1: Solubility of oleic acid in mole fraction as function of pressure (MPa), temperature (K) and CO_2 density ρ (kg.m^{-3}).

T (K)	P (MPa)	ρ (Kg.m^{-3})	yx10^{4}
313.15	10.0	628.61	2.57±0.40
	15.0	780.23	7.49±0.27
	20.0	839.81	8.99±0.57
	25.0	879.49	12.2±0.7
	30.0	909.89	14.07±0.40
333.15	15.0	604.09	2.59±0.10
	20.0	723.68	6.25±0.45
	25.0	786.55	19.7±1.7
	30.0	829.71	22.4±1.2
353.15	10.0	221.60	0.04±0.02
	15.0	427.15	0.19±0.05
	20.0	593.89	3.10±0.10
	25.0	686.22	10.9±1.8
	30.0	745.60	22.0±1.2

Table 4-2: Solubility of α-tocopherol in mole fraction as function of pressure (MPa), temperature (K) and CO_2 density ρ (kg.m^{-3}).

T (K)	P (MPa)	ρ (Kg.m^{-3})	yx10^4
313.15	10.0	628.61	1.81±0.40
	15.0	780.23	2.72±0.27
	20.0	839.81	3.65±0.57
	25.0	879.49	5.08±0.69
	30.0	909.89	5.45±0.40
333.15	10.0	289.95	0.09±0.10
	15.0	604.09	1.37±0.45
	20.0	723.68	3.22±1.65
	25.0	786.55	4.32±1.20
	30.0	829.71	5.537
353.15	10.0	221.60	0.06±0.02
	15.0	427.15	0.26±0.05
	20.0	593.89	2.53±0.10
	25.0	686.22	6.05±1.78
	30.0	745.60	10.3±1.2

Table 4-3: Solubility of squalene in mole fraction as function of pressure (MPa), temperature (K) and CO_2 density ρ (kg.m^{-3}).

T (K)	P (MPa)	ρ (Kg.m^{-3})	yx10^4
313.15	10	628.61	3.63±0.23
	15	780.23	8.37±0.99
	20	839.81	11.30±0.33
	25	879.49	14.53±0.95
333.15	10	289.95	0.02±0.01
	17.5	676.38	7.35±0.35
	25.0	786.55	8.35±0.68
	27.0	805.42	11.17±2.14
353.15	10.0	221.60	0.02±0.01
	17.5	505.88	2.69±0.13
	25.0	686.22	8.81±1.05
	27.0	712.61	11.47±0.01

Table 4-4: Solubility of pseudo component palm olein in mole fraction as function of pressure (MPa), temperature (K) and CO_2 density ρ (kg.m^{-3}).

T (K)	P (MPa)	ρ (Kg.m^{-3})	yx10^{4}
313.15	10.0	628.61	0.24±0.06
	15.0	780.23	0.39±0.04
	20.0	839.81	0.58±0.06
	25.0	879.49	1.20±0.20
	30.0	909.89	1.89±0.07
333.15	10.0	289.95	0.02±0.01
	15.0	604.09	0.11±0.01
	20.0	723.68	0.57±0.05
	25.0	786.55	0.68±0.11
	30.0	829.71	1.25±0.16
353.15	15.0	427.15	0.03±0.01
	20.0	593.89	0.23±0.02
	25.0	686.22	0.99±0.05
	30.0	745.60	1.04±0.21

Whereas the solubility isotherms of squalene and palm olein at 313 K is higher than 333 K and 353 K over the pressure range investigated. Nevertheless, at pressure of 25 MPa, the effect of temperature is nearly negligible over the solubility of squalene. At constant pressure, the solubility decreases with increasing temperature. This observation is in agreement with studies found in the literature on the solubility of squalene by Catchpole and Kamp (1997) and Brunner et al. (2009).

At the conditions studied the Sc-CO2 solubility of pseudo-component palm olein is low compared to other lipid components. In view of the fact that pseudo-component palm olein consists mainly of triglycerides, and it was determined that Sc-CO2 has very low affinity towards triglycerides (Markom et al. 2001).

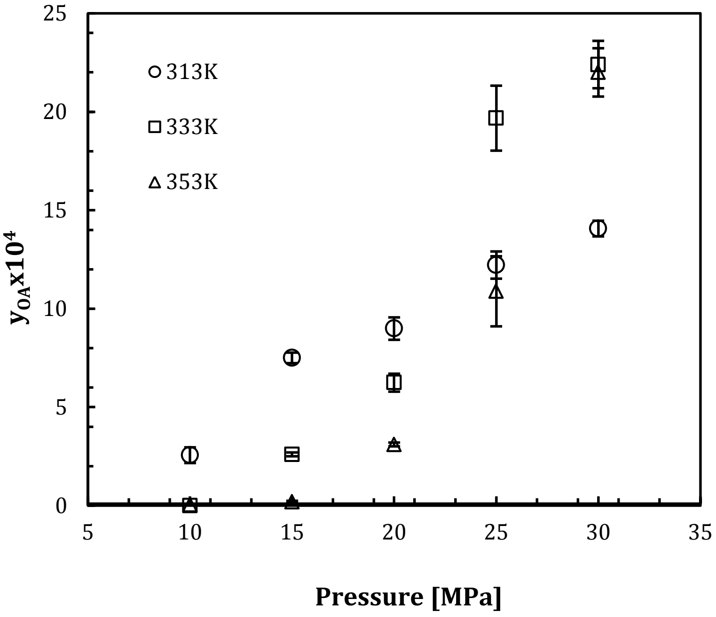


Figure 4.6: Solubility isotherms of oleic acid as function of pressure at temperatures of 313 K, 333K and 353 K.

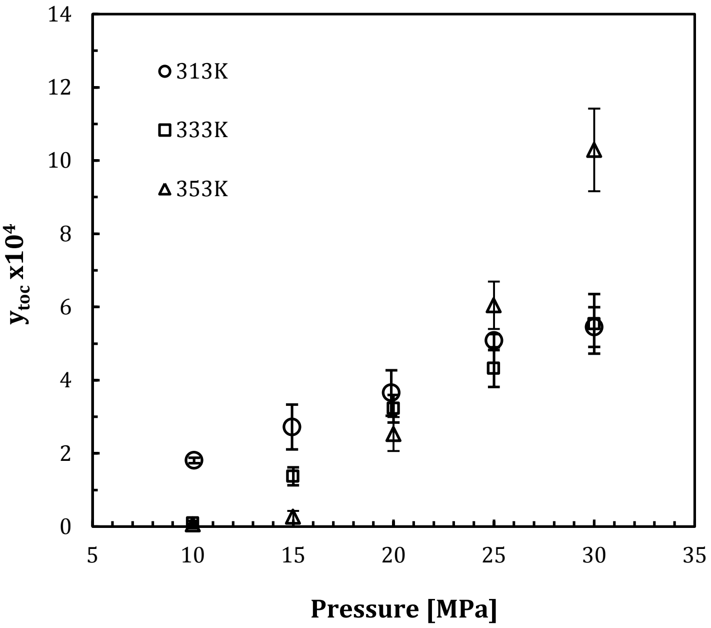


Figure 4.7: Solubility isotherms of α-tocopherol as function of pressure at temperatures of 313 K, 333K and 353 K.

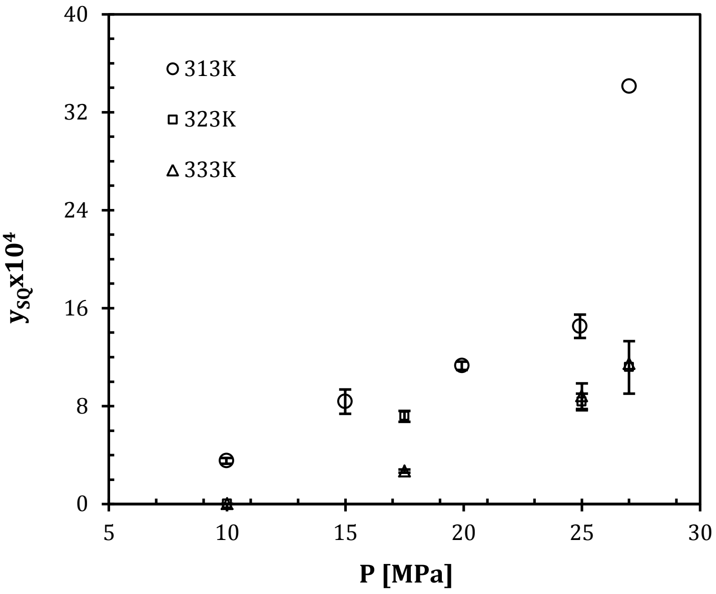


Figure 4.8: Solubility isotherms of squalene as function of pressure at temperatures of 313 K, 333K and 353 K.

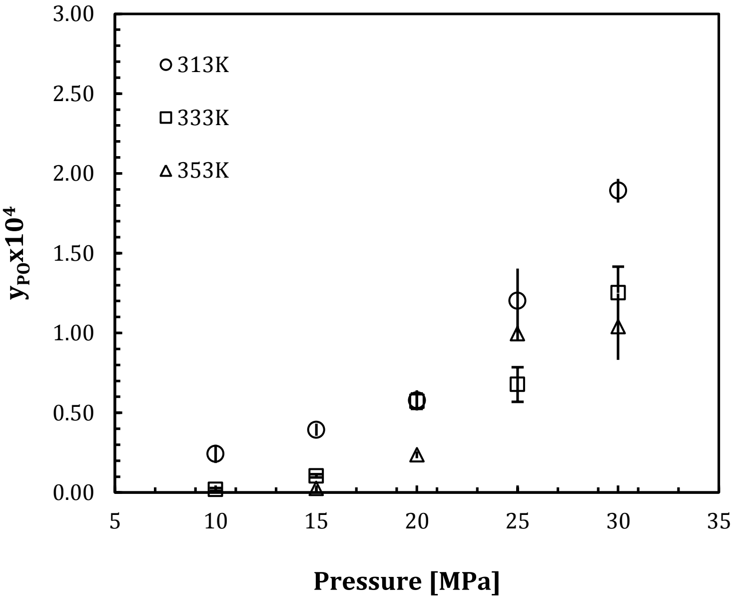


Figure 4.9: Solubility isotherms of pseudo component palm olein as function of pressure at temperatures of 313 K, 333K and 353 K.

In order to assess the reliability of the equipment and method used, OA solubility data were compared with the values found in the literature (Chrastil, 1982; Maheshwari et al., 1992; Skerget et al., 1995) and presented in Figure 4.10.

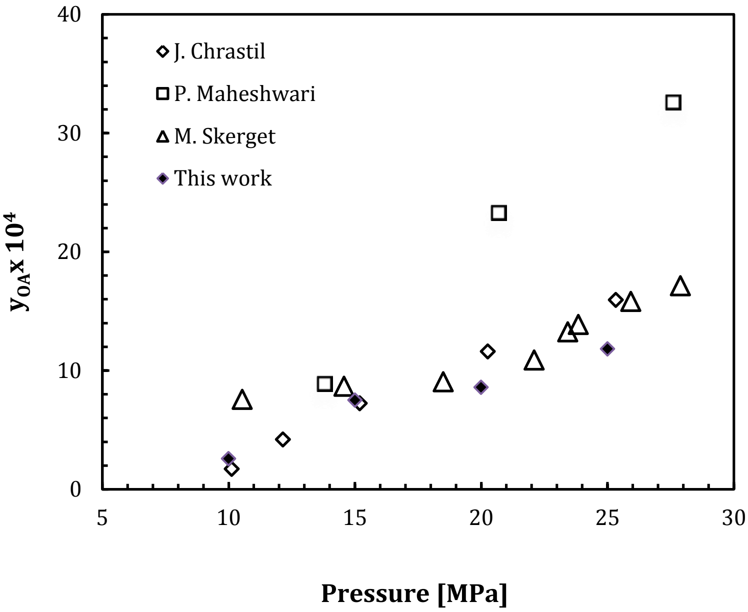


Figure 4.10: Solubility isotherms of oleic acid at 313 K.

The data for oleic acid solubility is in good concurrence with other investigators at pressure range between 10 to 20MPa. However, at higher pressure a discrepancy of approximately 35% was observed with the data obtained by Chrastil and Skerget. The solubility values reported by Maheshwari deviated significantly from the values found by Chrastil and Skerget and the data gathered by this investigation. This may be due to the impurities present in the reagent, which led to either positive or negative effects. Dobbs has found that when small amounts of entrainer are presenting, solubility in supercritical fluids can be affected by several orders of magnitude (Dobbs et al., 1987). The purity of the reagent used in this work was more than 98%. Another possible cause of these discrepancies is the sampling technique used during solubility measurements. During sampling, entrainment of the solute in the vapour phase can be the cause of a high degree of solubility. Similar discrepancies observed with the data of α-tocopherol (Chrastil (1982), Chen et al. (2000), Skerget et al. (2003), as shown in Figure 4.11. There is slight discrepancy between this work and the work obtained by Skerget, while Chen Data showed higher solubility at all pressure range studied compared to others.

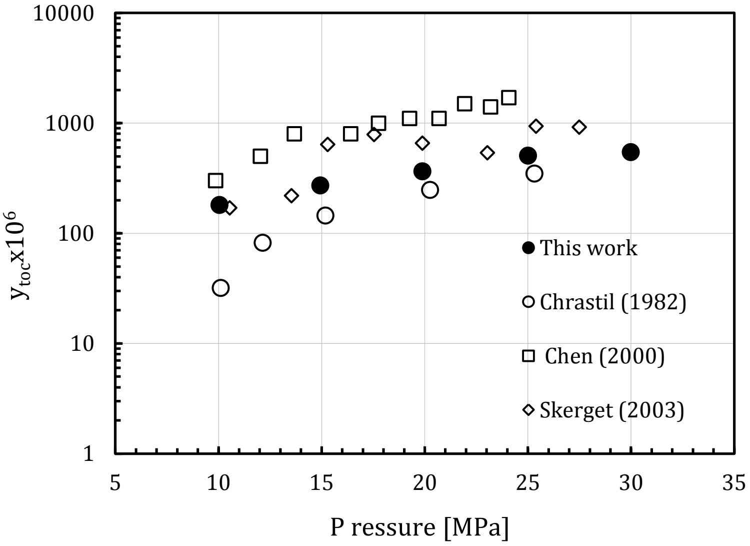


Figure 4.11: Solubility isotherms of α-tocopherol at 313 K.

The data were correlated using Chrastil's density related empirical equation, in order to examine the general trend of solubility behaviour, as follows:

$$\ln S = k \ln \rho_{CO_2} + \frac{a}{T} + b \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.5)$$

In this equation, S denotes the solubility of solute in Sc-CO2 (g.kg^{-1}), ρ is the density of CO2 (kg.m^{-3}), T is the temperature (K), *a* and *b* are constants, and k is the association number. Densities of CO2 were obtained from NIST Web-book. The fit of equation 4.5 to the experimental data is shown in Figure 4.12. There is nearly linear relationship between density of Sc-CO2 and logarithm of solubility of the compounds studied in the region of pressure investigated. Johannsen and Brunner (1997) have found similar trend in their investigation of the solubility of α-tocopherol in Sc-CO2.

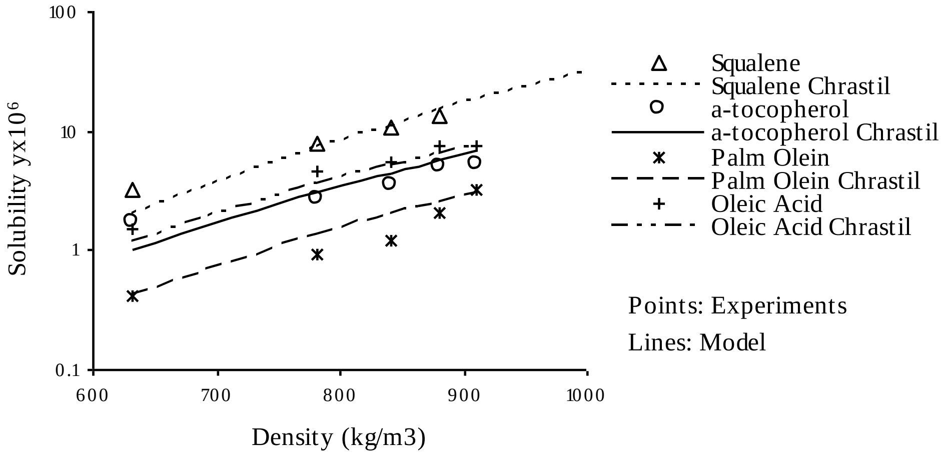


Figure 4.12: Solubility of lipid components as function of density at 313K.

It demonstrates that squalene has the highest solubility in CO2 among the lipid components in this study; while pseudo-component the lowest is shown by palm olein. This result matched expectations, since CO2 in its supercritical conditions is a non-polar solvent which preferably dissolves non-polar components. Squalene is a relatively non-polar hydrocarbon, while a palm olein structure has hydroxyl groups that enhance its polarity making it relatively more soluble in polar solvents. Meanwhile, oleic acid has a hydroxyl group attached to a non-polar chain, with the result that it is less polar than squalene (Reichardt, 2006). Model parameters for each lipid component are given in Table 4.5 with the average absolute relative deviation (AARD), defined as follows:

$$AARD\ (\%) = \frac{100}{n}\sum_{1}^{n}\frac{|S_{cal} - S_{exp}|}{S_{exp}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(4.6)$$

Obtained constants k, b and a were used to calculate the solubility and the calculated solubilities were in good agreement with the measured values with maximum deviation in terms of AARD is 18%, which is lower than the acceptable deviation values of 20% used for empirical equations (Jouyban et al., 2002).

Table 4-5: Model parameters of Chrastil's equation.

Lipid Component	***k***	***a***	***b***	***AARD***
Squalene	6.005	-2543.53	-29.913	12.1%
Oleic acid	5.056	-3566.41	-21.043	14.1%
α-tocopherol	5.209	-3628.39	-21.987	16.4%
Pal Olein	5.079	-731.212	-31.362	18.3%

4.4.2 Solubility of Solute Mixture System

4.4.2.1 Ternary Systems

The majority of the solubility data are presented as binary systems and there have been relatively few investigations into multi-components systems or real systems (refer to chapter 2 for more detailed information on literature found in this field of study). Knowledge of these kinds of complex systems is essential, however, in determining the selectivity of a specific solute presented in a mixture, as well as in understanding the intermolecular interactions in the solvation process. Squalene and other lipid components such as tocopherols and sterols are present in very small concentrations in palm oil refinery by-products. A mixture of 10 wt% squalene and 90 wt% palm olein (SQ +PO) was prepared to determine solubility in Sc-CO2 (ternary system) as function of pressure at a constant temperature of 313 K. The solubility data of the ternary mixture (SQ + PO +CO_2) are compared with their binary solubility data (SQ+CO2, PO+CO_2) are shown in Table 4.6.

Selectivity α is calculated as the ratio of respective mole fractions of squalene and palm olein in the solvent stream. It is clear that when pseudo component palm olein was added to squalene, squalene solubility showed a dramatic reduction, while palm olein solubility increased at pressures above 15 MPa. This finding is in agreement with the study obtained by Ruivo et al. (2007), the higher the content of more soluble component in Sc-CO2 the higher the solubility of the mixture. In this case, the addition of 10 wt% of squalene (more soluble) to palm olein (less soluble) has increased the solubility of the mixture. Ruivo has studied this effect with squalene concentration up to 75 wt%. However, this observation was found over all pressure range studied by Ruivo, while in this work only at pressure above 15 MPa. The reason might be behind the concentration of squalene used in both studies. The concentration of squalene used in this work is less than minimum concentration of 25 wt% used by Ruivo in his study.

Selectivity isotherms for squalene and palm olein as function of pressure are shown in Figure 4.13 and 4.14, respectively. There is significant reduction of squalene solubility in palm olein mixture compared to its solubility in the binary system squalene/Sc-CO2. Solubility diminution of a solute has been observed in various studies when another solute is added, as the vapour pressure of the solute is proportional to its concentration in the mixture, as stated in Raoult's law (Guclu-Ustundag and Temelli, 2007).

Table 4-6: Solubility data of squalene and palm olein in binary systems (SQ/PO +CO_2) and ternary systems (SQ +PO +CO_2) in Sc-CO_2 at constant temperature of 313.15 K.

P (MPa)	**SQ ($y \times 10^5$)**		**PO ($y \times 10^5$)**		**α (y_{SQ}/y_{PO})**
	SQ + CO_2	SQ+PO+CO_2	PO + CO_2	SQ+PO+CO_2	
10	35.35±0.07	7.55±0.09	2.43±0.23	0.33±0.25	23.1
15	83.69±0.12	8.15±0.21	3.94±0.09	3.54±0.38	2.30
20	113.0±0.03	9.96±0.15	5.78±0.02	11.29±0.14	0.88
25	145.3±0.07	12.96±0.11	12.01±0.17	15.14±0.27	0.86

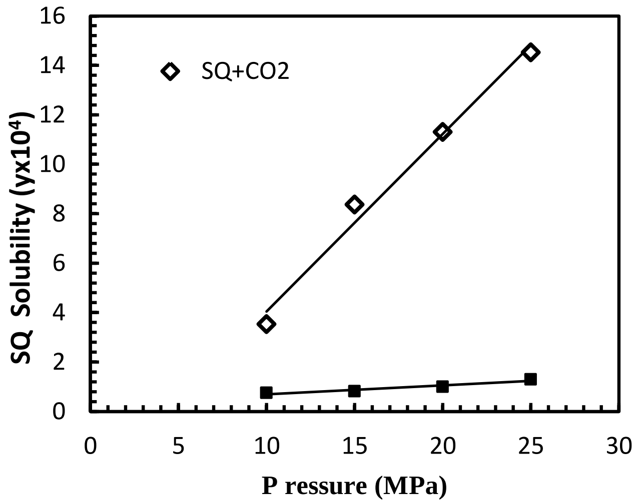


Figure 4.13: Solubility isotherms of Squalene at 313 K for the binary system (SQ+CO_2) and ternary system (SQ+PO+CO_2).

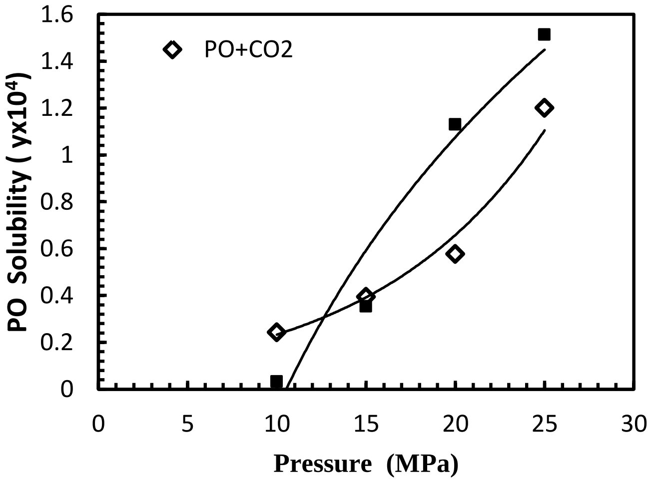


Figure 4.14: Solubility isotherms of pseudo component palm olein at 313 K for the binary system (PO+CO_2) and ternary system (SQ+PO+CO_2).

The behaviour of multi-component systems in Sc-CO2 is poorly understood. These multi-component systems are characterised by further non-ideality and more solute-solute interactions in their system than solute-solvent interactions in binary systems. However, it is possible to highlight that under high pressure the vapour pressure of the highly concentrated palm olein increases, leads to higher solubility, which is aided by the higher interactions with squalene and Sc-CO2 in the ternary mixture leading to further enhanced solubility of palm olein. This finding is in agreement with King et al. (1997) on his study of semi-batch extraction of mixture of glycerides, the solubilities of a component with low binary-solubility increase when it is present in a mixture with other component with high binary-solubility with Sc-CO2; in this case it is squalene. This is unlike the afore-mentioned, behaviour of squalene which exists at a much lower concentration. Although squalene solubility decreased dramatically after the introduction of palm olein into the system, it still dominated the extract composition at the lower pressure range (less than 15 MPa) as the selectivity shows its highest value at low pressures. This indicates that a one-step separation of squalene with a concentration of 90 wt% at a pressure of 10 MPa at temperature of 313.15 K is favoured.

It is clear that when a higher pressure is used, CO2 will dissolve more palm olein which effectively dilutes the concentration of squalene in the solvent phase. Catchpole reports similar results, finding that the selectivity toward squalene is best at lower pressure. Solubility data were predicted using Peng Robinson equation for mixture system (Catchpole and vonKamp, 1997).

4.4.2.2 Quaternary systems

Since FFA exists at a relatively high concentration in the deodorisation distillate of palm oil compared to squalene, the solubility behaviour in this work was studied in the presence of FFA for the quaternary systems (SQ + PO + OA + CO_2). The concentration of squalene was kept the same as in the ternary system at 10 wt%, with addition of 25 wt% OA (as FFA) and 65 wt% palm olein under the same conditions. The solubility data of the quaternary system SQ +PO +OA +CO_2 are provided in Table 4-7.

Table 4-7: Solubility data of squalene, palm olein and oleic acid in ternary systems (SQ+PO +CO_2) and quaternary systems (SQ +PO + OA+CO_2) in Sc-CO_2 at 313.15 K

P (MPa)	**SQ ($yx10^5$)**		**PO ($yx10^5$)**		**OA ($yx10^5$)**	**α**		
	SQ+PO+CO_2	SQ+PO+OA+CO_2	SQ+PO+CO_2	SQ+PO+OA+CO_2	SQ+PO+OA+CO_2	y_{SQ}/y_{PO}	y_{OA}/y_{SQ}	y_{OA}/y_{PO}
10	7.55±0.09	4.06±0.03	0.33±0.25	0.85±0.37	5.68±0.19	4.78	1.40	6.69
15	8.14±0.21	10.01±0.13	3.54±0.38	2.49±0.48	24.59±0.11	4.02	2.45	9.86
20	9.96±0.15	12.12±0.05	11.29±0.14	7.70±0.13	29.90±0.06	1.57	2.46	3.88
25	12.96±0.11	12.99±0.13	15.14±0.27	10.23±0.04	32.11±0.10	1.27	2.47	3.14

The concentrations in gaseous phase of the quaternary mixture of 10 wt% squalene, 25 wt% oleic acid and 65 wt% palm olein showed that oleic acid has the highest solubility in this quaternary system. As oleic acid is more soluble than squalene and palm olein, the overall amount of solute per kg CO2 increased. Squalene solubility shows relatively modest change in the presence of oleic acid in the quaternary system when compared to that in the ternary system, while the solubility of palm olein (the least soluble compound) decreased. Furthermore, the selectivity of squalene clearly reduced in the quaternary system. Raising the pressure the solubility of palm olein increased, causing a decrease in the concentration of both squalene and oleic acid in the gaseous phase. The results indicate that the presence of small concentrations of oleic acid in the feed lead to selectivity enhancement of squalene than that with higher concentrations of oleic acid. Selectivity of squalene in the ternary and quaternary systems as function of pressure is shown in Figure 4.15.

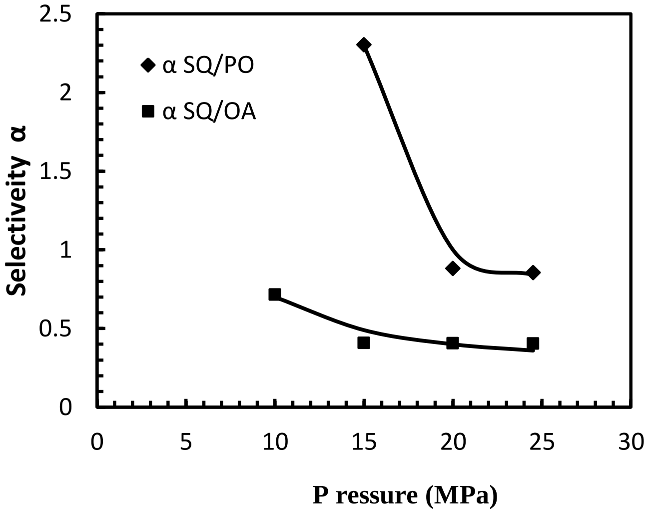


Figure 4.15: Selectivity of squalene in SQ+PO ternary mixture and SQ+OA+PO quaternary mixture as a function of pressure at 313 K.

Similar trends have been reported in the literature for different lipid systems. Where the presence of FFA is studied, FFA is enriched in the vapour phase resulting in selectivity of squalene being decreased, which indicates poor separation between oleic acid and squalene

(Simoes and Brunner, 1996; Ruivo et al., 2007). In order to investigate the distribution of lipid components in both gaseous and liquid phases, the distribution coefficient *Ki* of each component was calculated using equation (4.7):

Ki =yi/xi..Eq. 4.7

Here y_i is the mass fraction of component *i* in the gaseous phase and x_i is the mass fraction in the liquid phase. Distribution coefficients of squalene in the ternary system and quaternary system are presented in Figure 4.16. Distribution coefficients are higher than 1 in both systems which gives the indication that squalene is enriched in the gaseous phase. In ternary system, distribution coefficient of squalene is almost double of that in quaternary system. This result shows the importance of the consideration of the composition of the whole mixture when the behaviour of individual component is discussed (Stoldt and Brunner 1999).

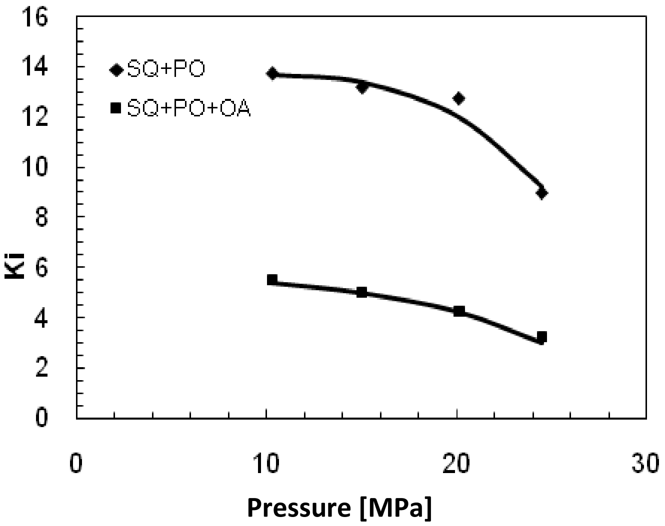


Figure 4.16: Distribution coefficients of squalene in SQ+PO+CO_2 ternary system and SQ+OA+PO+CO_2 quaternary system as function of pressure at 313 K.

Both squalene and FFA distribution coefficients were found to be higher than 1, therefore both components can be enriched in the gaseous phase. The distribution of squalene decreases with pressure, while that for Oleic acid has insignificant increase with the pressure, as shown in Figure 4.17. Similar trends were found in the study of ternary system of oleic acid and squalene by Ruivo et al. (2007).

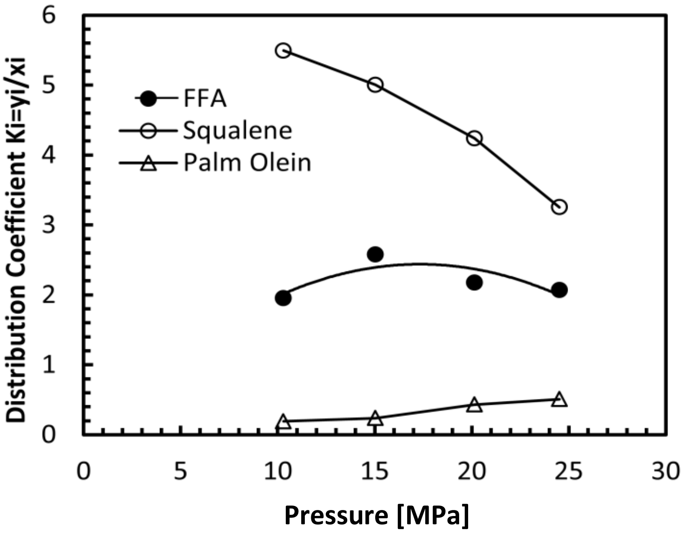


Figure 4.17: Distribution coefficients of squalene, FFA and palm olein as function of pressure at 313K in ternary system.

It is also clear that squalene was more enriched than oleic acid. Squalene was enriched in the gaseous phase, reaching its maximum value at lower pressure, and decreasing with increasing pressure. In contrast, palm olein was enriched in the liquid phase. Comparable trends were found by Gast et al. (2005) for the determination of solubility of complex mixtures of crude palm oil and soy oil deodoriser distillate. As shown in Figure 4.18, squalene and FFA were enriched in the gaseous phase; while triglycerides were enriched in the liquid phase expressed by distribution coefficient lower than 1. Other components such as tocopherols and sterols have shown relatively low solubility in Sc-CO2 and therefore they were remained in the liquid phase.

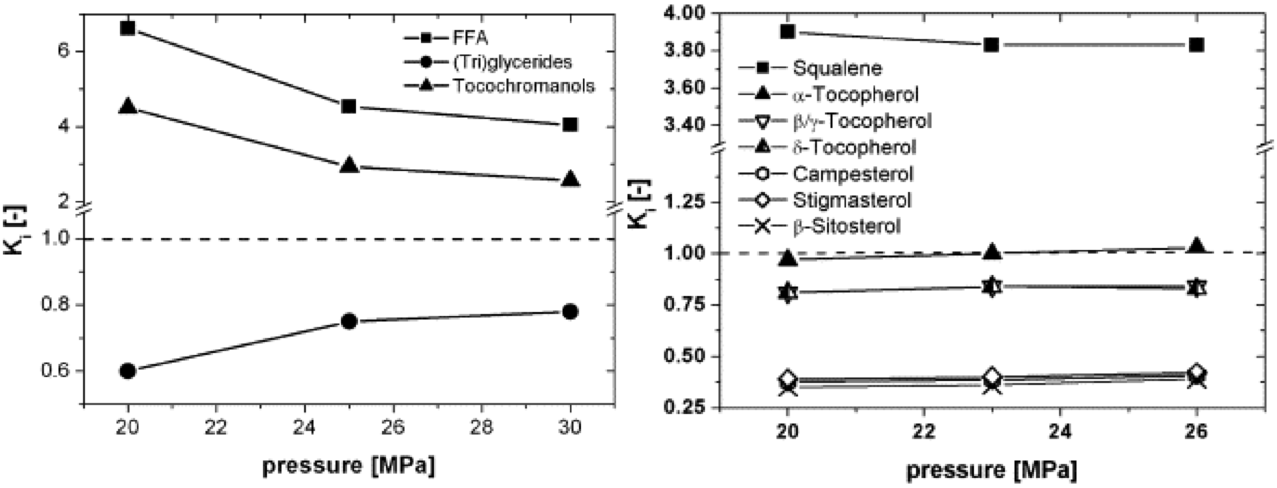


Figure 4.18: Distribution coefficients of lipid components of crude palm oil (Left) and soy oil deodorizer distillate (right) as function of pressure at 340K (Gast et al. 2005).

4.5 Conclusions

Squalene has been shown to have a higher solubility than the other lipid components in the binary systems studied due to its hydrocarbon and non-polar nature. Solubility investigation for the ternary and quaternary systems demonstrates the high selectivity of carbon dioxide towards squalene and free fatty acids. The presence of less soluble components, such as palm olein, affects the loading of the whole mixture, especially at a high concentration. The introduction of FFA into the SQ/PO mixture results in a decrease of carbon dioxide selectivity towards squalene. This is likely because the solubility of FFA is higher than the solubility of palm olein in CO_2, which reduces squalene vapour pressure, resulting in low selectivity. Solute-solute interactions have also been shown to affect the solubility in Sc-CO2. In conclusion this study demonstrates the feasibility of separating squalene and FFA from palm olein, by using the Sc-CO2 fractionation process.

Chapter 5

Counter-Current Supercritical Fluid Extraction and Fractionation of PFAD Components Using Sc-CO2

5.1 Introduction

There is paramount interest in the isolation of minor lipid components from their complex mixtures because of their potential health benefits. This work is devoted to study the extraction and recovery of squalene from PFAD mixture via the technology of continuous multistage Sc-CO2 extraction. The principles of the design of liquid-liquid extraction can be applied to the supercritical fluid extraction processes, as they are identical in concept. Liquid-liquid extraction is based on the relative solubilities of the compounds in two immiscible solvents, while in the case of supercritical fluid solvent extraction the separation of compound from a mixture is bases on its preferential solubility in supercritical fluid solvent. Multistage supercritical fluid extraction has several advantages and it can be a promising alternative to distillation and organic solvent extraction. This chapter explores the feasibility of squalene extraction and enrichment from PFAD using Sc-CO2 in counter-current fractionation column as function of different extraction conditions. Full presentation of the experimental apparatus and extraction procedures are stated in section 5.3 of this chapter, followed by a discussion of the main results in section 5.4, where the effect of pressure, temperature and solvent (S) to feed (F) ratio (S/F ratio) on the separation of squalene and FFA is presented. Finally this chapter closing stages are with an overview of the separation efficiency of both squalene and FFA, and final concluding marks.

5.2 Objectives

The overall objective of this chapter is to investigate the extraction and recovery of squalene from PFAD in counter-current extraction column runs isothermally by means of Sc-CO2 as extraction solvent. The investigation is carried out in terms of total yield measurements and concentration of squalene in the extract product, as function of state conditions; extraction pressure and temperature, and S/F ratio. Furthermore, the investigation covered the effect of state conditions on FFA presented in PFAD.

Extraction and fractionation experiments have been carried out at temperature of 313 K-353 K, and pressures between 10 and 20 MPa. Temperature lower than 313 K at pressures above 15 MPa, flooding was occurred in the column due to high solvent density. Sc-CO2 is known for its main advantage of having ambient critical temperature, therefore temperature above 353 K where not studied to avoid sample degradation. At pressure lower than 10 MPa, the solvent density was very low and there was no extraction occurred (extract mass was unquantifiable). The extraction column used in this project was capable to work at maximum pressure of 20 MPa. There is significant increase on Sc-CO2 density at pressures between 10 to 20 MPa. At pressure above 20 MPa, density isotherm showed insignificant changes (refer to Figure 2.21 in chapter 2 of this work; density isotherm as function of pressure).

5.3 Methodology

5.3.1 Counter-Current Fractionation Rig

A schematic diagram of the continuous counter-current extraction process used in this work is illustrated in Figure 5.1. Its maximum operating pressure and temperature were 20 MPa and 373, K respectively. It consists of a separation column, 2 m in height with 11.5 mm I.D., constructed with high pressure pipes. The separation column is equally divided into 4 sections; top three sections were packed with glass beads packing at effective height of 0.5m× 3. The left half a meter of the bottom of the column is packing free for raffinate collection.

Each section was heated individually using heating tape, and connected by cross fittings where thermocouples and feeding connections were installed. A three-channel temperature control unit (workshop, University of Birmingham) was used for maintain the temperature of the high pressure separation column. The column pipe lines were isolated to avoid heat lost.

Before starting extraction experiment, the heating system of the column was first turned on and regulated in order to achieve the desired extraction temperature. The compressed CO2 was passed through heat exchanger (HE 2) and heated to the designed process temperature (Thar Technologies Inc., Model HE) and introduced to the column. Carbon dioxide is introduced continuously between the third and bottom sections of the column via automated piston pump (Thar Technologies Inc., Model P-50) which delivers a constant flow rate. The flow of carbon dioxide enhances the column heating. The pressure inside the column was increased slowly in anticipation of the desired extraction temperature and pressure achievement.

When the operating pressure and temperature were reached, preheated PFAD was pumped to the column by HPLC pump (Waters 510 HPLC Pump) 0.5 m below the top section of the column, 1 m above carbon dioxide inlet. The feed location was chosen based on Fang et al. (2007) study of the effect of feed location on the separation of tocopherols from soybean oil deodorizer distillate. Although higher contact between feed material and solvent accelerates the extraction and makes fractionation process more efficient, there is insufficient height for rectification. Thus, insufficient rectification results in an increase of non-desired components in the top product. In this work, rectification and counter-current were taken in consideration; height of counter-current contact and height of rectification were 1 m and 0.5 m, respectively. The feed pipe line was heated via heating tape to prevent PFAD solidification. Also, the HPLC pump heads were manually heated continuously during the extraction run by means of heating gun.

The solute laden CO2 was depressurized through an automated back pressure regulator (BPR) with heating valve (Thar Technologies Inc., Model ABPR200) to a collection glass vessel with known weight. Subsequently, the extract was weighed and analysed. CO2 used in this work was in once-through mode, and its flow rate was regulated via solvent automated piston pump. The piston pump (Thar Technologies Inc., Model P-50) is designed to deliver up to maximum solvent flow rate value of 50g CO2 per minute. In all extraction experimental runs, a constant volumetric feed flow rate of 0.6 ml/min was used. The feed mass flow rate was calculated with the measured feed density multiplied by the volumetric flow rate. The feed volumetric flow was controlled by observing its level in the graduate cylindrical glass vessel.

Limitations in the available experimental device do not allow the extraction column to be operated with liquid external reflux. The length of experiments time was fixed to be 5 h for each run to ensure system reached equilibrium. However, some extraction runs can take up to 7 hours due to fluctuations on the feed pumping causing delay on time for the system to reach steady state. Starting time of each extraction experiment is the time when PFAD is introduced to the column. Samples of extract (top product) and raffinate (bottom product) were taken and measured by weighing every 1 hour time interval.

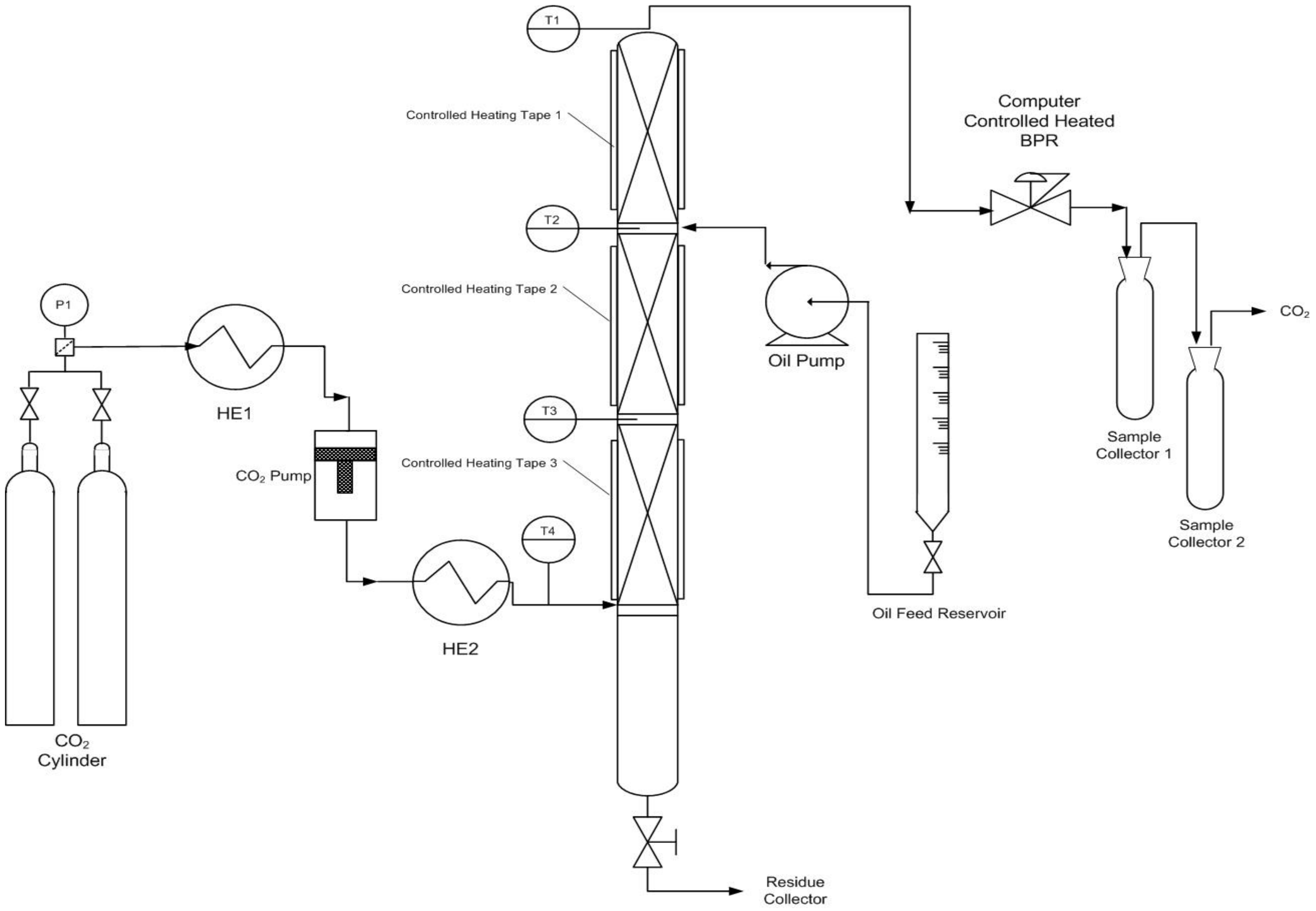


Figure 5.1: Schematic diagram of counter-current extraction and fractionation rig

5.3.2 Materials and Analysis

In this study, PFAD was used as feed material with certain composition of squalene, FFA, tocotrienols and other minor lipid components. More details about PFAD samples were discussed previously in Chapter 3; Materials and Analytical Techniques. The three main streams of the fractionation rig; feed, extract and raffinate were analysed using gas chromatography. Details on the analytical techniques used were discussed previously in Chapter 3.

5.3.3 Calculations

The analysis of counter-current Sc-CO2 extraction feasibility needs information regarding total product yield, product recovery and target component's concentration. During and subsequent to each fractionation run, certain calculations were performed. Throughout conducting an extraction experiment, certain data were recorded which were used afterwards to carry out the main calculations.

5.3.3.1 Overall Material Balance

An overall mass balance was calculated based on the following equation;

$$F = E + R \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (Eq. 5.1)$$

Where F is feed flow rate in g.h^{-1}, E is top product flow rate (Extract) in g.h^{-1} and R is bottom product flow rate (Raffinate) in g.h^{-1}. Furthermore, material balance for each component *i* of study was calculated subsequent to each run;

$$Fx_{iF} = Ex_{iE} + Rx_{iR} \dots\dots\dots\dots\dots\dots. (Eq. 5.2)$$

Where Fx_{iF} is the total mass of component *i* in the feed stream F. While x_{iF}, x_{iE} and x_{iR} are the mass fraction wt% of component *i* in the feed, extract and raffinate streams, respectively.

5.3.3.2 Product Yield and Recovery

Top mass yield of the extraction process, and squalene (or any component, *i*) recovery were calculated based on Eq. 5.3 and Eq. 5.4, respectively as following;

$$Yield\ (\%) = \frac{E}{F}\ \ x100\% \ldots\ldots\ldots\ldots\ldots..(Eq. 5.3)$$

$$Recovery\ (\%) = \frac{E\ x_{i_E}}{F\ x_{i_F}}\ \ x100\% \ldots\ldots\ldots(Eq. 5.4)$$

5.3.3.3 Standard Deviation Calculations

Each run of extraction measurements at certain conditions of state, was reproduced to and N number of times and variation of the results were calculated via standard deviation StDEV, see equation 5.5.

$$\text{StDEV} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots..Eq(5.5)$$

Where, (x_1, x_2,..., x_N) are the observed values of certain response (e.g. concentration, top and bottom mass) at each run of N, and $\bar{x}$ is the mean value of these responses.

5.3.3.4 Experimental Error

The experimental error of each extraction run was calculated based on total mass balance:

$$Error\ (\%) = 100\% - \frac{Extract\ Mass\ (g) + \ Raffinate\ Mass\ (g)}{Total\ Feed\ Mass\ (g)} \ldots\ldots\ldots(Eq. 5.6)$$

5.4 Results and Discussions

5.4.1 Calculation of Minimum Solvent to Feed Ratio

Prior to experimental initiation of the separation of squalene, the minimum S/F ratio was obtained based on the solubility data conducted previously, refer to Chapter 4. The separation experiments should be conducted at solvent flow rate greater to the minimum value to assure solvent saturation. Calculated S/F ratio is shown in Table 5.1 at different conditions of state. The minimum S/F ratio was estimated to be 8.8 g_{CO_2}/g_{Feed}, along for this range of temperatures, pressures and PFAD compositions. Thus, the minimum solvent flow rate used throughout this study was 5 g.min^{-1}.

Table 5-1: Calculated S/F ratios at different conditions of state; for feed flow rate of 0.5g.min^{-1} and squalene feed concentration of 2.2wt%.

T (K)	P (MPa)	Solubility (g_{SQ}/ kg_{CO_2})	M_{CO_2} (g.min^{-1})	S/F g_{CO_2}/g_{Feed}
313	10	3.3	3.5	6.7
	15	7.8	1.5	2.8
	20	10.6	1.1	2.1
	25	13.6	0.9	1.6
333	17.5	6.69	1.7	3.3
	25	7.79	1.5	2.8
	27	10.44	1.1	2.1
353	17.5	2.51	4.6	8.8
	25	8.23	1.4	2.7
	27	10.71	1.1	2.1

5.4.2 Preliminary Experiments

A first set of experiments was performed in order to estimate the time the extraction process when it reaches steady state; when no changes with time take place on outlet streams of the column and the composition of squalene is constant with time. The experiments were performed at temperature and pressure of 313 K and 10 MPa, respectively, with S/F ratio of 15 g_{CO_2}/g_{Feed}. Top and bottom streams were measured in 1hour time interval, along with concentration of squalene, as shown in Figure 5.2, and Figure 5.3. The process reached steady state approximately after 2 hours of continuous extraction.

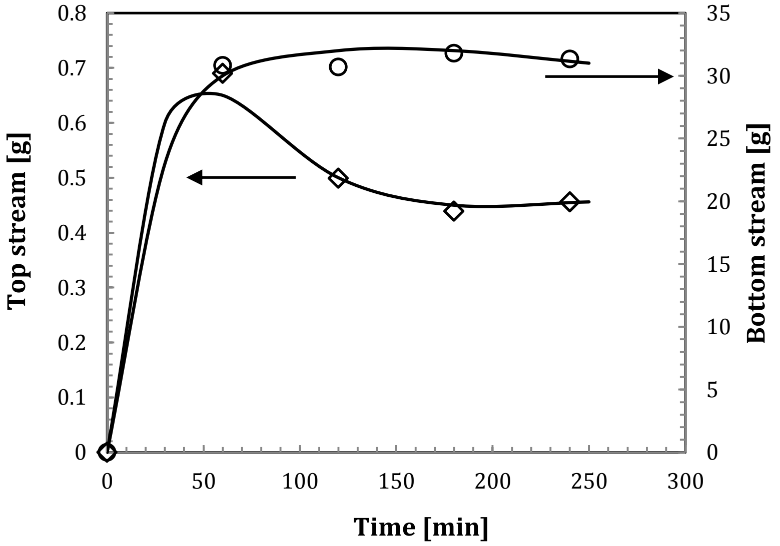


Figure 5.2: Top and bottom stream (g) of extraction process of PFAD as function of extraction time at temperature and pressure of 313K and 10MPa, respectively.

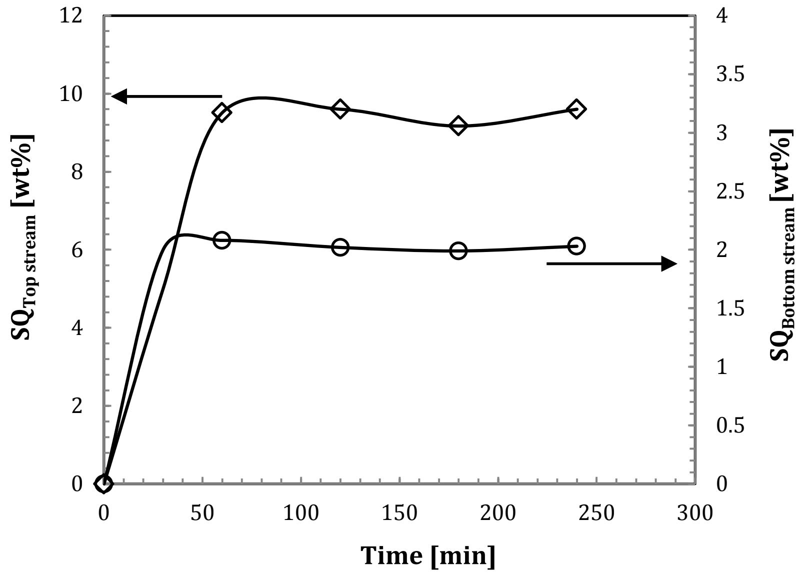


Figure 5.3: Squalene concentration (wt%) in top and bottom stream of extraction process of PFAD as function of extraction time at temperature and pressure of 313K and 10MPa, respectively.

5.4.3 Effect of Extraction Pressure

Fractionation experiments with PFAD were performed at temperatures between 313 and 353 K and pressures between 10 and 18 MPa. The S/F ratio was varied in the range from 10 to 35 g_{CO2}/g_{Feed}. The conditions of the experiments are listed in details in Table 5.2; each run was reproduced at least 3 times.

Table 5-2: Operating conditions of isothermal fractionation experiments.

Test	Pressure [MPa]	Temperature [K]	S/F Ratio [g/g]	Solvent flux [Kg.m^{-2}.h^{-1}]	Mean value of Top Product [g.h^{-1}]	gaseous phase loading with top product [g.kg CO2^{-1}]
1	10	313	15	4661	0.46	0.9680
2	12	313	15	4661	1.21	2.5229
3	15	313	15	4661	2.17	4.5302
4	18	313	15	4661	3.68	7.6576
5	15	323	15	4661	1.01	2.0976
6	15	333	15	4661	0.48	0.9953
7	15	353	15	4661	0.33	0.6840
8	15	333	10	2913	0.25	0.8422
9	15	333	25	7574	0.74	0.9491
10	15	333	35	11070	1.24	1.0868

5.4.3.1 Squalene Separation

Several experiments have been carried out at different extraction pressures in order to investigate the effect of pressure on the purification and recovery of squalene from PFAD with squalene concentration of 2.2 wt% (the value might slightly vary in each feed stock provided). This investigation has been carried out at constant temperature and S/F ratio of 313K and 15 $g_{solvent}/g_{oil}$, respectively. Figure 5.4 shows the total mass yield, squalene recovery and its concentration in the top product as function of different extraction pressures.

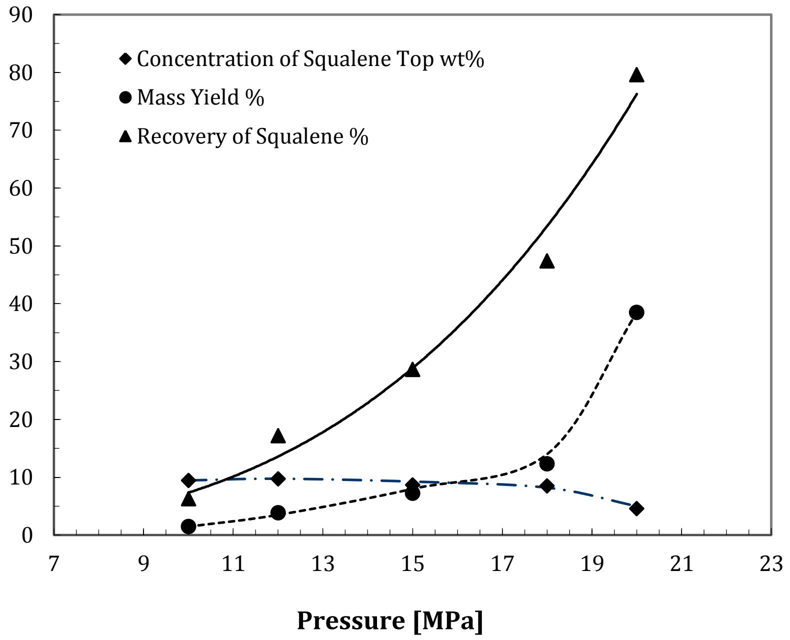


Figure 5.4: Total mass yield %, squalene recovery % and its concentration at the top product wt%: influence of fractionation pressure at constant temperature of 313 K and constant S/F ratio of 15 $g_{solvent}/g_{oil}$.

Results imply that pressure has high impact on the total mass yield as well as the recovery of squalene, both recovery and yield increased with increasing pressure, at 18 MPa and 313 K, squalene recovery and total mass yield of 50% and 15%, respectively. On the other hand, slight decrease on the concentration of squalene (wt% on solvent free basis) with the increasing of the pressure has been observed at pressure between 10 to 18 MPa. However, at 20 MPa squalene concentration showed about 50% decrease compared to its concentration at 10 MPa. This is due to the higher capacity (higher density) of Sc-CO2 with the increase of the pressure, therefore it can dissolve more of other PFAD components at these conditions. This observation is in agreement with the common rule that solubility generally increase with increasing pressure. This trend agreed with the solubility behaviour of ternary system of squalene/palm olein/CO2 performed in this work, selectivity of Sc-CO2 towards squalene inversely related to pressure, refer to Figure 4.15 in chapter 4. At elevated pressures, the solubility of lower volatility components increases more than that of higher volatility components, thus makes the Sc-CO2 less selective (Chao et al., 1993).

5.4.3.2 Free Fatty Acids Separation

The solubilities of triglycerides, di-glycerides and mono-glycerides (Hammam, 1992) in CO_2 are very low compared to FFA and squalene, and they are preferentially recovered in the raffinate. This also has been proved in the first part of this research project; palm olein is more likely to be enriched in the bottom stream, the distribution coefficient K of palm olein was less than 1. Refer to solubility studies on quaternary systems studied previously in chapter 4, section 4.4.2.2. Therefore, this work is focused on the separation of squalene and FFA only as they are enriched in the top stream. The physical properties of FFA and the composition of main FFA are given in Table 5.3.

Table 5-3: Molecular weight, melting point and composition of FFA presented in PFAD.

Common name	C:D	Mw	Melting point[a] [°C]	PFAD (feed) composition wt%
Palmitic Acid	C16:0	256.42	63	14.6
Stearic Acid	C18:0	284.48	71	0.96
Oleic acid	C18:1	282.46	13	7.79
Linoleic Acid	C18:2	280.45	-5	1.73

[a] Found by Rakshit (1981).

Figure 5.4 illustrates the recovery versus the extract concentration of total FFA as function of pressure. Experiments were conducted at constant temperature and S/F ratio of 313 K and 15 $g_{solvent}/g_{oil}$, respectively. Comparable results to squalene recovery, FFA recovery increased with the increase of extraction pressure at constant temperature. However, there is a significant decrease, compared to that for squalene, of the total FFA concentration at the top stream with pressure, the concentration dropped from about 20% with pressure increase of 5 MPa. Detailed concentration and recovery of each FFA component are presented in Figure 5.5 and Figure 5.6, respectively. As expected, extract concentration is highly dependent on the feed composition of each component as they follow similar pattern.

Ziegler stated that solubility of FFA in Sc-CO2 depends on the carbon chain length (molecular weight) of each FFA molecule; the solubility decreases with the increase in carbon chain (Ziegler and Liaw, 1993). The results of distribution coefficient k values (ratio of FFA concentrations of in top and bottom streams) showed a good agreement with this statement, see Table 5-4. Palmitic acid showed the highest separation efficiency compared to other FFA components. In the view of this investigation, concentration of ~50 wt% of FFA can be achieved at pressure of 18 MPa with recovery of ~43%.

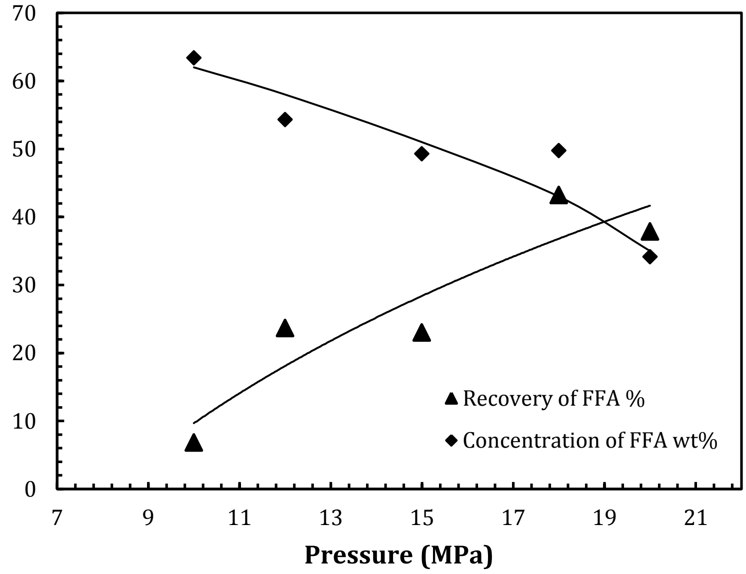


Figure 5.5: FFA Recovery (%) versus its concentration in the extract (wt%) as function of pressure at constant temperature and S/F ratio of 313 and 15 $g_{solvent}/g_{oil}$, respectively.

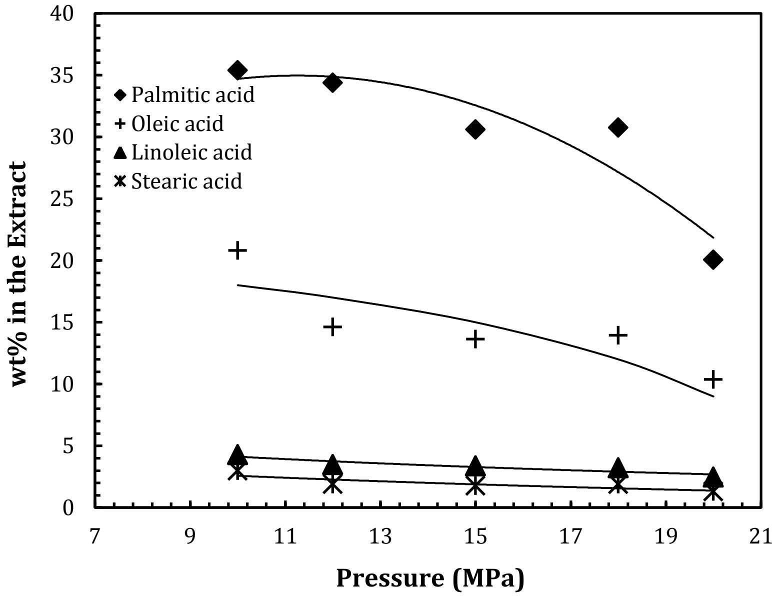


Figure 5.6: Top stream concentrations (wt%) of each FFA as function of pressure at constant temperature and S/F ratio of 313 and 15 $g_{solvent}/g_{oil}$, respectively.

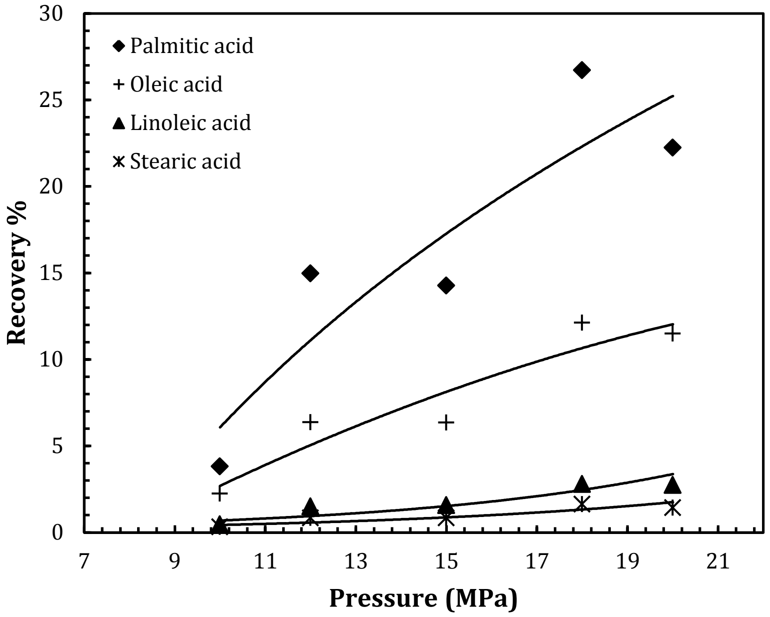


Figure 5.7: Recovery (w%) of each FFA as function of pressure at constant temperature and S/F ratio of 313 and 15 $g_{solvent}/g_{oil}$, respectively.

5.4.4 Effect of Extraction Temperature

5.4.4.1 Squalene Separation

To investigate the effect of extraction temperature, both pressure and S/F ratio were kept at constant value of 15 MPa and 15 gsolvent/goil, respectively. In contradictory to the effect of pressure, increasing temperature leads to decrease the yield and recovery of squalene along with insignificant decrease in the concentration of squalene in the top product, see Figure 5.8. Decreasing extract (top product) yield with increasing temperature has been also reported in the literature for the fractionation of canola oil deodorizer distillate (Guclu-Ustundag and Temelli, 2007) and esterified olive oil deodorizer distillate (Bondioli et al., 1993), at conditions of 343 K to 373 K at 25 MPa and 313 K to 333 K at 13 – 15 MPa, respectively.

Table 5-4: Distribution coefficient K of each component of FFA at different experimental runs; the conditions of each run are presented previously in Table 5.2.

Experiment No.	Ratio of component compositions in top and bottom streams, K									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
C16:0	2.61	2.74	2.52	2.60	2.67	3.03	3.13	2.41	2.98	3.09
C18:0	3.06	2.08	1.99	2.17	2.12	2.25	2.15	2.20	2.20	2.29
C18:1	2.72	2.04	1.97	2.04	1.96	2.10	1.97	1.79	2.05	2.12
C18:2	2.42	2.13	2.11	2.18	2.02	2.11	1.79	1.59	2.04	2.22

Squalene extraction dependence on solvent selectivity shows that with increasing solubility (by increasing pressure and decreasing temperature) selectivity of Sc-CO2 decreases towards squalene. According to the solubility measurement earlier in chapter 4 of this work supported with Hernandez measurements on the solubility of the binary system squalene and CO2, the results obtained showed strong dependence of solubility with pressure. Analogous to the presented work solubility of squalene increases with increasing pressure and decreasing temperature (Hernandez et.al, 2010).

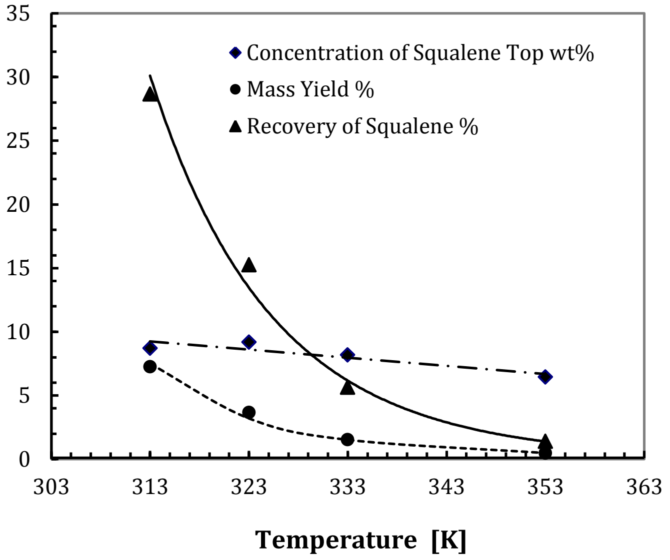


Figure 5.8: Total mass yield %, squalene recovery % and its concentration at the top product wt%: influence of fractionation temperature at constant pressure of 15 MPa and constant S/F ratio of 15 $g_{solvent}/g_{oil}$.

5.4.4.1 Free Fatty Acid Separation

While the concentration of squalene decreases with temperature at constant pressure, FFA showed the opposite effect as illustrated in Figure 5.9. FFA concentration in the top stream increased from ~50 wt% at 313 K to concentration of ~65 wt% at temperature of 333 K. At temperature higher than 333 K, the concentration of FFA showed no difference because of the cross over effect of solubility isotherms where no effect of temperature take place. At higher temperatures the density of CO2 decreases, therefore a reduction in the recovery of FFA was observed. In isobaric separation processes at relatively higher temperatures, Sc-CO2 is more selective to FFA. Similar investigations on FFA separation were obtained from soybean oil (Friedrich and List, 1982; Jokic et al., 2011) at pressures and temperature of 10 to 30 MPa and 313 to 323 K. They found there is clearly no effect of temperature on the concentration of Linoleic acid, Oleic acid and stearic acid.

Looking at the detailed FFAs compositions illustrated in Figure 5.10, comparable findings were obtained. However, comprehensible opposite effect on palmitic acid can be observed. At temperature from 313 K to 323 K at constant pressure of 15 MPa, palmitic acid in Jokic (2011) investigation increased from 13.47 wt% to 13.95 wt%, about 3.5% increase. While in this investigation at exact separation conditions of state, palmitic acid concentration increased from 30.58 wt% to 36.85 wt%, about 17% of increase. One can explain this phenomenon based on the variation of feed composition of palmitic acid in both systems, as PFAD consists of higher palmitic acid compared to soybean in Jokic study (Jokic et al., 2011). However, linoleic acid composition showed no change with temperature at isobaric conditions while it has the highest composition in Jokic system (Jokic et al., 2011).

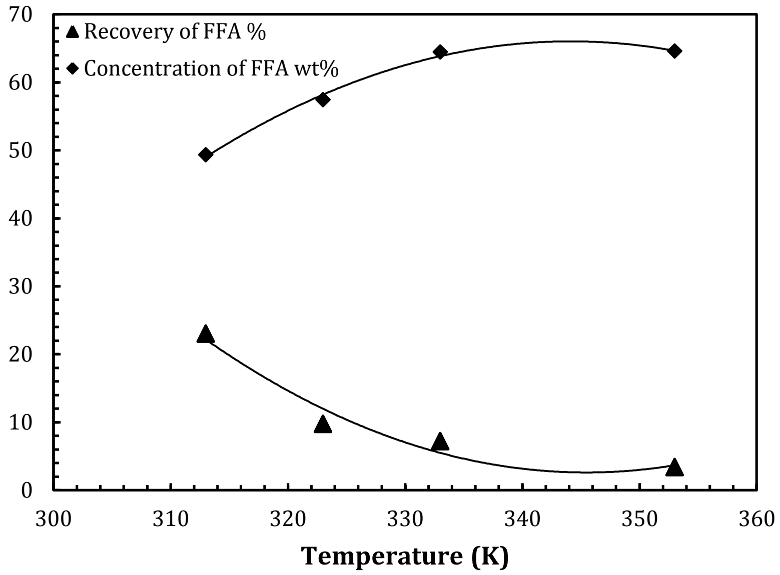


Figure 5.9: FFA total recovery % and its concentration at the top product wt%: influence of fractionation temperature at constant pressure of 15 MPa and constant S/F ratio of 15 $g_{solvent}/g_{oil}$.

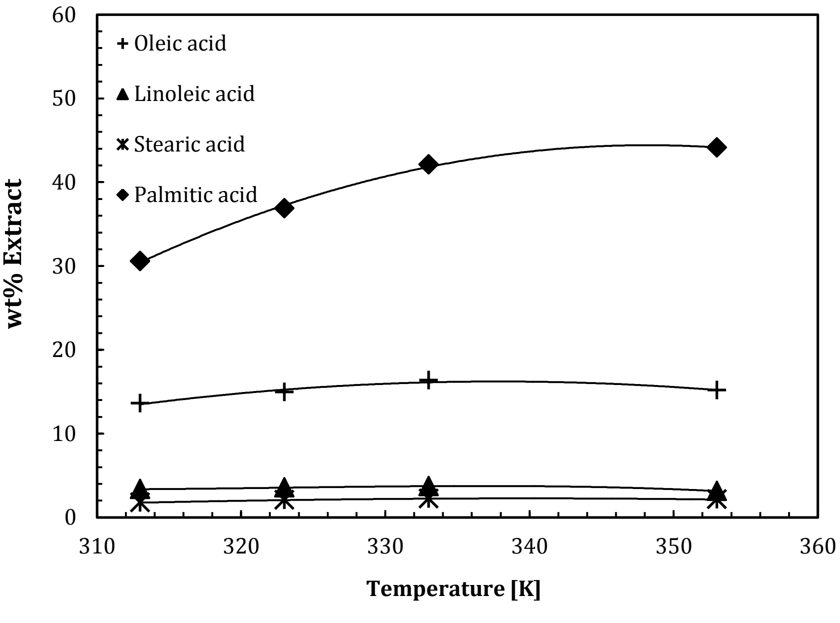


Figure 5.10: Detailed FFA concentrations at the top product wt%: influence of fractionation temperature at constant pressure of 15 MPa and constant S/F ratio of 15 $g_{solvent}/g_{oil}$.

5.4.3 Effect of Solvent to Feed Ratio

The recovery of squalene enhanced with increasing S/F ration from 5 to 35 by factor of 4 as shown in Figure 5.11. Total mass yield increased and squalene purity decreased with increasing S/F ratio. Contrary correlation was observed by Vazquez wt al. (2008) in the study of squalene isolation from esterified deodorizer distillate. This is due to the isolation of squalene in the raffinate, while in this work squalene is recovered in the extract.

There was a limitation on studying higher S/F ratios with column used in order to avoid flooding. Therefore, the upper bond was fixed to 35 S/F ratio. The capacity of Sc-CO2 strongly depends on pressure and temperature, higher pressures and lower temperatures enhance the capacity of the solvent. Recovery of squalene reached up to 50% at pressure and temperature of 18 MPa and 313 K, respectively. With the column used in this study at isothermal conditions, a higher enrichment of squalene was not possible. However, recovery of 50% squalene was achieved.

Analogous effect was observed according to FFA recovery, see Figure 5.12. Recovery increased from ~3% to ~15% with increase of S/F ratio. There is an increase observed on the FFA concentration between S/F of 10 to 20 $g_{solvent}/g_{oil}$. At S/F ratio more than 20 $g_{solvent}/g_{oil}$, no concentration enhancement of FFA at top product was observed. Analogous findings were observed by Dunford and King (2001) on their study on the deacidfication of rice bran oil by Sc-CO2: there was no significant effect on the extract composition with the increase of solvent flow-rate within the range of conditions of state investigated.

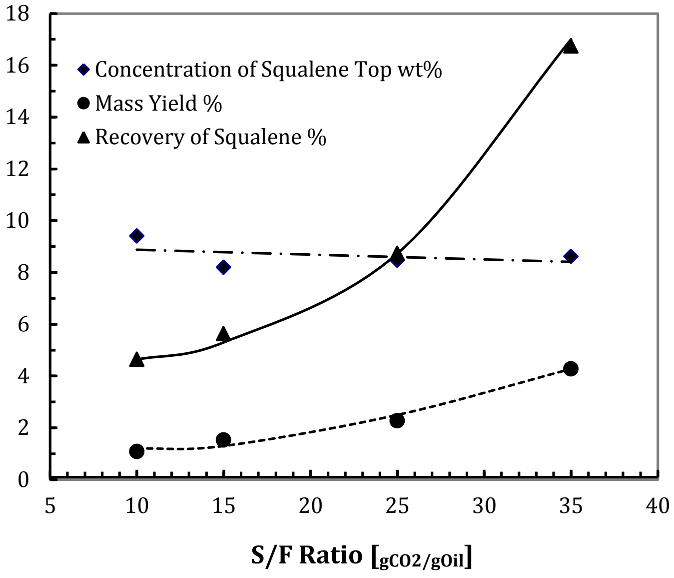


Figure 5.11: Total mass yield%, squalene recovery % and its concentration at the top product wt%: influence of S/F ratio at constant fractionation pressure of 15 MPa and constant temperature 333 K.

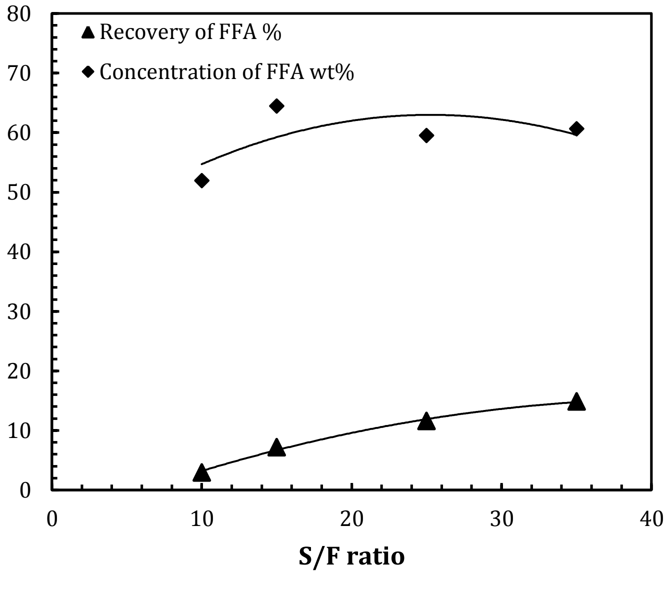


Figure 5.12: Total FFA recovery % and its concentration at the top product wt%: influence of S/F ratio at constant fractionation pressure of 15 MPa and constant temperature 333 K.

5.4.3 Overview of Squalene and Free Fatty Acids Selectivities

The effectiveness of the separation of the column was characterized by the ratios of the concentrations of squalene and FFA components in the top and bottom streams, distribution coefficient k. Distribution coefficients of each FFA component are given in Table 5.4. Figure 5.13 a–c reveals k values for both squalene and total FFA as function of pressure, temperature and S/F ratio. Squalene shows higher separation efficiency compared to that for the FFA at all conditions. At constant pressure of 15 MPa an increase of temperature reduces k value of squalene and gradually increases k value of FFA. This is due to the crossover of FFA solubility at pressure lower than 15 MPa as observed by Chrastil for oleic acid and stearic acid (Chrastil, 1982), while it is above 25 MPa for squalene (Hernandez et al., 2010).

At crossover point, the effect of temperature is negligible. Above the crossover point, solubility increases with increasing temperature. *k* values of squalene and FFA are almost equivalent at temperature of 353 K, which means that the separation of squalene and FFA are not achievable at these conditions. For each FFA component, palmitic acid has higher k value mostly at all conditions tested, as palmitic acid has low molecular mass compared to other FFA, therefore higher selectivity.

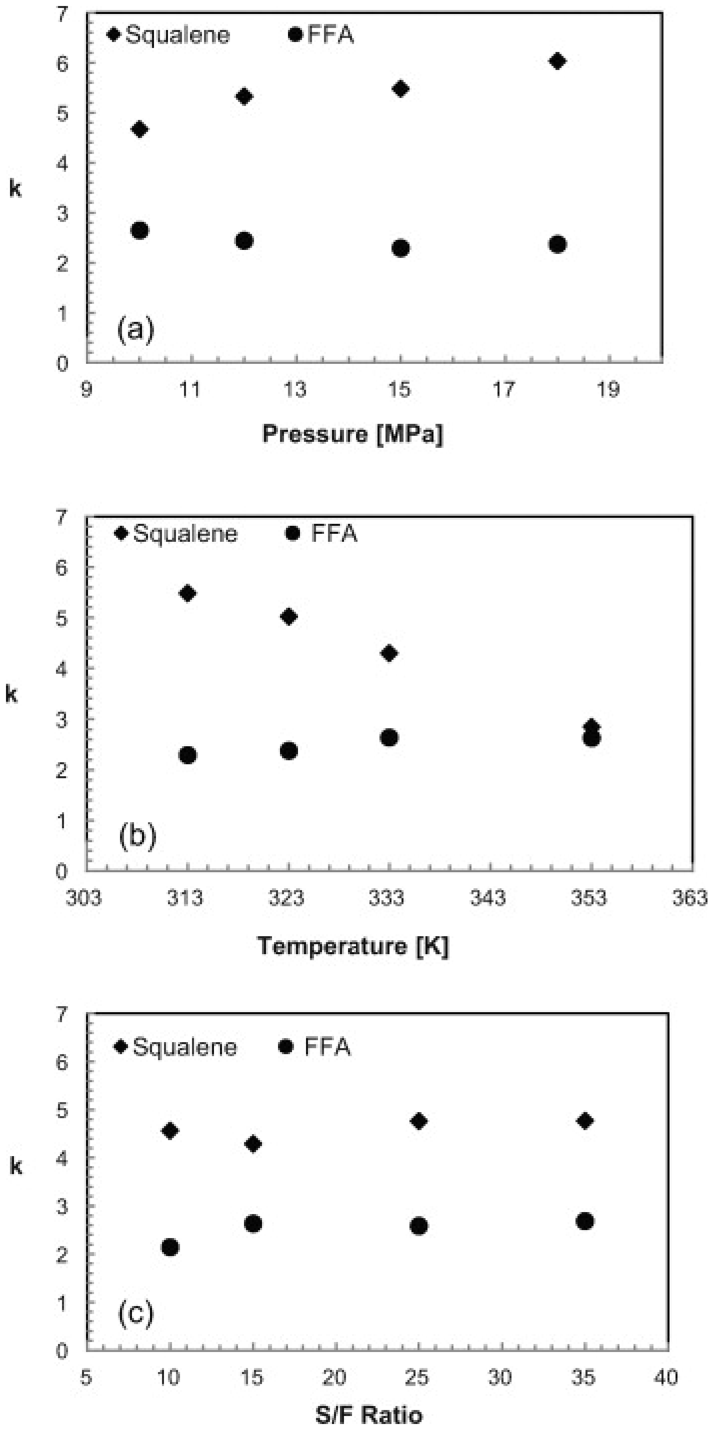


Figure 5.13: **(a)** Separation efficiency, *k*, ratio of squalene and FFA concentration in top and bottom streams as function of fractionation pressure at temperature of 313 K and S/F ratio of 15 $g_{solvent}/g_{oil}$. **(b)** Separation efficiency, *k*, ratio of squalene and FFA concentration in top and bottom streams as function of temperature at fractionation pressure of 15 MPa and S/F ratio of 15 $g_{solvent}/g_{oil}$. **(c)** Separation efficiency, *k*, ratio of squalene and FFA concentration in top and bottom streams as function of S/F ratio at fractionation pressure of 15 MPa and temperature of 333 K.

4.5 Conclusions

Squalene in PFAD could be significantly enriched by means of supercritical fluid fractionation. The process has shown higher sensitivity to pressure and temperature compared to solvent to feed ratio. The optimum conditions at isothermal fractionation experiments were found to be at 18 MPa and 313K with squalene concentration of 8 wt% from a 2.2wt% concentration in PFAD and recovery of 50%. FFA separation has shown diverse trend compared to squalene in the study of the effect of extraction temperature. Higher separation between squalene and FFA can be accomplished at relatively lower temperature at constant pressure. In general terms, Solvent power has an inverse correlation with the selectivity of squalene; therefore Sc-CO2 is less selective at higher extraction pressures. However, there is direct relationship between solvent power and the extraction yield has been observed. This relationship between solvent power and selectivity put in challenges on the developing of extraction processes, an efficient process requires high values of selectivity and total throughput.

Chapter 6

Longitudinal Thermal Gradient Counter-Current Supercritical Fluid Fractionation of PFAD Components Using Sc-CO2

6.1 Introduction

PFAD main components have considerable differences in their physical and chemical properties such as molecular weight and vapour pressures, along with their solubility parameters in Sc-CO2. These differences have a vital role on the distribution coefficient of these components in the liquid and co2 phases. Distributions of these components are also highly affected by the separation conditions such as pressure temperature and external flux ratio. As sighted on the previous study, chapter 5, there is a challenge in supercritical fluid extraction process to keep higher selectivities along with higher throughputs. Selectivity enhancement can be achieved by means of external flux. However, there is limitation of the Sc-CO2 experimental device column used which did not allow the use of the external reflux. In turn, this chapter explores the effect of longitudinal thermal gradient on the recovery of squalene. There is great interest on the study of thermal gradient in supercritical fluid separation processes. Thermal gradient along the column results on density gradient of CO2 within the extraction column where the bottom zone of the column is used for the extraction due to high CO2 density and the top zone is used for fractionation and rectification of high Sc-CO2-affinity components due to the internal reflux phenomena. This chapter contains description of the introduction of thermal gradient on the column used in this study. The effect of thermal gradient was investigated at various separation pressures.

6.2 Objectives

This chapter is aimed to achieve three main investigations; first to study and investigate the effect of thermal gradient along the column on total yield and concentration of squalene in the extract product, as function of extraction pressure. Second objective is to conduct full study on the effect of each temperature zone along the extraction column. Ultimately, the investigation covered the effect of state conditions on other components presented in PFAD; such as FFA components. Extraction and fractionation experiments have been carried out at temperature of 313K- 363K at different column zones, and pressures between 15 and 20 MPa.

6.3 Methodology

6.3.1 Counter-Current Fractionation Rig

A schematic diagram of the continuous counter-current extraction process used in this work is illustrated in chapter 5, Figure 5.1. For Full presentation of the experimental apparatus and extraction procedures refer to chapter 5, section 5.3. For thermal gradient attainment, each section was heated and controlled individually using heating tape. The main zones of the fractionation column where the temperature was measured and controlled are illustrated in Figure 6.1.

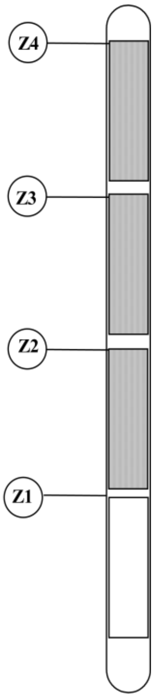


Figure 6.1: Main zones of the fractionation column, from bottom to top, z1 to z4.

6.3.2 Materials and analysis

In this study, the same stock material of PFAD used in the isothermal fractionation studies was used for this investigation. In order to attain full comparison between the effect of isothermal fractionation with that at thermal gradient conditions. More details about PFAD samples were discussed previously in Chapter 3; Materials and Analytical Techniques. The three main streams of the fractionation rig; feed, extract and raffinate were analysed using gas chromatography. Details on the analytical techniques used were discussed previously in Chapter 3.

6.3.3 Calculations

Full details on the calculations carried out during this investigation are presented previously in chapter 5, section 5.3.3.

6.4 Results and Discussions

Pressures of above 15 MPa and S/F ratio of 35 g/g were used since higher selectivity, higher throughputs and moderate yield and recovery were achieved at these conditions; detailed discussions are presented in chapter 5. Different temperature gradient were used for this investigation are illustrated in Table 6.1 and Figure 6.2. ThG1, ThG2 and ThG3 are isothermal conditions to be compared with the thermal gradient conditions. The increase of temperature is linear along the column from bottom to top zones.

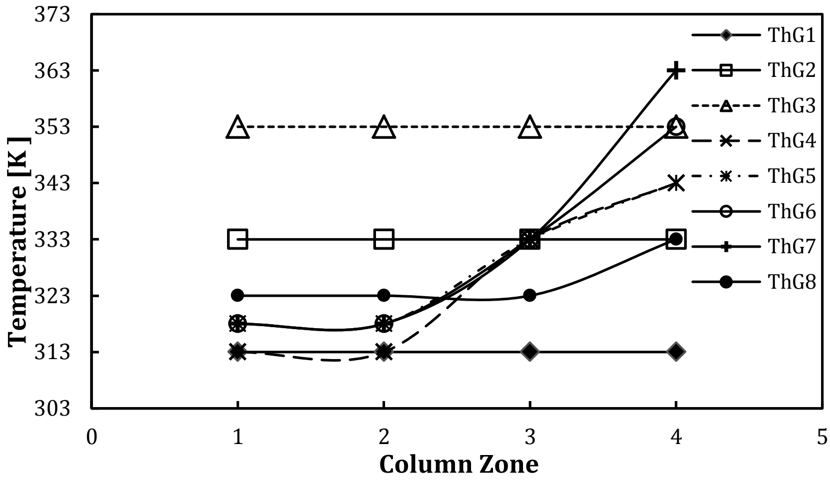


Figure 6.2: Types of temperature profile used.

Table 6-1: Temperature profile used along the column from zone 1 (bottom) to zone 4 (top).

Column zone	**Temperature profile [K]**									
Bottom to Top	**ThG1**	**ThG2**	**ThG3**	**ThG4**	**ThG5**	**ThG6**	**ThG7**	**ThG8**	**ThG9**	**ThG10**
1	313	333	353	313	318	318	318	323	323	323
2	313	333	353	313	318	318	318	323	323	323
3	313	333	353	333	333	333	333	323	323	323
4	313	333	353	343	343	353	363	333	343	353

6.3.2 Effect of Pressure

The effect of pressure under thermal gradient mode was studied at temperature profile of ThG6, by increasing the pressure at constant thermal gradient along the fractionation column, the squalene recovery was increased significantly as illustrated in Figure 6.3. Temperature gradient profiles ThG8, ThG9 and ThG10 are designed to study the temperature of the top zone of the column.

The process shows higher squalene recovery of approximately 90% compared to that at isothermal conditions 50% recovery at 20 MPa. Distribution coefficients of squalene increased significantly when thermal gradient applied to the fractionation column, see Figure 6.4. It is clear that non-isothermal process shows significant improvement in the squalene recovery and concentration. Vazquez studied recently the thermal gradient effect on the separation of fatty acid methyl esters, parallel to this study; there was significant positive effect on the separation observed (Vazquez et al., 2008). Although higher throughputs can be attained at higher pressure, however divergence effect was observed on the extent of squalene concentration at the top stream.

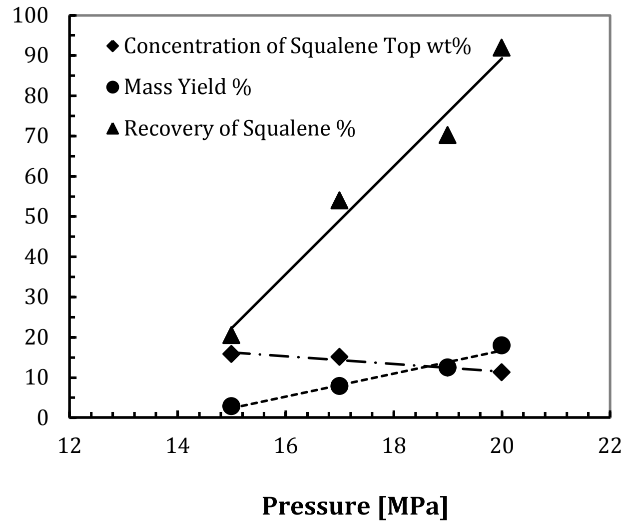


Figure 6.3: Effect of extraction pressure on the recovery of squalene and its concentration in the top stream at temperature profile ThG6.

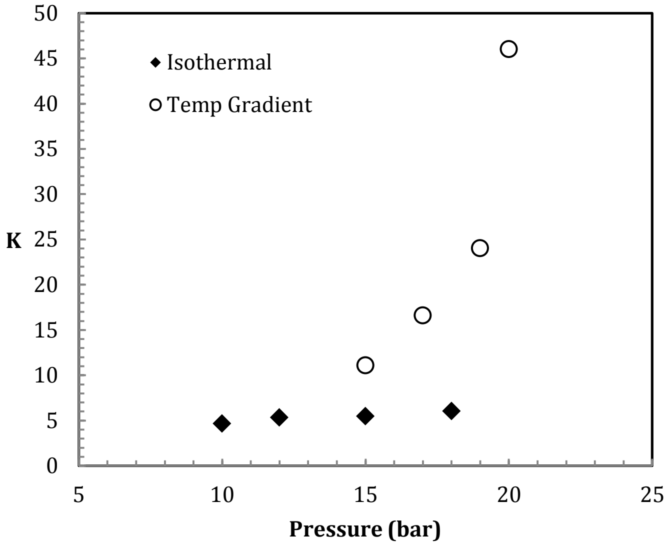


Figure6.4: Distribution coefficients of squalene as function of pressure at the application of isothermal process ThG1 and thermal gradient process ThG6.

6.3.2 Effect of Temperature Profile

Further temperature gradient profiles were tested at pressure of 15 MPa; results are illustrated in Figure 6.5. From the results it is very obvious that changing the temperature over the column stages shows significant increase in the concentration of squalene in the top product, 16 wt% at temperature gradient profile ThG6. Constant temperature profile ThG1 of 313K produced squalene recovery of 27% similar to that at thermal gradient profile ThG4, no significant change on the recovery was observed at 15MPa. Furthermore, isothermal condition at 313K appears to give higher top mass yield than other temperature profiles. Similar findings were observed by King (King et al., 1997) on the study of the fractionation of glycerides mixtures.

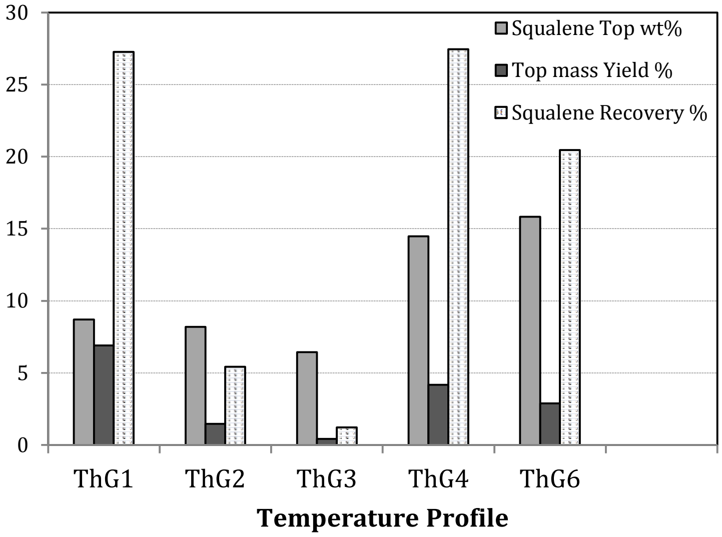


Figure 6.5: Squalene recovery %, top mass yield % and squalene concentration wt% at different thermal gradient profiles at pressure of 15 MPa.

Similar studies were carried out in this work at higher pressure of 20 MPa, see Figure 6.6. Top zone temperature has clear effect on the squalene concentration at the top stream, concentration increased with increasing top zone temperature while there was no significant change observed on recovery and overall throughput. Contradictory to the observation of Hernandez et al. (2010) on the phase equilibrium study of binary system squalene and CO2, where temperature is of minor influence at pressure lower than 25MPa. There are solute-solute interactions involved in this situation as this work is dealing with much complex multi-component system compared to the binary system studied by Hernandez.

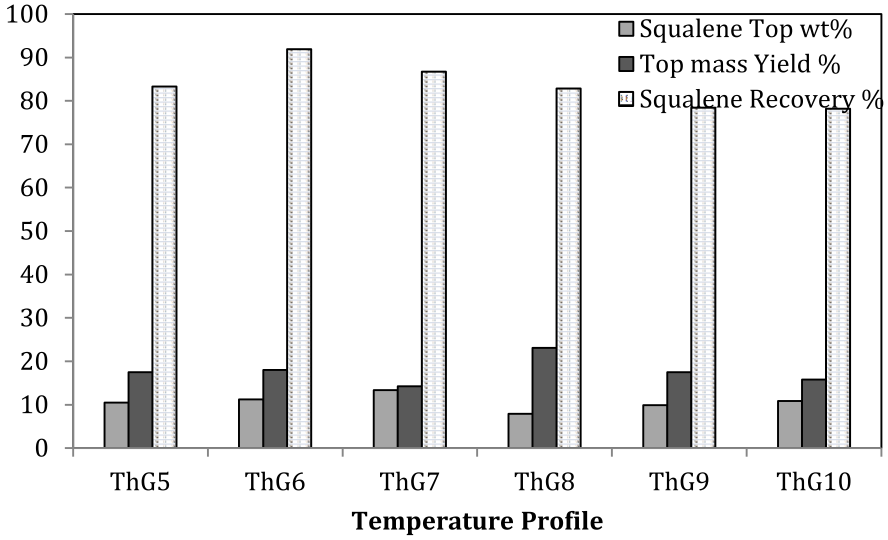


Figure 6.6: Squalene recovery %, top mass yield % and squalene concentration wt% at different thermal gradient profiles at pressure of 20 MPa.

Also, by imposing thermal gradient conditions, at the bottom and middle zones of the fractionation column where the density is high (at low temperature and high pressure of 20 MPa), the extract is produced at this stage after that it is followed by a refining step at higher temperature (top zone) where the squalene is concentrated. At these conditions, internal reflux pattern is formed. Lower section of the column is operated near to higher density of CO2, therefore higher solvent loading while upper section operated at relatively lower density where the selectivity of squalene is enhanced relative to that of heavier molecules. Under these conditions both extraction and distillation are met. Bondioli et al. (1993) studied thermal gradient for the purpose of squalene enrichment enhancement from olive oil deodorizer distillate. Total yield and purity of squalene were improved at temperature profile from bottom to top of 318:333:353 K compared to that of 318:333:363 K.

6.3.3 FFA Recovery and Concentration Under Thermal Gradient

The recovery of FFA at temperature profile follows similar trend at isothermal conditions as function of pressure, comparison is shown in Figure 6.7. However, the recovery slop at thermal gradient ThG6 is steeper than that of isothermal temperature of ThG1. That's mean a small increase in the pressure can enhance the recovery more compared to the isothermal conditions. Pressure increase from 15 to 18 MPa at isothermal conditions lead to an increase of FFA recovery from 23% to 43%, while an increase from 15 to 17 MPa under thermal gradient lead to an increase from 15% to 40% recovery with almost no influence on the FFA concentration.

In the case of the concentration of FFA in the top stream at isothermal conditions there was a linear decrease with increase of pressure. Similar to what have been explained earlier; the column under thermal gradient (from lower temperature at the bottom) is basically divided to two main sections; extraction section and enrichment section. In the extraction section most of FFA along with other components is extracted because of the high density of the solvent. Moving toward the top of the column, the density of CO2 decreases associated with the increase of temperature. The less soluble components start to precipitate causing an enrichment of FFA.

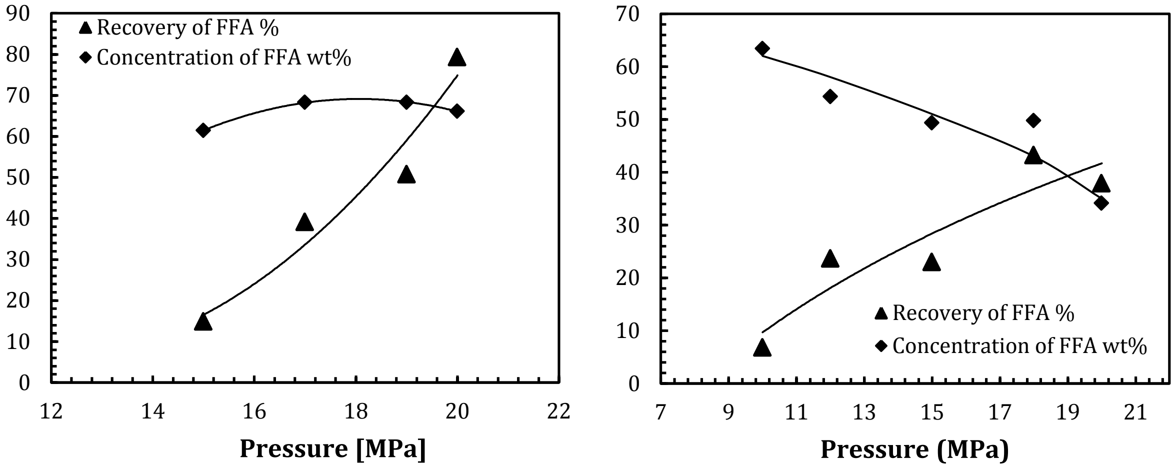


Figure 6.7: Effect of extraction pressure on the recovery of FFA and its concentration in the top stream at temperature profile ThG6 (left) and at isothermal temperature ThG1 (right).

6.5 Conclusions

It can be concluded that internal reflux effect, by employing thermal gradient along the column, was significant. The employment of temperature gradient in the separation of squalene and FFA increased the separation factor. Higher internal reflux can be achieved at wider temperature gradient along the fractionation column. Thus, overcoming the challenge between selectivity and solvent power. Implying thermal gradient in supercritical fractionation has shown a significant increase in the recovery of squalene, more than 95%, at pressure of 20 MPa combined with temperature gradient profile of ThG6. Therefore, Supercritical fluid extraction is a viable alternative for the current techniques used because it can produce solvent free products.

Chapter 7

Effect of Feed Composition on the Extraction and Fractionation of Squalene

7.1 Introduction

In the view of previous results chapters, the application of supercritical fluid extraction (isothermally and under thermal gradient) on the separation and enrichment of PFAD components, both squalene and FFA showed higher enrichment in the top stream of the extraction column. As sighted on the earlier study of this research project, chapter 4, there is a challenge in the separation of FFA and squalene, especially with higher FFA concentration in the feed system. Squalene selectivity enhancement was achieved by means of external flux. However, FFA also enhanced along with squalene. This chapter explores the effect of feed compositions on the recovery of squalene. There is great interest on the study of different feed materials of PFAD in supercritical fluid separation processes. This chapter contains description of the introduction of different PFAD feed material and its effect on the separation and enrichment of squalene. All experiments were performed at optimum conditions of 20 MPa and temperature gradient profile ThG6.

7.2 Objectives

This chapter is aimed on the study of the effect of PFAD feed compositions mainly squalene and FFA content on the recovery and enrichment of squalene in the top product. In this investigation four different PFAD stocks were used at different compositions of FFA and squalene.

7.3 Methodology

7.3.1 Counter-Current Fractionation Rig

A schematic diagram of the continuous counter-current extraction process used in this work is illustrated in chapter 5, Figure 5.1. For full presentation of the experimental apparatus and extraction procedures refer to chapter 5, section 5.3. For the study of different PFAD feed stocks, each run of extraction experiment was conducted three times. The first run was for the purpose of cleaning. Since PFAD feed stocks consist of similar component but with variant compositions, a cleaning step of the pipe lines of the extraction column by the means of organic solvent was skipped. Alternatively, each PFAD stock was used for this purpose.

7.3.2 Materials and Analysis

In this study, four different PFAD were used. More details about PFAD samples were discussed previously in Chapter 3; Materials and Analytical Techniques. The three main streams of the fractionation rig; feed, extract and raffinate were analysed using gas chromatography. Details on the analytical techniques used were discussed previously in Chapter 3.

7.3.3 Calculations

Full details on the calculations carried out during this investigation are presented previously in chapter 5, section 5.3.3.

7.4 Results and Discussions

The effect of feed compositions on the squalene extraction was studied. Four different feed materials were used PFAD1, PFAD2, PFAD3 and PFAD4, which had different composition of squalene and FFA. Compositions are illustrated in Table 7-1. All experiments were performed at optimum conditions of 20 MPa and temperature gradient profile ThG6 (318-353 from bottom to top of the column).

Table 7-1: Squalene and FFA compositions in different PFAD Stocks.

Feed	Composition wt%	
	Squalene	FFA
PFAD 1	1.7	5
PFAD 2	2.2	25
PFAD 3	3.7	46
PFAD 4	3.7	96

In all cases squalene concentration was successfully increased, however as the feed FFA concentration increased, the recovery of squalene at the top stream decreased as shown in Figure 7.1. Almost 99% of squalene was recovered from the feed material that has 5wt% FFA with squalene enrichment from 1.7 wt% in the feed to 16 wt% in the extract.

Comparable findings were found on the investigation of soy oil deodorizer distillate as feed material for supercritical fluid extraction conducted by Gast et al. (2005). Soy oil deodorizer distillate consisted of 3.1 wt% squalene and 2.7 wt% FFA, comparable to low-FFA raw material PFAD 1 used in this work. Gast recovered up to 99% of squalene in the extract and squalene was enriched from 3 wt% to 18 wt% in the extract at extraction pressure of 23 MPa and temperature of 353 K. The results indicate that a FFA separation step prior to the fractionation process will benefit the recovery of squalene from high FFA content raw material.

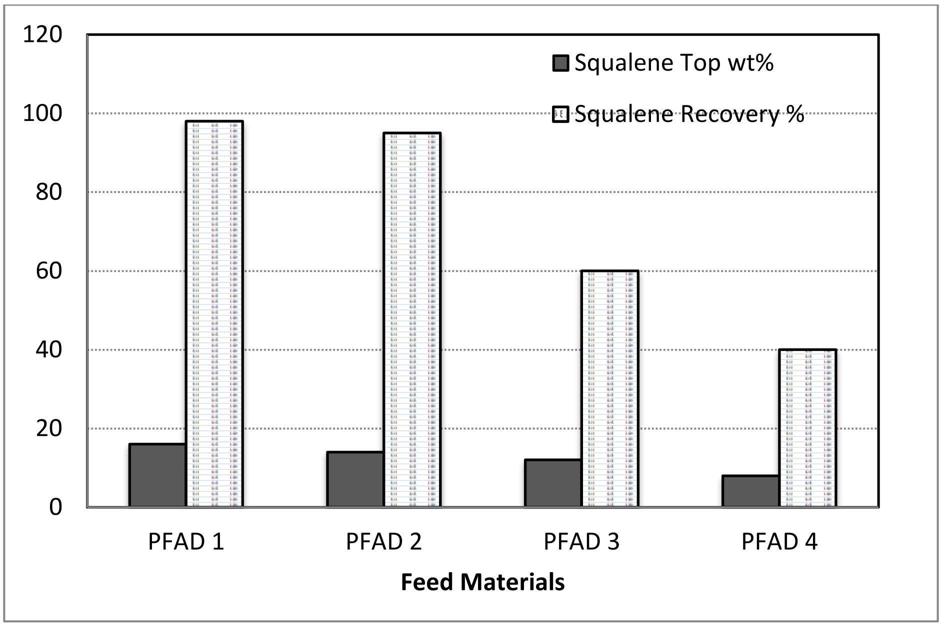


Figure 7.1: Squalene content in the top product and its recovery obtained for different feed materials at 20 MPa and gradient temperature profile of ThG6.

The composition of squalene in the feed and top stream is presented in Figure 7.2. Squalene has the highest feed composition in Feed stock 4; however the application of SCF separation on this feed lead to low separation enrichment compared to the other feed stocks due to the high FFA concentration (almost pure FFA sample with 96%). In contrast, in Feed 1 where it contains the lowest concentration of squalene and lowest FFA content, it showed higher enrichment of squalene in the extract. Therefore, the composition of FFA in the feed has significant effect on the enrichment of squalene in the top product. Catchpole et al. (2000) has studied the effect of feed composition of vitamin A palmitate extraction. The investigation concluded that the concentration in the top product is linearly dependent on feed concentration. This finding is not valid in this case where both squalene and FFA compositions vary in the feed material.

Chua et al. (2007) established a method to separate squalene from PFAD using adsorption chromatography. In Chua study squalene was concentrated by removing extraneous substances such as FFA. PFAD sample used in the study have similar concentration of PFAD4 in this work 3.7-wt% squalene and 83.8-wt% FFA. Squalene content concentrated up to 15.8-wt% after two pre-treatment steps of PFAD: neutralization and hydrolyzation. Although, squalene content is almost double the concentration achieved in the present work, SCF extraction can be a reliable alternative method for squalene. Taking into consideration the addition of two reaction steps for the removal of FFA required in Chua method, which is not favoured in food and pharmaceutical industries. SCF extraction is not chemical intensive and yet produced competitive results.

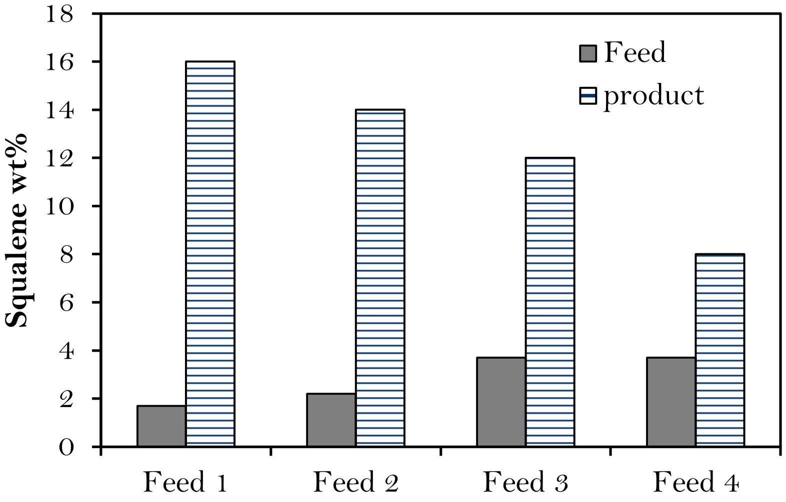


Figure 7.2: Squalene compositions in the feed and top stream.

Fang et al. (2007) and Bondioli et al. (1993) have proposed esterification of deodorizer distillates to convert FFA and glycerides to fatty acid ester prior to the SCF counter-current extraction. The combination of pre-esterification step followed by SCF extraction column can enhance the enrichment of squalene in the extract. The addition of external reflux to the fractionation column can also enhance the top product concentration of squalene. It is needed to create counter-current flow regime inside the extraction column (Riha and Brunner 2000). Investigations on the enrichment of squalene from soy oil deodorizer distillate were conducted by Gast el al. (2005) at fractionation column of 6m height coupled with external reflux. Nevertheless, the concentration of squalene was found to 6 times more than its concentration in the feed material, while in this research project with elimination of external reflux squalene enriched 10 times its concentration in the feed PFAD1. This might be because of high concentrations of other components such as tocopherol and sterols in soy oil deodorizer distillate and/or the effect of internal reflux due to thermal gradient along the column.

7.4 Conclusions

The effect of feed composition was investigated in this chapter. The results showed that there is a significant effect of FFA content on the enrichment of squalene. Almost 99% of squalene was recovered from the feed material that has 5 wt% of FFA only. The results indicate that a FFA separation step prior to the fractionation process will benefit the recovery and the enrichment of squalene.

Chapter 8

Conclusions and Future Work

8.1 Main Concluding Marks

The main objectives of this research project were fulfilled. Solubility of main PFAD components was measured at various pressures and temperatures. Fractionation and enrichment investigations were conducted on PFAD components under isothermal conditions along the extraction column. The use of temperature gradient throughout the fractionation column was also investigated. Effect of PFAD feed compositions were studied. The following are the main concluding marks of this research project:

8.1.1 Solubility Studies

Binary systems of CO2/squalene, CO2/oleic acid, CO2/α-tocopherol, and CO2/pseudo-component palm olein were measured at temperatures of 313, 333 and 353 K, and at a pressure range of 10 to 30 MPa. A ternary system of CO2/squalene/palm olein and a quaternary system of CO2/squalene/ palm olein/ oleic acid were also investigated at 313 K and pressures of 10 to 25 MPa. Among lipid components mentioned above, squalene has been shown to have a higher solubility in the binary systems studied due to its hydrocarbon and non-polar nature, while palm olein has the lowest solubility within the studied state of conditions. The data of binary solubility measurements were correlated using Chrastil's density related empirical equation, and calculated solubilities were in good agreement with the measured values with maximum deviation in terms of AARD is 18%.

A mixture of 10 wt% squalene and 90 wt% palm olein (SQ +PO+ CO_2) was prepared to determine solubility in Sc-CO2 (ternary system) and for the quaternary systems (SQ + PO + OA + CO_2), squalene was kept at 10 wt%, with addition of 25 wt% OA (as FFA) and 65 wt% palm olein under the same conditions. Solubility investigations for the ternary and quaternary systems exhibit the high selectivity of carbon dioxide towards squalene and free fatty acids. The presence of less soluble components, such as palm olein, affects the loading of the whole mixture, especially at high concentration. The introduction of FFA into the mixture results in a decrease of carbon dioxide selectivity towards squalene. This is likely because the solubility of FFA is higher than the solubility of palm olein in CO2, which reduces squalene vapour pressure, resulting in low selectivity. Solute-solute interactions have also been shown to influence the solubility in Sc-CO2. In conclusion, solubility studies demonstrate the feasibility of separating squalene and FFA from palm olein. Squalene and FFA distribution coefficients were found to be higher than 1, therefore both components can be enriched in the gaseous phase. Squalene was enriched in the gaseous phase, reaching its maximum value at lower pressure, and decreasing with increasing pressure.

8.1.2 Separation and Fractionation Studies

In the separation experiments, the optimum operating conditions were determined by studying the effect of pressure, temperature and solvent to feed ratio (S/F). Fractionation experiments with PFAD were performed at temperatures between 313 and 353K and pressures between 10 and 18MPa. The S/F ratio was varied in the range from 10 to 35 gsolvent/goil. The process has shown higher sensitivity to pressure and temperature compared to solvent to feed ratio. The optimum conditions at isothermal fractionation experiments were found to be at 18 MPa and 313K with squalene concentration of 8 wt% from a 2.2wt% concentration in PFAD and squalene recovery of 50%.

FFA separation has shown different trend compared to squalene in the study of the effect of extraction temperature. Higher separation between squalene and FFA can be accomplished at relatively lower temperature at constant pressure. In general terms, solvent power has an inverse correlation with the selectivity of squalene; therefore Sc-CO2 is less selective at higher extraction pressures. However, there is direct relationship between solvent power and the extraction yield has been observed. This relationship between solvent power and selectivity put in challenges on the developing of extraction processes, an efficient process requires high values of selectivity and total throughput. Squalene in PFAD could be significantly enriched by means of supercritical fluid fractionation.

The employment of temperature gradient in the separation of squalene and FFA increased the separation factor, due to the internal reflux effect. Higher internal reflux can be achieved at wider temperature gradient along the fractionation column. Thus, overcoming the challenge between selectivity and solvent power which occurred when applying isothermal fractionation measurements. Implying thermal gradient in supercritical fractionation has shown a significant increase in the recovery of squalene, more than 95%, at pressure of 20MPa combined with temperature gradient profile of ThG6.

8.1.3 Effect of Feed Composition

Although squalene showed higher distribution coefficients compared to FFA, the high concentration of FFA present in PFAD has an influence on the selectivity of squalene. Therefore, the effect of feed composition was investigated in this project. The results showed that there is a significant effect of FFA content on the enrichment of squalene. Almost 99% of squalene was recovered from the feed material that has 5 wt% of FFA only. The results indicate that a FFA separation step prior to the fractionation process will benefit the recovery and the enrichment of squalene.

In conclusion, squalene and FFA in PFAD could be significantly enriched by means of supercritical fluid fractionation. Different fractions with various concentration of squalene can be achieved by tuning the conditions of state. Supercritical fluid extraction is a viable alternative for the current techniques used because it can produce solvent free products especially for food and pharmaceutical industry where the quality of the product is required.

8.2 Suggestions and Future Work

This research project has recognized several areas of interest that require further investigation in the lipid components fractionation from real PFAD systems.

8.2.1 The Study of the Effect of Co-Solvents Addition

In this work it was found that the high selectivities of Sc-CO2 at low pressure come at the expense of throughput, the product yield decreased at lower pressure. Therefore the use of organic co-solvent may enhance the selectivity and total throughput. Catchpole studied the effect of ethanol addition on the solubility of squalene and his results showed solubility enhancement of squalene (Catchpole et. al, 2000). However, an additional operating unit will be required for the removal of the organic solvent from the final product.

8.2.2 The Effect of External Reflux and Feed Introduction Location

The extraction and enrichment of squalene investigation in this research project was conducted without the use of external reflux. More detailed knowledge of the effect of external reflux would allow more knowledge on the purification of squalene extracted from PFAD. All fractionation experiments conducted at certain feed sample introduction point into the extraction column. Further work with varying sample introduction position would be required to fully compare the extraction results.

8.2.1 Optimization Using Response Surface Methodology

Optimization supercritical fluid extraction using response surface methodology RSM has great interest among researchers, as it can minimize the number of experimental runs. Summary of recent studies on this area is given in Table 8-1. All of the research studies on the SCF extraction where applied on batch system mode. To our knowledge, there is no study applying this method on a continuous SCF fractionation process. It is suggested to discuss the advantages and limitation of the use of RSM analysis on continuous SCF extraction process. The experimental design using a computer program Design Expert 7.1 was applied on the fractionation experiments in this work; however time constraints prohibited the investigations on its application as statistical analytical tool for the optimization of the process. Detailed work on this area is presented in the Appendix A.

Table 8-1: Literature review on the use of RSM methodology for predicting and optimization of extraction processes.

Target compound(s)	Matrix Phase	Factors	Responses	Extraction Mode	Statistical Method	Reference
Vitamin E, oil	Solids (wheat germ), Kalahari melon and roselle seeds	3Factors and 3levels Pressure temperature, Solvent flow rate	Concentration of VE	Batch	Central Composite rotate design CCRD and RSM	(GE, et.al,2002), (Shao et al., 2008), (Nyam et al, 2010)
Oil, tocopherol	Solids; (Olive Husk), microalgae Spirulina	P, T, Solvent flow rate, particle size	Oil yield %, tocopherol concentration	Batch	RSM Full 2 factorial design	(de Lucas, et.al, 2002), (de Lucas, et.al, 2003), (Mendiola et.al, 2008), (Gracia et. Al, 2011)
Beta-carotene	Solids (apricot pomace)	P, T, Ethanol %	Beta-carotene concentration	Batch	2^3 full factorial central composite design	(Sanal. et.al, 2005)
oil	Solids (cottonseeds)	P, T, extraction time	Yield%	Batch	CCRD and RSM	(Bhattacharjee. Et.al, 2007)
FFA, triglycerides, orizanols	Solids (rice bran)	P, T	concentrations	Batch	2 factor central composite design, RSM	(Wang et al., 2008)
triglycerides	Solids (Jatropha seeds, Aquilaria crassna seeds, soybean	P, T, solvent to solid rate, extraction time	concentration of triglycerides, total yield %	Batch	CCRD, RSM	(Chen et.al, 2009), (Chen et.al, 2010), (Jokic et al, 2010)
phytosterol	Solids, Roselle seeds	P, T, Solvent flow rate	Yield% and concentration	Batch	RSM	(Nyam et al, 2010),
arotenoids, squalene and sterols	Solids, lotus bee pollen	P, T	Yield% and concentration	Batch	two-factor central composite rotatable design	(Xu et al, 2011)
FFA	Boletus edulis	P, extraction time	Yield %	Batch	RSM	(Vidovic et.al, 2011)
Squalene	Liquid (olive oil deodorizer distillate)	P, T, extraction time	Squalene concentration	Batch	RSM	(Akgun, 2011)

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APPENDIX

Appendix A

Application of central composite design in the Optimization of Sc-CO2 Extraction of Squalene from PFAD Using Response Surface Methodology RSM

A.1 Introduction

Optimization of a process is basically determining the required and necessary inputs (variables) to obtain the desired outputs (responses). Response surface methodology (RSM) is a powerful statistical tool used widely in the optimization of supercritical fluid extraction batch processes. It is basically used for experimental design, building efficient models, factors and variables evaluation and optimum conditions calculation. RSM was successfully applied in supercritical fluid extraction processes by various researchers in the field (refer to table 8.1 in chapter 8 for all references). However, all the studies found were based on batch mode extraction. This work discusses the advantages and limitation of the use of RSM analysis on a ***continuous*** SCF extraction process. The overall aim was to investigate the applicability of the statistical analysis in the optimization of the Sc-CO2 extraction of squalene from PFAD via the application of RSM, in order to find the best conditions of the main variables.

A.2 Methodology

A.2.1 Experimental Design DoE

A central composite design (CCD) based response surface methodology (RSM) was implanted to determine optimal squalene extraction conditions. DoE and statistical analysis was achieved by means of Design Expert Program version 7.1. Response surface methodology RSM was employed to analyse the separation and enrichment of squalene based on the effect of four main factors; extraction temperature, extraction pressure, solvent and feed flow rates. The response is namely recovery of squalene in the extract Y_1 in %.

The corresponding ranges (low and high value) of the main variables were as follows: pressure of 10 and 20 MPa; temperature of 40 and 80 °C; solvent flow rate of 8 to 24 g/min and feed flow rate of 0.6 to 2 ml/min. all the factors were coded at three levels: -1, 0, and +1 in order to be optimized, un-coded and coded levels are illustrated in Table A-1. The experimental domain of the extraction process for a 4 factors and 4-level CCD is illustrated in Figure A.1. There are total of 30 experiments consisted of; 6 central points, and 24 factorial points. Six replicates of the centre points were used in order to estimate the experimental error.

Table A-1: Independent factors and their levels for central composite design.

Factors	*Symbol*	*Factor levels*		
		-1	*0*	*+1*
Extraction pressure, bar	X_1	10	150	20
Extraction temperature, °C	X_2	40	60	80
Solvent flow rate, g/min	X_3	8	16	24
Feed flow rate, ml/min	X_4	0.6	1.3	2

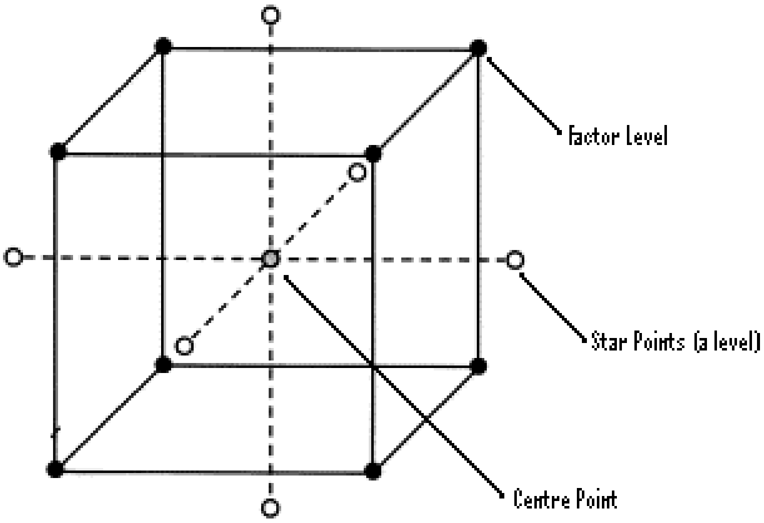


Figure A.1: Graphical representation of factors levels, center point and star points α-level of the CCD of three variables.

A.2.2 Statistical analysis

For the responses prediction of optimization of the fractionation of squalene, Design Expert 7.1 software was used and a quadratic polynomial regression model was used. The model proposed for each response Y was as following:

$$Y = A_\circ + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j$$

Where Y represents response, X_i and X_j are independent variables, $A_\circ$ is constant, A_i is linear coefficient of the model, A_{ii} representing quadratic coefficient and A_{ij} is interactive coefficient of the model. The model was evaluated by comparing the experimental and predicted responses and analysis of variance (ANOVA).

A.2.3 Counter-current continuous fractionation rig

Full description of counter-current SCF extraction and separation of squalene is discussed intensively previously in chapter 5, section 5.3.1.

A.2.4 Materials and analysis

In this study, the same stock material of PFAD used in the isothermal fractionation studies was used for this investigation. More details about PFAD samples were discussed previously in Chapter 3; Materials and Analytical Techniques. The three main streams of the fractionation rig; feed, extract and raffinate were analysed using gas chromatography. Details on the analytical techniques used were discussed previously in Chapter 3.

A.2.5 Calculations

Full details on the calculations carried out during this investigation are presented previously in chapter 5, section 5.3.3.

A.3 Results and Discussions

A.3.1 Design of Experiments DoE

A.3.1.2 Full Type Design

Experiments were designed statically using computer programme Design Expert 7.1 after defining the main variables and their levels. As a result, a total of 30 experiments were designed for full design type with alpha (axial point) value of 1.414. The four factors values for each run are represented in Table A-2. Centre point was designed to be at Pressure, temperature, solvent flow rate and feed flow rate at their zero level. Axial points designed by the software were difficult to be achieved experimentally. For example Run 2, the feed flow rate FF was at value of -0.1. In this case, the value was changed to be at a minimum value possible to conduct experimentally. Similar changes were made for other axial points. For Run 7, at pressure of 50 bar, solvent is not at its supercritical conditions therefore the pressure was changed to be 80 bar. For Run 10, the maximum pressure of the extraction column used in this work was 200 bars, pressure above that was not possible to be studied. Therefore pressure was changed from 250 bars to 200 bars. The main obstacle of this design was to conduct the axial points (star points) and most of the responses were assumed and not measured. Primarily experiments are important in this case.

Table A-2: Full experimental runs for the optimization of squalene enrichment.

Run	P	T	SF	FF
1	150	60	16	1.3
2	150	60	16	-0.1
3	150	60	16	1.3
4	200	40	8	0.6
5	150	60	16	1.3
6	150	60	16	1.3
7	50	60	16	1.3
8	100	80	8	0.6
9	150	20	16	1.3
10	250	60	16	1.3
11	150	60	16	2.7
12	100	80	8	2
13	150	100	16	1.3
14	100	40	8	0.6
15	200	80	8	2
16	150	60	32	1.3
17	100	40	24	0.6
18	100	80	24	2
19	200	40	8	2
20	100	40	8	2
21	200	80	8	0.6
22	200	40	24	0.6
23	150	60	0	1.3
24	150	60	16	1.3
25	200	80	24	0.6
26	200	40	24	2
27	200	80	24	2
28	150	60	16	1.3
29	100	40	24	2
30	100	80	24	0.6

Responses of the experiments are given in Table A-3. For certain experimental runs at given conditions, there were either no extraction occurred or the column reached flooding point. In these two main cases assumption were made for the values of the responses; at flooding point squalene SQ recovery was assumed to be 100% and at low extraction points SQ recovery was assumed to be 0.001%.

Table A-3: Response (SQ Recovery) at each run of the full type design.

Run	Factor 1 A:P bar	Factor 2 B:T oC	Factor 3 C:SF g/min	Factor 4 D:FF ml/min	Response 1 SQ Recovery %
1	150.00	60.00	16.00	1.30	10.1
2	150.00	60.00	16.00	0.30	38.8
3	150.00	60.00	16.00	1.30	10.6
4	200.00	40.00	8.00	0.60	100
5	150.00	60.00	16.00	1.30	10
6	150.00	60.00	16.00	1.30	8.7
7	80.00	60.00	16.00	1.30	0.001
8	100.00	80.00	8.00	0.60	0.001
9	150.00	40.00	16.00	1.30	62
10	200.00	60.00	16.00	1.30	33.7
11	150.00	60.00	16.00	2.70	3.6
12	100.00	80.00	8.00	2.00	0.001
13	150.00	100.00	16.00	1.30	0.9
14	100.00	40.00	8.00	0.60	12.8
15	200.00	80.00	8.00	2.00	3.9
16	150.00	60.00	32.00	1.30	3.6
17	100.00	40.00	24.00	0.60	18.9
18	100.00	80.00	24.00	2.00	0.001
19	200.00	40.00	8.00	2.00	100
20	100.00	40.00	8.00	2.00	2.7
21	200.00	80.00	8.00	0.60	13
22	200.00	40.00	24.00	0.60	100
23	150.00	60.00	2.00	1.30	0.001
24	150.00	60.00	16.00	1.30	10.8
25	200.00	80.00	24.00	0.60	41
26	200.00	40.00	24.00	2.00	100
27	200.00	80.00	24.00	2.00	13
28	150.00	60.00	16.00	1.30	8.5
29	100.00	40.00	24.00	2.00	4.6
30	100.00	80.00	24.00	0.60	0.001

The software analyse different models and test the lack of fit of the each model in order to find the best fit model for the tested design. A summer y of the model statistics is shown in Table A-4 below. Quadratic model were suggested with R^2 of 0.96. Analysis of variance (ANOVA) of the statistic model is given in Table A-5.

Table A-4: Model Summary Statistcs

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-2
Linear	20.36	0.6843	0.6338	0.5147
2FI	16.12	0.8495	0.7702	0.5995
Quadratic	9.19	0.9614	0.9254	0.7879
Cubic	2.52	0.9988	0.9944	

Table A-5: ANOVA for response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	31547.70	14	2253.41	26.69< 0.0001significant	
A-P12283.15	*1*	*12283.15*	*145.49*	*< 0.0001*	
B-T11086.53	*1*	*11086.53*	*131.32*	*< 0.0001*	
C-SF	*138.75*	*1*	*138.75*	*1.64*	*0.2193*
D-FF	*729.45*	*1*	*729.45*	*8.64*	*0.0101*
AB5260.02	*1*	*5260.02*	*62.31*	*< 0.0001*	
AC52.93	*1*	*52.93*	*0.63*	*0.4408*	
AD10.08	*1*	*10.08*	*0.12*	*0.7345*	
BC52.93	*1*	*52.93*	*0.63*	*0.4408*	
BD10.08	*1*	*10.08*	*0.12*	*0.7345*	
CD33.35	*1*	*33.35*	*0.40*	*0.5391*	
A^2*717.14*	*1*	*717.14*	*8.49*	*0.0107*	
B^2*1746.54*	*1*	*1746.54*	*20.69*	*0.0004*	
C^2*186.21*	*1*	*186.21*	*2.21*	*0.1582*	
D^2*139.00*	*1*	*139.00*	*1.65*	*0.2189*	
Residual	1266.36	15	84.42		
Lack of Fit	*1261.69*	*10*	*126.17*	*135.13< 0.0001significant*	
Pure Error	*4.67*	*5*	*0.93*		
Cor Total	32814.05	29			

Final Equaltion of SQ Recovery based on quadratic model is given below:

SQ Recovery = +38.37841 +0.41372 * P -1.80816* T + 0.70149 * SF - 12.12163 * FF - 0.018131 * P * T+ 4.54688E-003 * P * SF-0.022679 * P * FF +0.011367 * T * SF- 0.056696 * T * FF-0.25781 * SF * FF +3.81499E-003 * P^2 + 0.026623 * T^2 -0.044438* SF^2 +5.61547 * FF^2

Model Graphs

Responce Y (SQ recovery) is plotted agains two main factors temperature and pressure. The remaining two factors were located at certain values; FF at center value and SF at its maximum level. Figure A-2 reperesent SQ recovery as finction of temperature and pressure.

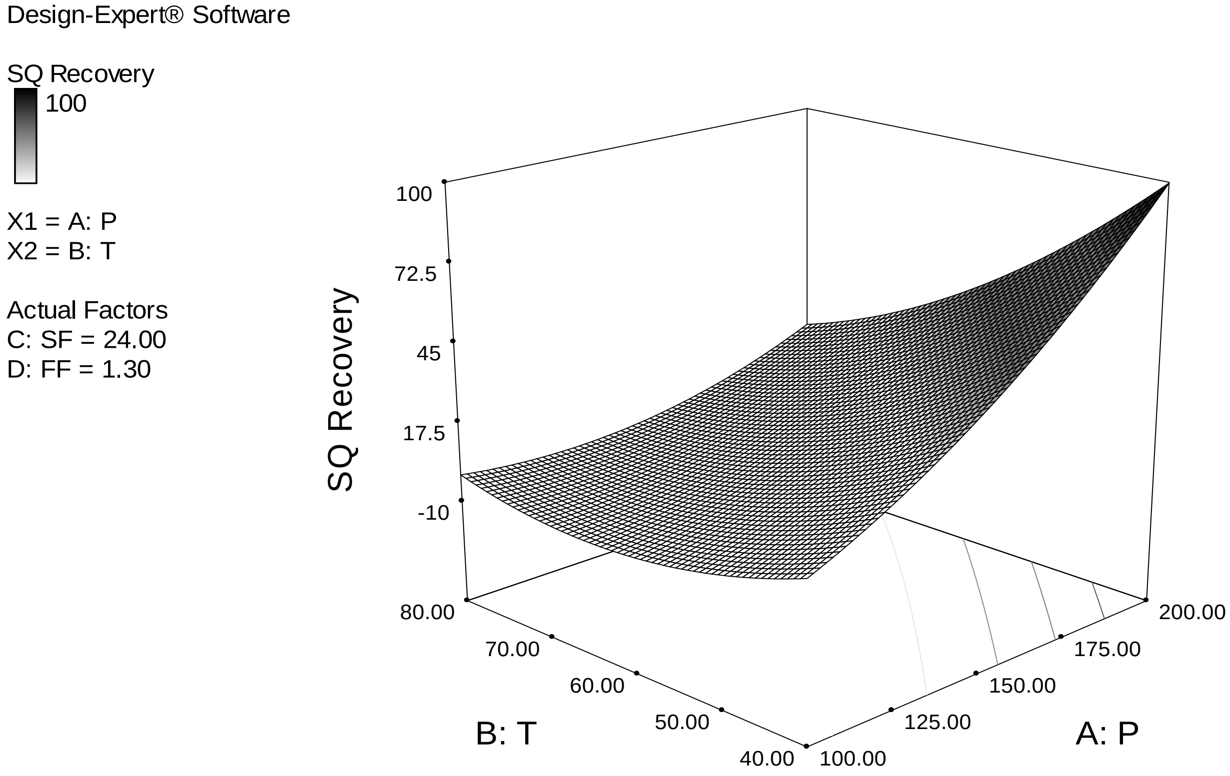


Figure A-2: Graphic model 3D surface of SQ recovery as function of Temperature and pressure.

Its clear from the Figure A-2 that pressure has significant effect on SQ recovery from PFAD. Higher extraction pressureand lower extraction temperature lead tohigher SQ recovery based on the model.

A.4 Conclusions

Modeling and optimization using responce surface methodolgy is very promising statistical tool in the filed of supercritical extraction. However, full examination and study is required for its application in extraction processes conducted in countinous mode. Based on this investgation it was found that experimnetation perior to DoE was vital to evaluate each factor and define significant variables. Moreover, a full study on the extraction column and predection of flooding points are required perior to statistical design in order to avoid incorrect assumptions.

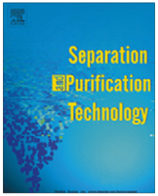
Appendix B: Publications

Separation and Purification Technology 83 (2011) 189–195

Contents lists available at SciVerse ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur



Solubility measurements and analysis of binary, ternary and quaternary systems of palm olein, squalene and oleic acid in supercritical carbon dioxide

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ABSTRACT

Through the application of a dynamic method, the solubility of three lipid components with different volatilities and polarities was measured in supercritical carbon dioxide (SC-CO_2). Binary systems of CO_2/squalene (SQ), CO_2/oleic acid (OA) and CO_2/pseudo-component palm olein (PO) were measured at temperatures of 313, 333 and 353 K, and at a pressure range of 10–30 MPa. A ternary system of CO_2/SQ/PO and a quaternary system of CO_2/SQ/PO/OA were also investigated at 313 K and pressures of 10–25 MPa. Comparison of the ternary system with the binary system showed a decrease in the solubility of SQ, with a corresponding rise in the solubility of PO. In the quaternary system, the presence of OA decreased the selectivity of SQ. The data were correlated using Chrastil's equation in order to examine the general trend of solubility behaviour.

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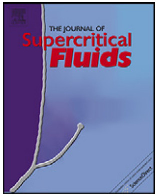

Isothermal and temperature gradient supercritical fluid extraction and fractionation of squalene from palm fatty acid distillate using compressed carbon dioxide

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ABSTRACT

Currently the extraction of valuable components from oils is of growing interest. Palm fatty acid distillate (PFAD) is a by-product from palm oil refining process which contains valuable minor components. This work investigated the extraction and recovery of squalene using supercritical carbon dioxide (Sc-CO_2) as solvent from PFAD. The process has been carried out on a counter-current glass beads packed column with the dimensions of 11.45 mm internal diameter (I.D.) and 1.5 m of effective height. The effect of the experiment conditions on the squalene purity and recovery has been studied. The pressure and temperature were the operating conditions investigated and they varied from 10 to 20 MPa and 313 to 353 K, respectively. Squalene and free fatty acids (FFA) content in the extract increased, and triglycerides content decreased during most of the fractionation runs. Longitudinal thermal gradient profiles along the column was investigated for further recovery of squalene from PFAD, results showed the highest squalene recovery of more than 95% was reached and concentration of squalene was increased from 2 wt% in the feed to 16 wt% in the top product.