DEVELOPMENT OF IDENTIFICATION TECHNIQUE BY FTIR-PCA FOR SUPERCRITICALLY EXTRACTED METABOLITES FROM *PARKIA SPECIOSA* (HASSK) SEEDS

by

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Thesis submitted in fulfillment of the
requirement for the degree
of Masters of Science

February 2007

ACKNOWLEDGEMENT

All praises to Allah the Almighty who help me and gave me strength, inspiration and patience to finish this research. It is only with His help, blessings and guidance and only by His will that I finally managed to complete my thesis.

Several people have directly or indirectly contributed to my work. I would like to thank them all, with special thanks and my sincere gratitude to the following persons: My supervisor, Prof. Ir Dr. Mohd Omar Abdul Kadir for his guidance with helpful discussion, comments and constant support during the work. To my beloved father and mother, Prof. Dr. Zhari Ismail and Sa'diah Bt Abdullah for giving valuable advice, financial support, encouragement and personally to my father for allowing me to use the facilities in his laboratory, thanks a lot.

I also wish to express my heartiest thanks and deepest gratitude to my kind-hearted friend En Mohd Azizi Che Yunus and En Wahyu for their constructive comments, guidance and assistance especially during my experiment and thesis writing. Thanks to Mr. Mohd Razak Hamdan and Mrs. Azizan from School of Pharmaceutical Sciences for their assistance at the Herbal Laboratory. I would like to give my deepest thanks to Mr. Amin from KBioCorp Sdn. Bhd. for his guidance on the basic of chromatography and spectroscopy.

To my brothers and sisters, I love you all. Lastly to all my friends from SPG, thanks for all your encouragement and support. To Syidee, Azren, Akram, Al-Adrian, Hafidz, Noordin, Nazrul, Mohd Firdaus, Shuhaib, Khusnizaim, Hafis, Firal, Fairos, Zul, Azhar and Faizal, without all of you, it's hard for me to complete this thesis. Thank you.

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LIST OF ABBREVIATIONS

SC-CO_2	Supercritical Carbon Dioxide
SF or SCF	Supercritical Fluid
HPLC	High Performance Liquid Chromatography
GC-TOFMS	Gas Chromatography-Time of Flight Mass Spectrometry
TLC	Thin Layer Chromatography
NMR	Nuclear Magnetic Resonance
NIR	Near Infrared
FTIR	Fourier Transform Infrared
ATR	Attenuated Total Reflection
PCA	Principal Component Analysis
ROSE	Residium Oil Supercritical Extraction
NCL	Near Critical Liquid
CP	Critical Point
TP	Triple Point
L-S	Liquid Solid
GRAS	Generally Regarded As Safe
SD	Standard Deviation
MeOH	Methanol
EtOH	Ethanol
M	Modifier
TPH	Total Petroleum Hydrocarbons
LSD	Lysergic Acid Diethylamide
DTGS	Deurated Tri-Glycine Sulphate

amu	Atomic Mass Unit
v_{asym}	Asymmetric Vibration
v_{sym}	Symmetric Vibration
PSU	Possible Structural Unit
AIC	Abundance Ion Chromatogram

LIST OF SYMBOLS

		Unit
T_c	Critical Temperature	^{O}C or K
P_c	Critical Pressure	^{O}C or K
T_R	Reduced Temperature	^{O}C or K
P_R	Reduced Pressure	MPa or bar or psi or atm
ρ	Density	kg/ m^3
ρ_R	Reduced Density	kg/ m^3
ρ_C	Critical Density	kg/ m^3
F	Flow Rate	mL/ min
t_e	Extraction Time	sec or min
dP	Particle Size	μm

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Pembangunan Teknik Pengenalpastian Metabolit Dari Biji *Parkia speciosa* (Hassk) Yang Diekstrak Secara Lampau Genting dengan Menggunakan Kaedah FTIR-PCA

ABSTRAK

Analisis kualitatif dan kuantitatif terhadap hasil ekstrak bendalir lampau genting karbon dioksida (BLG-CO_2) telah dilakukan pada beberapa keadaan pengekstrakan. Keadaan pengekstrakan tersebut ialah suhu pada 313 K, 323 K, 333 K, 343 K, 353 K and 363 K dan tekanan pada 20.68 MPa, 27.58 MPa, 34.47 MPa, 41.37 MPa, 48.26 MPa and 55.16 MPa. Tempoh pengekstrakan telah ditetapkan pada 50 minit dan saiz partikel ditetapkan pada saiz 250 - 300 µm. Suatu sifat yang menarik telah diperhatikan iaitu pada tekanan rendah, hasil yang telah diekstrak berkurang dengan peningkatan suhu, manakala pada tekanan tinggi, hasil yang diekstrak bertambah dengan peningkatan suhu. Sifat ini yang dikenali sebagai “retrograde vaporization” telah dikaji secara mendalam dengan meneliti nilai-nilai kebolehekstrakan sampel-sampel terekstrak SF. Nilai kebolehekstrakan tertinggi bagi *Parkia speciosa* dalam CO_2 adalah 0.458 g hasil/100 g CO_2 pada keadaan interaksi 34.47 MPa/363 K.

Cara-cara pengenalpastian, pengelasan dan pengasingan yang pantas menggunakan spektroskopi Penukaran Fourier Inframerah (FTIR) digabungkan dengan analisis kemometrik telah digunakan untuk menentukan profil ekstrak genting lampau di dalam setiap keadaan pengekstrakan. Analisis komponen utama (PCA) telah dijalankan keatas spektrum FTIR bagi kajian metabolit. Plot skor dan ”loading” menunjukkan kaedah metabolomik ini sesuai bagi analisis

ekstrak genting lampau dari produk tumbuhan. Keputusan yang didapati dari kaedah ini mampu mengenalpasti dan mengasingkan sampel yang mengandungi kepekatan asid karboksilik tak tepu yang tinggi dari sampel-sampel yang lain.

Analisis komponen menggunakan Kromatografi Gas-Spektrometri Jisim (GC-TOFMS) telah dilakukan berdasarkan peratusan kesamaan yang melebihi 75 % dan luas puncak melebihi 0.3 %. Asid thiodipropionic, didodesil ester dikenalpasti mempunyai peratusan luas puncak yang tertinggi pada kebanyakan sampel. Komponen yang lain adalah asid linoleik klorida, asid palmitik, asid linoleik, asid miristik, asid arakidonik dan asid undekanoik. Komponen terpenoid yang terdiri dari β-sitosterol, kampesterol dan stigmasterol dikenalpasti pada semua keadaan pengekstrakan manakala komponen lain seperti skualene dan lupeol dikenalpasti pada keadaan pengekstrakan tertentu sahaja. Gabungan pemisahan kromatografi dengan teknik pengenalpastian seperti yang didapati pada kaedah GC-TOFMS memudahkan pengesanan kepelbagaian komponen dari keadaaan pengekstrakan genting lampau CO_2 yang berbeza-beza dan pengasingan komponen kimia yang berbeza dari biji *Parkia speciosa*.

Pengesahan keputusan didapati dari kaedah FTIR-PCA menggunakan GC-TOFMS berjaya mengenalpasti kandungan asid karboksilik tak tepu dengan peratusan luas puncak tertinggi pada keadaan yang terasing dari kaedah FTIR-PCA.

Development of Identification Technique by FTIR-PCA for Supercritically Extracted Metabolites from *Parkia speciosa* (Hassk) Seeds

ABSTRACT

The qualitative and quantitative analysis on the yield of *Parkia speciosa* seeds extracted by Supercritical Carbon Dioxide Extraction (SC-CO_2 extraction) were performed under various extraction conditions. The combined conditions were interactions of temperatures at 313 K, 323 K, 333 K, 343 K, 353 K and 363 K and pressures at 20.68 MPa, 27.58 MPa, 34.47 MPa, 41.37 MPa, 48.26 MPa and 55.16 MPa. Extraction time and particle size were fixed to 50 minutes and 250 - 300 μm respectively. An interesting behavior was found where at low pressure, extracted yield decreased with increasing temperature while at high pressure, the extracted yield increased with increasing temperature. This behavior known as the retrograde vaporization was further studied by examining the extractability values of SF extracted samples. The highest extractability value of *Parkia speciosa* in CO_2 was 0.458 g yield/100 g CO_2 at the interaction condition of 34.47 MPa/363 K.

A rapid identification, classification and discrimination tools using Fourier Transform Infrared (FTIR) spectroscopy combined with chemometrics analysis was applied to determine the profile of SF extracts in each extraction conditions. Principal Component Analysis (PCA) was applied to the FTIR spectra for further analysis of metabolites. Scores and loadings plot show that this metabolomics method is suitable for the analysis of SF extracted samples from natural products. The results obtained by this method successfully discriminate samples with high concentration of unsaturated carboxylic acid from other samples.

The analysis of compound by Gas Chromatography-Time of Flight Mass Spectrometry (GC/TOF-MS) was done based on percentage of similarity and peak area of more than 75 % and 0.3 % respectively. Thiodipropionoic acid, didodecyl ester was present with highest percentage area in most sample conditions. Other main compounds were linoleic acid chloride, palmitic acid, linoleic acid, myristic acid, arachidonic acid and undecanoic acid. Terpenoids compound of β-sitosterol, campesterol and stigmasterols were identified at all conditions, while some other compounds such as squalene and lupeol were identified only at certain condition. The combination in chromatographic separation with an identification technique as in GC/TOF-MS has made it possible to detect the variability obtained by different SC-CO_2 extraction condition and separation of different chemical compounds in *Parkia speciosa* seeds.

Verification of results from FTIR-PCA methods by GC/TOF-MS successfully identified the unsaturated carboxylic acids with the highest percentage area at conditions discriminated by FTIR-PCA methods.

CHAPTER 1

INTRODUCTION

1.1 Introduction

The studies on natural products are one of the most active research areas in the world today. Clinical tests have indicated that certain natural products do contain active ingredients that are effective for treating some difficult diseases. Since active compounds in natural products usually are in low concentrations, a great deal of research has been done to develop more effective and selective extraction methods for recovery of these compounds from the raw materials. Therefore, developing alternative extraction techniques with better selectivity and efficiency are highly desirable and consequently, Supercritical Carbon Dioxide (SC-CO_2) extraction as an environmentally responsible and efficient extraction technique for solid materials was extensively studied for separation of active compounds from natural products. Advantages of SC-CO_2 have been discussed in the literature which are worth mentioning; rapid, simple, good analyte selectivity, efficient, suitable for thermally labile compound, near solvent free character and reduced environmental hazard (Goncalves et al, 2005). CO_2 remains the most commonly used fluid because of its low critical parameters (T_c = 304.04 K, P_c = 7.38 Mpa), non-toxic, has non-flammable properties and are available in high purity at low cost. SC-CO_2 too has good solvent properties for extraction of non-polar components such as hydrocarbons (Vagi et al, 2004).

Petai or scientifically known as *Parkia speciosa* Hassk. is a tropical leguminous tree in the family of *Leguminosae* found in most of South East Asian countries. The seeds have been eaten as food either cooked or raw due to its high nutritional value. It is known to have important chemical and medicinal compounds such as several cyclic polysulfides which are used for treatment of antibacterial activity on kidney, ureter and urinary bladder infections (Gmelin et al, 1981), thiazolidine-4-carboxylic acid for anticancer activity (Suvachittanont et al, 1996) and have a hypoglycaemic effect due to synergistic action of β-sitosterol and stigmasterol (Fathaiya et al, 1994).

In this project, carbon dioxide (CO_2) with 99.99% purity was used. Of all the gases and liquids studied, CO_2 remains the most commonly used fluid for SFE applications of its low critical constants (T_c = 304.04 K, P_c = 7.38 Mpa), its non toxic and non flammable properties and its availability in high purity with low cost. Supercritical CO_2 has good solvent properties for extraction of non-polar compounds such as hydrocarbons, while its large quadrupole moments also enables it to dissolve some moderately polar compounds such as alcohols, esters, aldehydes and ketones. Supercritical CO_2 is also suitable for the extraction of heat sensitive compounds (Anne et al, 2000), leaves no solvent residue in the products, odourless, colourless, and has low surface tension and viscosity. The diffusivity of supercritical CO_2 is one to two orders of magnitude higher than for other fluids, which permits rapid mass transfer, resulting in a larger extraction rate than that obtained by conventional extraction method (Roy et al, 1996).

Unlike synthetic drugs, extracts from natural products contains a complicated system of mixtures. Thus, the methods of choice for identification of ‘botanical drug’ are mainly intended to obtain a characteristic fingerprint of a specific plant that represent the presence of a particular quality defining its chemical constituents. For such purposes, chromatographic techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and thin layer chromatography (TLC) were used widely as reported in numerous publications (Sim, 2006).

Initially, the use of infrared (IR) spectroscopical method is restricted only for structural elucidation of isolated compounds from the herbal matrices. It is also found useful in phytochemical studies as a ‘fingerprinting’ device, for comparing natural with synthetic sample (Harbone, 1998). Due to the inherent complexity of the IR spectrum, the actual interpretation may be difficult and the operation requires much experience. Indeed, slight differences in the spectra within the same plant species may not be obvious and generally not visible to the naked eye. Thus, the application of IR spectroscopy in herbal analysis is still very limited compared to its applications in other areas (food and beverage industry, microbiology, pharmaceutical etc).

With the advance of computer technology, chemometric methods have become a leading tool among the scientific communities towards faster result analysis and shorter product development time (Seasholtz, 1999). Among others, an unsupervised pattern recognition technique such as Principal

Components Analysis (PCA) are frequently used in handling multivariate data without prior knowledge about the studied samples (Miller et al, 2000).

A natural product including plant seed extracts undergoes natural variation. The quality of extracted samples from different SF extraction conditions may vary. Thus, the identification of extracts based on different SF extraction conditions is crucial in order to ensure authenticity, quality, safety and efficacy of the extracted products. The manufacturer of plant extracts always seeking for a faster and cost-effective verification method since the traditional wet chemistry analysis are too laborious, time consuming and expensive. A rapid quality verification method with the integration of statistical and mathematical modeling for extracting relevant information base on the infrared spectroscopy data was developed.

1.2 Objectives

The main objective of this research is to develop a technique of using Supercritical CO_2 (SC-CO_2) extraction on bioactive compounds from *Parkia speciosa* seeds. The optimized extraction factors that gives the best product yield (qualitative and quantitatively) is expected to be achieved and a rapid quality verification method with the integration of statistical and mathematical modeling for extracting relevant information based on the infrared spectroscopy data will be developed.

This main objective could be further divided into other objectives, which are:-

1. To determine the SC-CO_2 condition effect (pressure and temperature) on the extraction yield and composition distribution and to study the interactions between this two independent variables
2. To determine the most dominant independent variables that produce the maximum yield at the optimum SC-CO_2 condition
3. To extend the use of FTIR transmission spectroscopy associated with the appropriate chemometric method (Principal Component Analysis) in discrimination and classification of *Parkia speciosa* seeds based on its supercritical fluid extraction condition.
4. To explore the active compounds in *Parkia speciosa* seeds using SC-CO_2 selected conditions and identify the active compounds available by using Gas Chromatography (Time of Flight) – Mass Spectrometry (GC TOF-MS)

CHAPTER 2

LITERATURE REVIEW

2.1 Supercritical Fluid Extraction

2.1.1 History of Supercritical Fluid Extraction

The first observation of the occurrence of a supercritical phase was first reported by Baron Cagniard de la Tour in 1822 (Mc Hugh and Krukonis, 1986). In his report, he noted that the gas-liquid boundary disappeared when the temperature of certain materials was increased by heating each of them in a closed glass container. The critical point of a substance was first discovered from these early experiments.

In 1879, Hannay and Hogarth demonstrated the solvating power of supercritical fluids for solids and studied the solubilities of cobalt (II) chloride, iron (III) chloride, potassium (III) bromide and potassium iodide in supercritical ethanol ($T_c = 243^{O}C$, $P_c = 63$ atm). They concluded that the solubility of substances having low vapor pressure in supercritical fluids conditions were dependant on pressure (Stahl et al, 1988).

Later, Buchner reported that the solubilities of certain non-volatile organic materials in CO_2 under supercritical conditions were also orders of magnitude higher than would be expected from vapor pressure considerations alone.

The interest shown in supercritical fluids during the first half of the twentieth centuries involved process operations and not analytical chemistry. Wilson et al. devised a propane deasphalting process for refining lubricating oils in 1936. Although the process was not considered a supercritical fluid extraction, propane deasphalting does make use of the change in solvent power of a liquid with changes in temperature/pressure in the vicinity of its critical point.

A few years later, purification and separation process of vegetables and fish oils was developed. The process concentrated the polyunsaturated triglycerides in vegetable oils and the vitamin A from fish oils using propane as a selective solvent.

Residium oil supercritical extraction (ROSE) process was designed by Kerr McGee Corp for the removal of lower boiling products from the residue of crude oil distillation in order to improve petroleum-refining technology in 1970s. Subcritical pentane was used in the process to isolate lighter components from the heavier asphaltenes. Separation of the lighter component by heating the pentane to supercritical temperature at fixed temperature led to a lowering of the pentane density and separation initially of a highly viscous fraction and then a heavier oil fraction. (Taylor, 1996)

Zosel (1971) reported the decaffeination of green coffee with CO_2. His famous method has provided significant changes in supercritical fluid extraction technology. The process was accomplished by soaking the beans in water and then immersing them in supercritical CO_2. The presence of water was essential for the efficient extraction of the caffeine from within the bean and as results of that, currently over a dozen of patents have been issued that concerns the decaffeination of coffee.

Since 1980, there has been rapid development of supercritical fluid extraction (SFE) from natural products, polymers and fish oils. The use of supercritical fluids in chemical reaction and synthesis of polymers and organic chemicals and removal of nicotine from tobacco are under study. (Taylor, 1996)

2.1.2 Principles of Supercritical Fluids (SCFs)

A supercritical fluid (SCF) is any compound at a temperature and pressure above the critical point. Above the critical temperature (T_c) of a compound, the pure, gaseous component cannot be liquefied regardless of the pressure applied. The critical pressure (P_c) is the vapor pressure of the gas at the critical temperature. In the supercritical environment only one phase exists. The fluid, as it is termed, is neither a gas nor a liquid. This phase retains solvent power approximating liquids as well as the transport properties common to gases.

By increasing the pressure of the gas above the critical point (Figure 2.1), it is possible to give liquid-like densities and solvating strengths. Near the critical point, the density of the gas will increase rapidly with increasing pressure. Here, the solubility of many compounds is several orders of magnitude greater than predicted from the classical thermodynamics of ideal gases. As the average distance between molecules decreases, non-ideal gas behavior will begin to govern the interactions between the solvent and the sample accounting for a tremendous enhancement in solubility. In supercritical region, solvating strength is a direct function of density, which in turn is dependent on system pressure (at constant temperature). Solvating strengths can be fine-tuned by adjusting the pressure and/or temperature, using the solvent anywhere in the range of ideal gas to nearly pure liquid. Because of the non-compressibility of liquids, this phenomenon is unique to supercritical fluids. It is even possible, by adding small quantities of co-solvent, to custom design a supercritical fluid for a specific application.

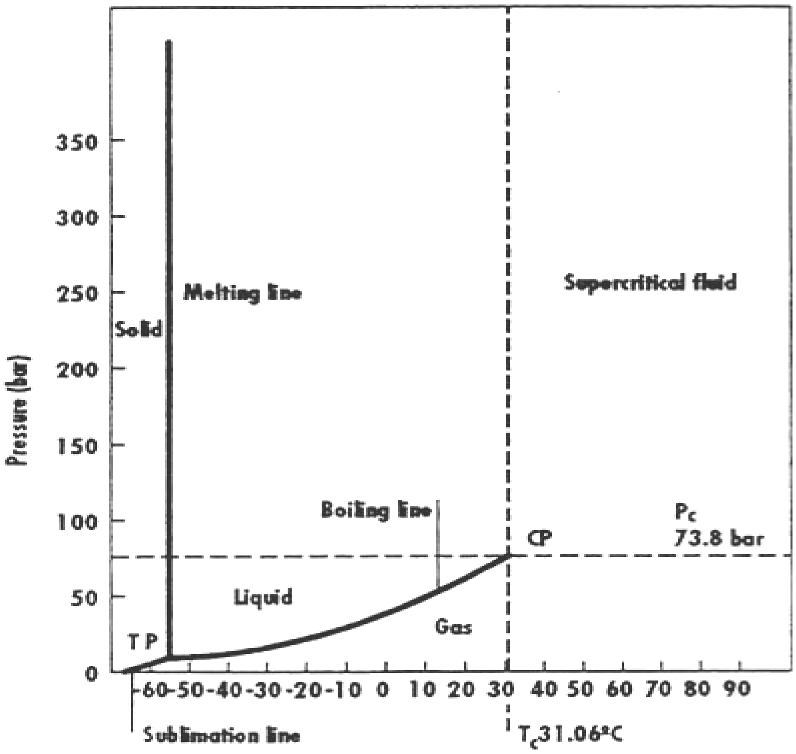

Figure 2.1: Phase (pressure-temperature) diagram for CO_2: CP=critical point, TP=triple point, P_c=critical pressure, T_c=critical temperature. (Source: Brogle, 1982)

2.1.3 Properties of Supercritical Fluid

2.1.3.1 Density and Solvent Power

Brogle (1982) discovered that the solvating power of the supercritical fluid is dependant on temperature and pressure. At low pressure, the solvent power of CO_2 surprisingly decreases with rising temperature whereas at high pressure it increases as measured by his experiment on naphthalene solubility (Figure 2.2).

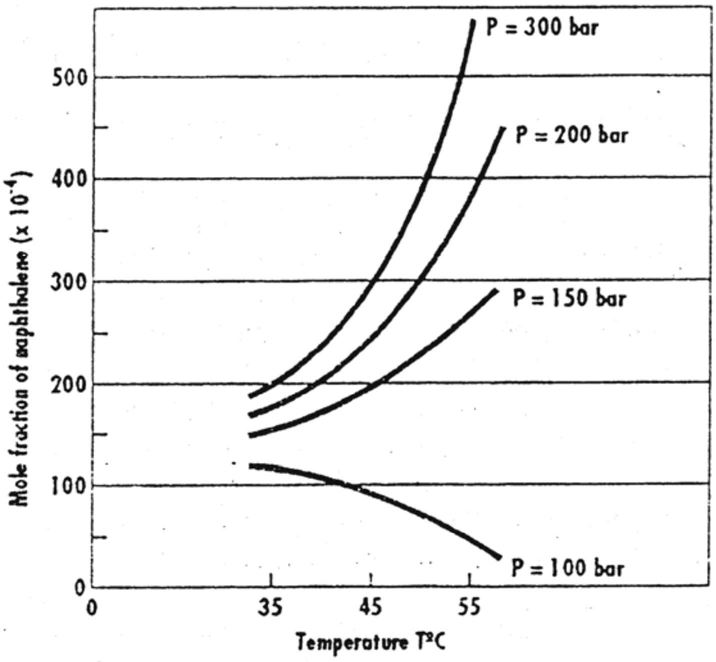

Figure 2.2: Solubility (mole fraction) of Naphtalene in CO_2 as a function of temperature at various pressures (Source: Brogle, 1982)

As for solubility-temperature relationship being much simpler, the pressure was replaced by density. This is because density decreases with an increase in temperature at low pressure and still changes in temperature haven't much effect on density at high pressure (Figure 2.3). Thus density, not pressure, is proportional to the solvent power of the supercritical fluid. It is based on many solubility measurements in the region from ambient condition to 1000 bar and 100^{O}C that solvent power of a supercritical fluid increases with; density at a given temperature; and temperature at a given density.

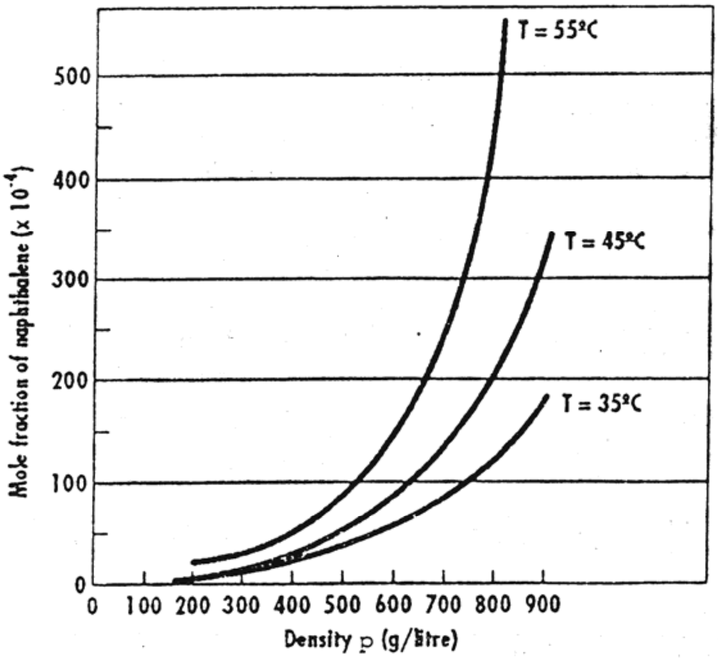

Figure 2.3: Solubility (mole fraction) of Naphtalene in CO_2 as a function of density at various temperatures (Source: Brogle, 1982)

Table 2.1 gives a detailed listing of the pressure (in MPa) requirements to achieve specific CO_2 densities at various temperature. The listing emphasizes the need for higher pressure at higher temperature to achieve specific density.

Table 2.1: Density-Temperature-Pressure Relationship for CO_2

Density (g/mL)	313	323	333	343	353	363	373	383	393
1.000	52.6	61.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.95	38.3	46.3	54.4	64.4	68.0	n.a.	n.a.	n.a.	n.a.
0.90	28.1	35.0	42.0	48.9	51.8	n.a.	n.a.	n.a.	n.a.
0.85	21.1	26.9	32.9	40.1	44.7	n.a.	n.a.	n.a.	n.a.
0.80	16.4	21.3	26.4	31.4	36.5	41.6	46.7	n.a.	n.a.
0.75	13.4	17.5	21.8	26.1	30.5	34.8	39.2	43.6	51.0
0.70	11.5	15.0	18.7	22.3	26.0	29.7	33.4	37.2	42.5
0.65	10.4	13.3	16.5	19.6	22.7	25.9	29.0	32.2	35.4
0.60	9.7	12.2	14.9	17.6	20.3	22.9	25.6	28.4	31.1
0.55	9.3	11.5	13.8	16.1	18.3	20.6	23.0	25.2	27.6
0.50	9.1	10.9	12.9	14.8	16.8	18.8	20.7	22.7	24.6
0.45	8.9	10.4	12.2	13.8	15.5	17.2	18.8	20.5	22.1
0.40	8.7	10.0	11.5	12.9	14.3	15.7	17.1	18.5	19.7
0.35	8.4	9.6	10.8	12.0	13.2	14.4	15.5	16.7	17.8
0.30	8.1	9.0	10.1	11.1	12.1	13.0	14.0	14.9	15.8
0.25	7.7	8.4	9.3	10.0	10.8	11.6	12.3	13.0	13.7
0.20	7.0	7.5	8.2	8.8	9.4	9.9	10.5	11.0	11.6

Source: Hewlett Packard Co. (Wilmington, DE, USA)
n.a = data not available
pressure is given in MPa
temperature is given in K

Source: Taylor (1996)

As for relationship between density and pressure, at a reduced temperature ($T_R = T/T_c$), from 0.9 to 1.2, small increase in reduced pressure ($P_R = P/P_c$) will result in a dramatic increase in solvent reduced density ($\rho_R = \rho/\rho_c$) from 0.1 (near gas density) to 2.5 (near liquid density) (Figure 2.4). As solvent reduced density became near liquid density, supercritical fluid will acted as a liquid solvent. By altering pressure and temperature values, the properties of supercritical fluid could change from gas which has low solvent power to liquid (higher solvent power).

Solvent with high solvent power have a lower selectivity because more compounds from a mixture of components are soluble. Whereas solvent with low solvent power have higher selectivity

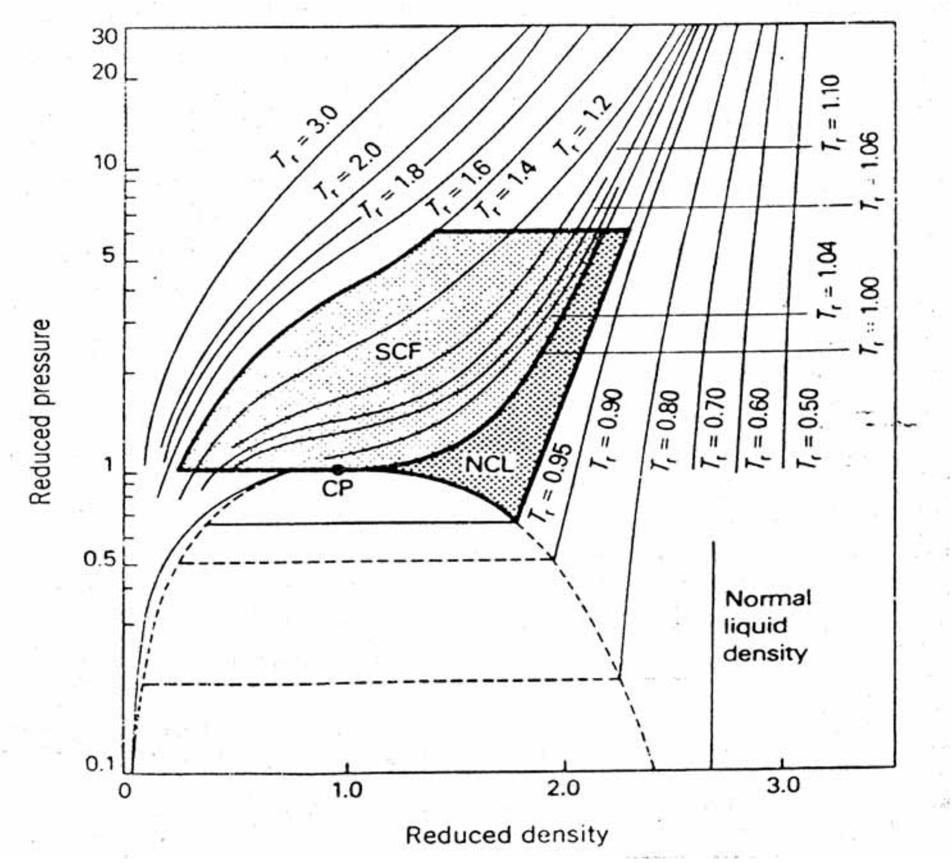

Figure 2.4: Reduced pressure (P_R) – reduced density (ρ_R) diagrams at various temperatures (T_R). SCF = supercritical fluid region; NCL = near critical liquid region. (Source: de Filippi, 1982)

2.1.3.2 Diffusivity and Viscosity

Characteristic values for the gaseous, supercritical fluid, and liquid state are listed in Table 2.2. In the supercritical state, liquid-like densities are approached, while viscosity is near that of normal gases, and diffusivity is about two orders of magnitude higher than in typical. Table 2.3 shows a more specific comparison of supercritical CO_2 with the properties of other organic liquid solvents.

Table 2.2: Comparison of Physical Properties for Gaseous, Supercritical Fluid and Liquid CO_2

	Density (g/mL)	Dynamic Viscosity (g/cm-sec)	Diffusion Coefficient (cm^2/sec)
Gas (ambient)	0.0006-0.002	0.0001-0.003	0.1-0.4
Supercritical Fluid (T_c, P_c)	0.2-0.5	0.0001-0.0003	0.0007
Liquid (ambient)	0.6-1.6	0.002-0.03	0.000002-0.00002

Source: Stahl et al (1988)

Table 2.3: Comparison of Physical Properties of Supercritical Fluid CO_2 with Liquid Solvents at 298 K

	CO_2*	n-Hexane	Methylene Chloride	Methanol
Density (g/mL)	0.746	0.660	1.326	0.791
Kinematic viscosity ($m^2/s \times 10^7$)	1.00	4.45	3.09	6.91
Diffusivity of benzoic acid ($m^2/s \times 10^9$)	6.0	4.0	2.9	1.8

*20.27 MPa, 328 K

Source: Lee and Markides (1990)

As was the case for density, values for viscosity and diffusivity are dependant on temperature and pressure. The viscosity and diffusivity on the supercritical fluid approach those of a liquid as pressure is increased, whereas an increase in temperature leads to an increase in viscosity of a gas, the opposite is true in the case of supercritical fluids. Diffusivity, on the other hand will increase in temperature. As shown in Figure 2.5 and Figure 2.6, changes in viscosity and diffusivity are most pronounced in the region about the critical point. Even at high pressures (300-400 atm) viscosity and diffusivity of supercritical fluids differ by 1-2 orders of magnitude from normal liquids.

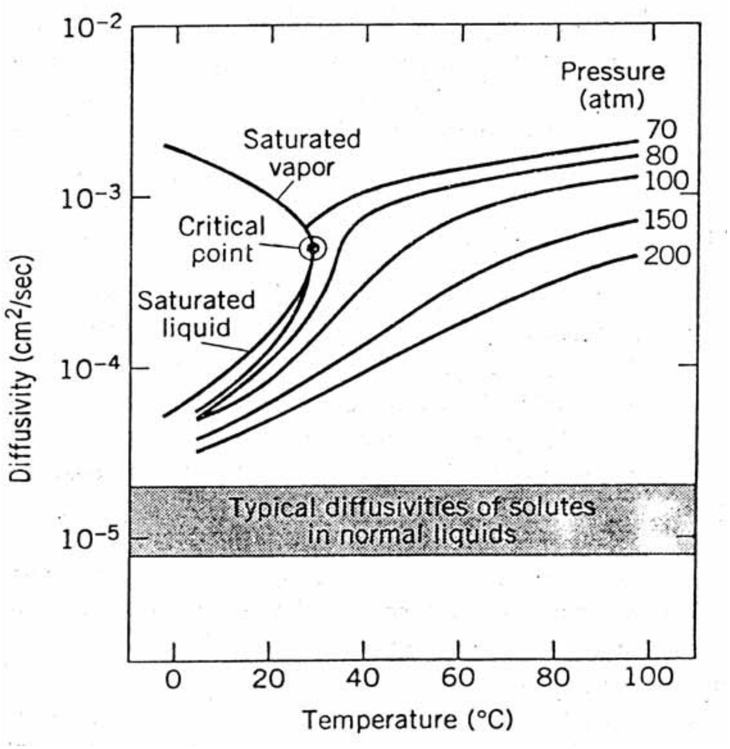

Figure 2.5: Diffusivity of CO_2 versus temperature at various pressures (Source: McHugh and Krukonis, 1986)

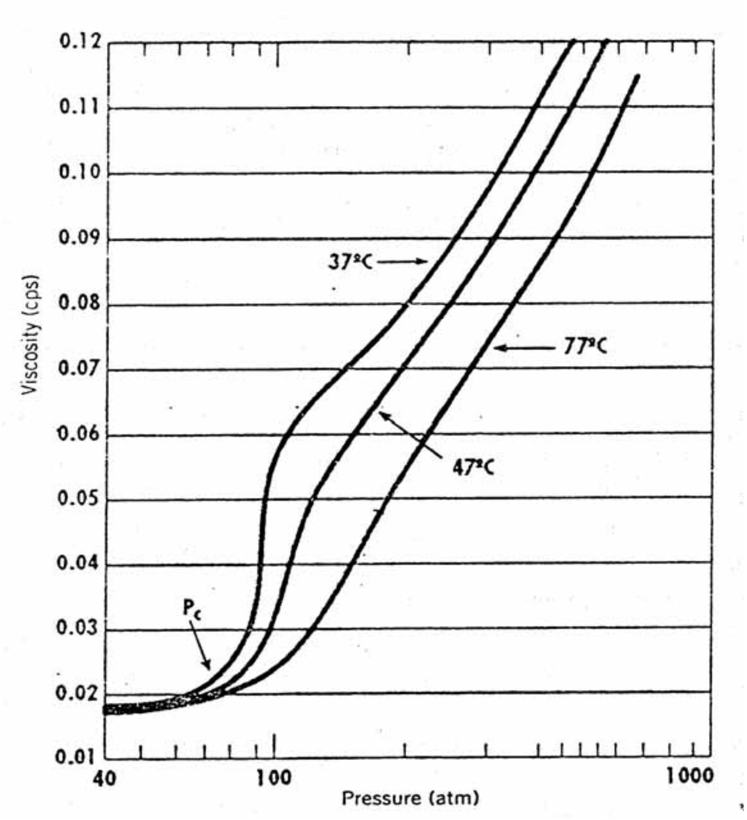

Figure 2.6: Viscosity behaviour of CO_2 at various temperatures and pressures.
(Source: Taylor, 1996)

2.1.4 Supercritical Fluid Extraction (SFE)

2.1.4.1 Supercritical Fluid Extraction and its advantages

Supercritical fluid extraction utilizes the ability of certain chemicals to become excellent solvents for certain solutes under a combination of temperature and pressure. Other major advantages of supercritical fluid extractions as reported by Taylor (1996) are summarized as follows:

1. Provide quantitative or complete extraction – in SFE, a fresh fluid is continuously forced to flow through the samples
2. Remarkably high selectivity – in SFE, the solvation power of the fluid can be manipulated by changing pressure (P) and/or temperature (T). This tunable salvation power of supercritical fluids is particularly useful for the extraction of complex samples such as plant material
3. Eliminate sample concentration process – solutes dissolved in supercritical CO_2 can be easily separated by depressurization. Therefore sample concentration process, which is usually time consuming and often results in loss of volatile components, could be eliminated
4. Ideal technique to study thermally labile compounds and may lead to the discovery of new natural compound – as SFE could be performed at low temperature. Many undesirable reactions such as hydrolysis, oxidation, degradation and rearrangement could be effectively prevented, and common difficulties for quality assessment in classical hydro distillation could be avoided

5. Compared with the 20 – 100g of samples typically required in liquid-solid extraction (L-S methods), as little as 0.5-1.5 g of samples are needed in SFE methods. It has been reported that from only 1.5 g of fresh plant samples, more than 100 volatile and semi volatile compounds could be extracted and detected by gas chromatography-mass spectrometry (GC-MS), of which more than 80 compounds were in sufficient quantity for accurate quantifications.
6. Supercritical fluids use no or significantly less environmentally hostile organic solvents. A SFE method may need no or only a few milliliters of an organic solvent while a typical liquid-solid extraction method would require tens to hundred of milliliters.
7. SFE may allow direct coupling with a chromatographic method, which can be useful means to extract and directly quantify highly volatile compound.
8. The fluid, usually CO_2, in large scale SFE processes, can be recycled or reused thus minimizes waste generation.
9. Supercritical fluids have relatively lower viscosity and higher diffusivity (the diffusivity for supercritical fluids is 10^{-4} cm^2s^{-1} and for liquid solvents is 10^{-5} cm^2s^{-1}). Therefore, it can penetrate into porous solid materials more effectively than liquid solvents and, consequently, it may render much faster mass transfer resulting in faster extractions. For example, with comparable or better recoveries, the extraction time could be reduced from hours or days in a liquid-solid extraction method to a few tens of minutes in SFE

2.1.4.2 Carbon Dioxide (SC-CO_2) as Supercritical Fluid Extraction solvent

CO_2 is the solvent of choice for use in supercritical fluid extraction because it is Generally Regarded as Safe (GRAS), nonflammable, non-corrosive and inexpensive. CO_2 has low critical temperature, T_c (31.06^{O}C) and low critical pressure, P_c (7.386 MPa) as perceived from Table 2.4, which is adapted from a list of 73 pure supercritical fluids by Rizvi et al (1994).

Products of biological origin are often thermally labile, lipophilic, non-volatile and required to be kept and processed around room temperature. As CO_2 has a critical temperature of 31^{O}C, it is a particularly attractive medium for the extraction of biological material. From the table, fluids other than CO_2 showing critical temperature in its vicinity are often difficult to handle and to obtain in a pure form, may be toxic or give rise to explosive mixtures, ecologically prohibited or highly reactive chemicals (Rizvi et al, 1994).

Other factors of using CO_2 as a solvent are since it is relatively nontoxic, does not support combustion, exhibit readily attainable critical parameters, is commercially available in high purity and is environmentally compatible.

SFE methods employing SC-CO_2 can result in reduction extraction times and can be automated (Lehotay et al, 1995). In addition, SC-CO_2 can be removed easily from the extract (no solvent residue) and there are no costs associated with solvent waste disposal (Stahl et al, 1980)

Table 2.4: **Critical points of gases**

Fluid	Critical Temperature (K)	Critical Pressure (MPa)
Helium 3	3.311	0.115
Helium 4	5.188	0.227
Hydrogen	33.25	1.297
Neon	44.40	2.6545
Nitrogen	126.24	3.398
Carbon monoxide	132.85	3.494
Argon	150.66	4.860
Oxygen	154.58	5.043
Methane	190.55	4.595
Krypton	209.46	5.49
Carbon tetrafluoride	227.6	3.74
Ethylene	282.35	5.040
Xenon	289.7	5.87
Chlorotrifluoromethane	302.0	3.92
Carbon dioxide	**304.17**	**7.386**
Ethane	305.34	4.871
Acetylene	308.70	6.247
Nitrous oxide	309.15	7.285
Sulfur hexafluoride	318.82	3.765
Hydrogen chloride	324.55	8.263
Bromotrifluoromethane	340.08	3.956
Propylene	365.05	4.600
Chlorodifluoromethane	369.27	4.967
Propane	369.85	4.247
Hydrogen sulfide	373.40	8.963

Source: Rizvi et al (1994)

2.1.4.3 Review on Supercritical Fluid Extraction of Seeds

Supercritical fluids have been used to extract wide range of analytes from botanical samples. These analytes range from essential oils to phytochemicals, and can include lipid extraction. These extracts have been used for analytical, supplementation and flavor and fragrance purposes. Among this, seeds are a part of products that are most commonly extracted. For example, cloudberry seed produce oil that is low in saturated fats and contains relatively high amounts of tocopherols and carotenoids. The extraction of cloudberry seed oil by supercritical fluid extraction was compared to that of a Soxhlet extraction using diethyl ether as the solvent. The range of extraction conditions consisted of 9-30 MPa at either $40^{O}C$ or $60^{O}C$. The extracts were analyzed by GC-FID and HPLC for their fatty acid and carotenoid/ tocopherol content respectively. There was no significant difference in the fatty acid composition of any of the extracts; the carotenoid content did not increase at pressures over 15 MPA, and the amount of tocopherols extracted decreased with increasing pressure (Manninen et al, 1997).

Apart from that, lipids have been extracted from a series of different types of nuts and oil seeds. An example for this is pecan seeds. A reduction in the lipid content of pecans can increase their storage life. It was determined that the optimum extraction conditions for pecan oil were 66.8 MPa and $75^{O}C$. The kinetics of this extraction was also determined to be limited by the solubility of pecan oil in supercritical CO_2 for the initial part of the extraction, while it was diffusion limited for the second half of the extraction (Alexander et al, 1997).

In the case of other extraction conditions, in SFE of β-carotene from paprika, Weathers et al (1999) observed that when pressure decreased from 338 to 250 bar, which corresponded to a decrease of the fluid density, the extraction efficiency was dramatically reduced. However, in SFE of lignans, schisandrol A, schisandrol B, schisandrin A, schisandrin B and schisandrin C, from *Schisandra chinensis*, Choi et al (1998) found that if the extraction time was sufficiently long (e.g. 30 min 100 mg^{-1} plant material), the temperature (40 - $80^{O}C$) and pressure (135 - 340 bar) of the SC-CO_2 had no distinct effect on the extraction yields of the targeted lignans. But if the extraction time was 6 min, increasing the pressure greatly enhanced the yields of the lignans.

Other examples of supercritical fluid extraction of seeds are extraction of triglycerides from different plant seeds; soybeans, canola seeds (Stahl et al, 1980; Friedrich et al, 1982, Taylor et al, 1993), corn (List et al, 1984; Taylor et al, 1993), cottonseed (List et al, 1984; Snyder et al, 1984) and peanuts (Snyder et al, 1984). Taylor et al, (1993) provided an example of SFE for three different oilseed types (Table 2.5). The SFE results agree well with those obtained by Soxhlet extraction method using gravimimetry to determine the final oil yield.

Table 2.5: Extraction of Various Seed Oil: SFE versus Soxhlet

Sample	Weight percent of recovery (±SD)	
	SFE[a]	Soxhlet[b]
Soybean flakes	20.6 (±0.2)	20.5 (±0.2)
Canola	39.8 (±0.5)	40.5 (±0.5)
Wet-milled corn germ	48.9 (±0.5)	50.4 (±1.3)

[a]n = 4; [b]n = 5

Source: Taylor et al (1993)