การใช้คาร์บอนไดออกไซด์ภาวะเหนือวิกฤตในการสกัดน้ำมันหอมระเหยจากไม้กฤษณา *Aquilaria subintegra*

นางสาวธัญญาภรณ์ รำเพย

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UTILIZATION OF SUPERCRITICAL CO_2 IN ESSENTIAL OIL EXTRACTION FROM AGARWOOD *Aquilaria subintegra*

Miss Thanyaporn Rumpoei

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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Thesis Title	UTILIZATION OF SUPERCRITICAL CO_2 IN ESSENTIAL OIL EXTRACTION FROM AGARWOOD *Aquilaria subintegra*
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Field of Study	Petrochemistry and Polymer Science
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ธัญญาภรณ์ รำเพย : การใช้คาร์บอนไดออกไซด์ภาวะเหนือวิกฤตในการสกัดน้ำมันหอมระเหยจากไม้กฤษณา *Aquilaria subintegra* (UTILIZATION OF SUPERCRITICAL CO_2 IN ESSENTIAL OIL EXTRACTION FROM AGARWOOD *Aquilaria subintegra*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.ศุภวรรณ ตันตยานนท์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.ดร.สมเกียรติ งามประเสริฐสิทธิ์, 78 หน้า.

งานวิจัยนี้ศึกษาการสกัดไม้กฤษณา *Aquilaria subintegra* ด้วยคาร์บอนไดออกไซด์ภาวะเหนือวิกฤต พบว่าไม้กฤษณาบดละเอียดให้ปริมาณน้ำมันหอมระเหยที่มากกว่าไม้กฤษณาบดหยาบ 4 เท่า โดยสภาวะที่ใช้ในการทดลองเพื่อศึกษาผลของปัจจัย คือช่วงอุณหภูมิ 40-70 °C ความดัน 12-25 MPa และอัตราการไหลของคาร์บอนไดออกไซด์ 1-4 กรัม/นาที ทำการวิเคราะห์หาองค์ประกอบทางเคมีด้วยเทคนิคแก๊สโครมาโทกราฟี และแก๊สโครมาโทกราฟีร่วมด้วยแมสสเปกโทรเมตรี เทียบกับน้ำมันหอมระเหยเชิงพาณิชย์ พบว่าที่อุณหภูมิ 65 °C ความดัน 12 MPa และอัตราการไหลของคาร์บอนไดออกไซด์ 3 กรัม/นาที เป็นภาวะที่เหมาะสมที่สุด โดยพิจารณาจากการพบสารกลุ่ม sesquiterpenoid เช่นเดียวกับที่พบในน้ำมันหอมระเหยเชิงพาณิชย์ จากนั้นศึกษาผลของตัวทำละลายร่วม (เมทานอล, เอทานอล และเอทิลแอซิเตท) ที่ใช้ในการสกัดด้วยคาร์บอนไดออกไซด์ภาวะเหนือวิกฤต และเติมลงไปในปริมาณ 5-10%v/v พบว่าถึงแม้จะให้ปริมาณผลิตภัณฑ์สูงขึ้น แต่เมื่อนำไปวิเคราะห์หาองค์ประกอบทางเคมีพบว่ามีสารกลุ่ม sesquiterpenoid เพียงเล็กน้อย ส่วนมากเป็นสารกลุ่ม chromone นอกจากนี้พิจารณาการสกัดไม้กฤษณาโดยการกลั่นด้วยไอน้ำ และการสกัดด้วยตัวทำละลายแบบซอกส์เลต พบว่าแม้การสกัดด้วยตัวทำละลายแบบซอกส์เลตจะให้ปริมาณผลิตภัณฑ์สูงสุด แต่องค์ประกอบทางเคมีที่ได้ประกอบด้วยคาร์บอกซิลิกสายโซ่ยาว และสารในกลุ่ม chromone

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต........................
ปีการศึกษา..........2552.......... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก
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THANYAPORN RUMPOEI : UTILIZATION OF SUPERCRITICAL CO_2 IN ESSENTIAL OIL EXTRACTION FROM AGARWOOD *Aquilaria subintegra*. THESIS ADVISOR: ASSOC.PROF. SUPAWAN TANTAYANON, Ph.D., THESIS CO-ADVISOR: ASSOC.PROF. SOMKIAT NGAMPRASERTSITH, Ph.D., 78 pp.

The supercritical CO_2 extraction (SC-CO_2) of agarwood *Aquilaria subintegra* was studied. Extraction of agarwood in fine-milled form with 35 mesh size gave 4 times higher the extraction yield than cut dry agarwood. Various extraction conditions; temperature 40-70 °C, pressure 12-25 MPa, and CO_2 flow rate 1-4 g/min, were investigated. The chemical compositions were analyzed by gas chromatography (GC) and gas chromatography with mass spectrometry (GC/MS). The results were compared with the commercial products. It was found that the optimal condition for extraction was 65 °C, 12 MPa, and CO_2 flow rate of 3 g/min. Considering the extract contained sesquiterpenoid compounds as also found in the commercial ones. Three different co-solvents: methanol, ethanol, and ethyl acetate, were used in SC-CO_2 extraction and the additional volume was varied 5-10%v/v. Although the extraction gave higher yield, their GC chromatogram showed few sesquiterpenoid derivatives whereas many chromone derivatives were obtained. In addition, the extraction by the steam distillation and soxhlet extraction of agarwood were investigated. Although the soxhlet extraction gave the highest yield among all methods, the chemical compositions consisted of long chain carboxylic acid and chromone derivatives.

Field of Study : Petrochemistry and Polymer Science
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Student's Signature
Advisor's Signature
Co-Advisor's Signature

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LIST OF ABBREVIATIONS

°C	: Degree Celsius
MPa	: Mega Pascal
g	: Gram
h	: Hour
mL	: Milliliter
w/w	: Weight Percentage

CHAPTER I

INTRODUCTION

1.1 The Statement of problem

Agarwood or aloewood, is a botanical plant in several countries. It is classified under the family Thymelaeaceae. In Thailand, there are five indigenous species of agarwood, i.e., *Aquilaria subinteqra*, *A. crassna*, *A. malaccaensis*, *A. hirta*, and *A. rugosa*. The *A. subinteqra* is native to Rayong, Chantaburi, Trad, and Chonburi provinces [1]. Its heartwood is fine, black or brown in color and fragrant. The aroma of the wood comes from sesquiterpene compounds [2]. The plant synthesizes these aromatic terpenes when it is injured by insects, physical cuts, bacterial infections and chemical stimulation. The agarwood is sold in 3 forms as pieces of heartwood, heartwood powder and oil. The heartwood is burned for aromatic vapor in houses and shrines and carved for art and the Holy Spirit image. The powder is used for making incense and medicine [3]. The agarwood is claimed to possess aphrodisiac, flatulence and diuretic properties. It is one of the ingredients used in the treatment of smallpox, rheumatism, spasm of bronchous and respiration, abdominal cramp, diarrhea, nausea, vomiting, anxiety, elderly fatigue, pregnancy and post partum illness [4]. The agarwood oil is used in perfumery and incense industries. The classical method that is currently used in commerce for the agarwood oil extraction is hydrodistillation. These procedures performed at high temperature can lead to degradation of thermally labile compounds resulting in the formation of undersirable and unpleasant compounds. Moreover, this method consumes 7-15 days and high energy for extraction [5].

The supercritical fluid carbon dioxide (SC-CO_2) extraction is known as non-flammable, non-toxic, chemically stable and less energy consumption method. It provides some advantages over the classical method, since supercritical carbon dioxide (SC-CO_2) has liquid-like density, low viscosity, high diffusivity, good transport properties and gives faster extraction and high yields [6]. Nowadays SC-CO_2 method has been used to extract volatile components from various kinds of spices and plants for flavor and fragrance ingredients in pharmaceutical, cosmetic, and food industries [7]. The aim of this research is the investigation of various extraction parameters such as pressure, temperature, particle size, CO_2 flow rate, and the type and amount of co-solvent on the extraction yield (in % weight) and the chemical composition (in % peak area). The results are then compared with the extraction agarwood with steam distillation and soxhlet extraction method.

1.2 Objectives of the research work

1. To study the extraction of agarwood oil by using SC-CO_2 as a solvent.
2. To study the effect of temperature, pressure, CO_2 flow rate, and different co-solvent on oil extraction from agarwood and find the optimal condition.

1.3 Scope of the research work

1. Literature survey.
2. The investigation of the effect of various extraction parameters on agarwood oil extraction by using SC-CO_2.
    - The effect of extraction temperature: 40-70 °C
    - The effect of extraction pressure: 12-25 MPa
    - The effect of CO_2 flow rate: 1-4 g/min
3. The investigation of the effect of co-solvent on agarwood oil.
4. The study of the extraction of agarwood oil by using soxhlet extraction and steam distillation for comparison.
5. Gas-chromatography and Gas-chromatography with mass spectrometer analysis for agarwood oil compositions obtained by SC-CO_2 extraction compared with agarwood oil obtained by soxhlet extraction.
6. The summary of the results and write thesis.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Agarwood plant [8-9]

Native name: crissna (East of Thailand)
Gayucaru, gayucahy, gayudeungpoo (Malaysia, Pattanee province of Thailand)
Maihom(East and South of Thailand)
Acaru, takorn (Bali)
Timheang, Chen Xiang (China)
Seeseadnam (Bureerum Province of Thailand)
Tagraunam (Chantaburi Province of Thailand)
Maipoumgmaprao (South of Thailand)
Gaharu, kikaras, mengkaras (Indonesia)
Gaharu, tengkaras, karas (Malaysia)
Agar (Burma)
Eagle Wood, Aglia, Lignum Aloes, Agarwood, Calambac, Akyaw, Aloewood, Calambour (English)
Sasi (Assamese)

Thymelaeaceae genus: Aquilaria

Five type of aquilaria can be found in Thailand:

A. crassana: in the Middle, North, North-East
A. malacensis: in the South
A. subintegra: in the East
A. hirta: in the South
A. rugosa: in the North

2.1.1 General characteristic

From Figure 2.1, aquilaria is medium to large tree, non-shed leaves. It is 8-21 meters up stall. Its circumference is about 1.5-4.5 meters. Peak of the tree is cone shape. Trunk is straight and usually has section at base when it is old. Outer shell is smooth and gray-white color. Shell thick about 5-10 millimeters. It has holes to release air in general area. Outer shell will crack when it is old. Inner shell is white-yellow and has hair, which look like silk, at peak.

Figure 2.1 Aquilaria tree.

2.1.2 Characteristic of wood

Aquilaria wood has both of normal wood and fragrant wood which has Aquilaria oil. Normal wood normally is white-cream color on newly cut.After that, it will change to light brawn. Splinter is straight. Wood is medium rough. It can be easily saw. Rubbing to be shadow is bad. It is not durable. Staying in water is medium durable. When transformation, it should be dry suddenly. In expose to the air, it is easily split and hold fungus. This make loss color. Specific gravity is 0.52 and natural durable is 0.5-7.5 years. The average of durable is 1.1 year.

Wood which has Aquilaria oil is black, heavy and drown, Quality of wood depends on accumulating of Aquilaria oil in cells of wood. Chemical component of aroma oil from Aquilaria compose of much resin, fragrant substance (Sesquiterpene alcohol: Dihydroagarofuran, Agarofuran, Agarofan, Agarospirol, Eudesmane, Valencane, Eromphilane, Vetispirane) and derivative of Chromone.

Quality checking of Aquilaria wood is let it into water. If it flush with water, it is excellent and be called “Gharki”. This wood type is black and very fragrant. If it soar, usually light brawn or dark blue, its quality inferior to the black and be called “Neem Ghaki”. If it drawn, it is low quality and be called “Samaleh”.

2.1.3 Important substance

Essential oils Agarol; agarospiol; agarofuran; dihydroagarofuran; 4-hihydroxydihydroagarofuran,3,4-hihydroxydihydroagarofuran; nor-ketoagarofuran; baimuxinol; dehydrobaimuxinol; baimuxinic acid; bimuxinal; dehydromaimuxinaol; sinenofuranal; sinenofuranol; agarotetrol; aquillochin; benzyl acetone; dehydrojinkoh-eremol; 3,5-dinitrobenzoate; (-)-1,11-epoxyguaia-11-ene; (-)-guaia-1-diene-15 ol; (-)-guaia-1,11-diene-15-carboxylic acid; (1)-quaia-1,11-diene-9-one; (-)-quaia-1,11-diene-15,2-olide; (-)-quaia-1,11,dien-15-al; hydroxycinnamic acid; hydroxyisoagarotetrol; 9-hydroxyselina-4,11-dien-14-oic acid; isoagarotetrol; liriodenine; methoxyguaia-1,11-dien-15-carboxylate; 2-(2-phynylethyl)-7-methoxychromonyl-6-oxychromone; 2-(2-phenylethyl)-6,7,8-trihydroxy-5,6,7,8 tetrahydro5-2-(2-phenylethyl) chromonyl-6-oxy chromone; 2-(2-phenyl)5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydro-chromone; rotundone; (-)-selina-3,11-dien-14 al; (+)-selina-4,11-dien-14-al; selina-3,11-dien-14-oic acid; selina-4,11-dien-14-oic acid; (-)-selina-3,11-dien-9-one; (+)-selina-3,11-dien-9-ol, 1,3-dibehenyl-2-ferulyl glyceride, 12-O-n-deca-2,4,6-trienoylyphorbol-13-acetate; 5-hydroxy-6-methoxy-2-(2-phenylethyl)chromone; 6-hydroxy-2-(2-hydroxy-2-phenylethyl) chromone; ; abietane ester 6-methoxy-2-(2-phenylethyl)chromone; 8-chloro-2-(2-phenylethyl)-5,6,7-trihydroxy-5,6,7,8-tetrahydrochromone; 6,7-dihydroxy-2-(2-phenylethyl)-5,6,7,8-tertahydrochromone; 7,8-dimethoxy-2-[2-(3’-acetoxyphenyl)ethyl]chromone; 6,7-dimethoxy-2-(2-phenylethyl)chromone;

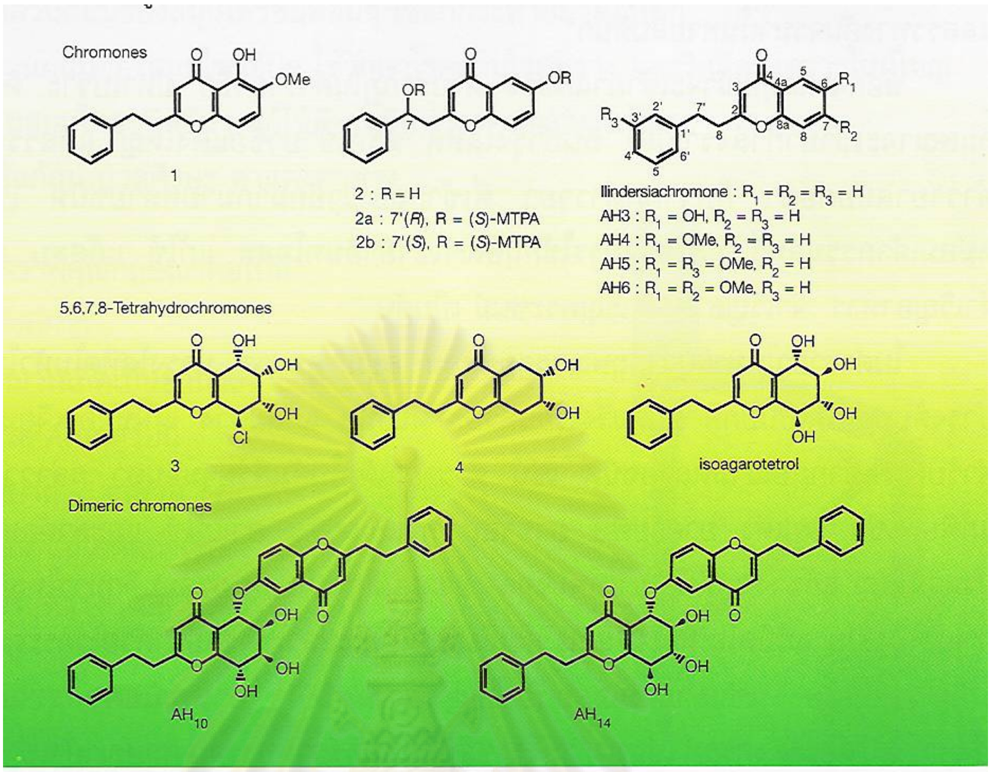


Figure 2.2 Some structural formula of chromone derivatives found in Aquilaria.

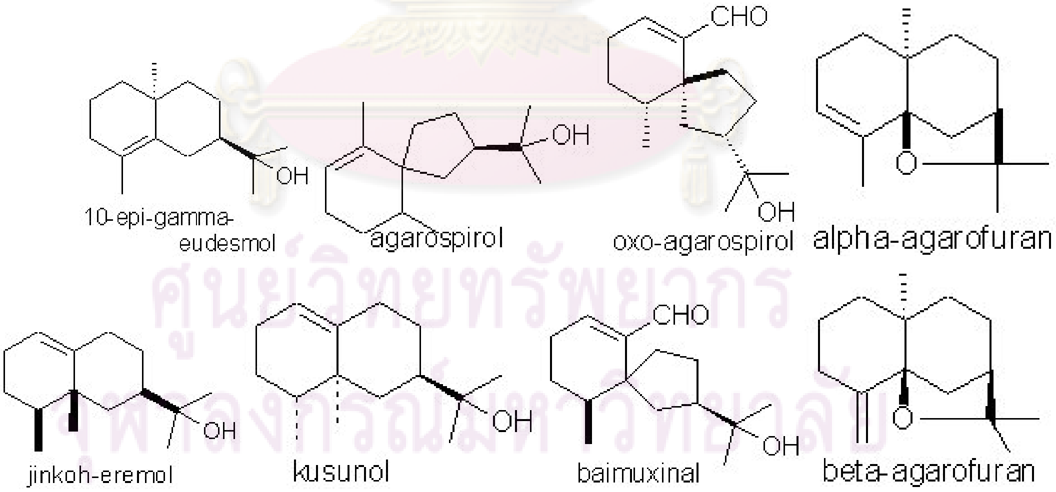


Figure 2.3 Some structural formula of sesquiterpene derivatives found in Aquilaria.

2.2 Essential oil

Essential oils are the volatile, aromatic oils obtained by steam or hydrodistillation of botanicals. Most essential oils are primarily composed of terpenes and their oxygenated derivatives. Different parts of the plants can be used to obtain essential oils, including the flowers, leaves, seeds, roots, stems, bark, wood, etc. Certain cold-pressed oils, such as the oils from various citrus peels, are also considered to be essential oils but these are not to be confused with cold-pressed fixed or carrier oils such as olive, grapeseed, coconut etc. which are non-volatile oils composed mainly of fatty acid triglycerides. Other aromatic, plant-derived oils, which technically aren't essential oils because they are solvent extracted, include Absolutes (hexane followed by ethanol extraction), CO_2 (liquid carbon dioxide used as the solvent) and Phytols or Florosols (fluoro-hydrocarbon solvent).

2.2.1 Agarwood essential oil

Agarwood oil is extracted from the wood of Agar. There are different grades of Agarwood oil. Agarwood comes in solid or liquid form. Solids are only solid at room temperature, and if warmed slightly, it turns to mobile liquid. It is an anti-asthmatic and can be applied directly to the skin as it is non-irritating. The oil is very tenacious and only the tiniest of drops is needed to fill the air with its soul evoking aroma. It is a complex aroma with many nuances, deep and ethereal. The aroma takes about 12 hours to unfold and it will last on the skin for more than a day, and if placed on any material, the scent can last for months. It can be used as a perfume, an aroma therapy and an essential oil or as an aid for the deepest meditation. This fragrance will unlock the subconscious and allow you to go deep into your memories.

2.2.2 Benefits of essential oil

Essential oils is the primary ingredients in aromatherapy which are safe and simple, natural products. They can be used just for pleasure, or to help individual heal physical and emotional ailments. It can be a complete which is holistic and natural form of therapy, taking into account the effect of the treatment on the body, the mind and the emotions of the person receiving it. The effectiveness of essential oils usage has been proven by scientific analysis, confirming the intuitive link, understood by our ancestors, between nature and general well-being. The dynamics of aromatherapy enable us to bring the essence of nature into our everyday lives.

2.2.3 Physical properties of essential oil

The first and foremost physical properties of essential oil are the highly fragrant, concentrated, and potent substances. Essential oil has a liquid-soluble molecular structure which allows them to pass easily through the skin. They penetrate into the fat layers of the skin quickly, which is why massage is such an effective treatment. Other than that, it is volatile, so that it is easily evaporate into air. The storage of essential oils is usually in a dark bottle because of it is sensitive to light. Essential oils are not standard product and will vary from batch to batch. Chemical variations will occur based on the time of day, harvest time, growing location and part of the plant to be extracted. They are cytophylactic which regenerate new cells and enhance the function of our organs.

2.2.4 Chemical properties of essential oil

Essential oils may have two major components which are terpene hydrocarbon, and oxygenated compounds. Terpene hydrocarbon can be divided into two group; monoterpenes and sesquiterpenes. While oxygenated compounds are phenols, monoterpenes, and sesquiterpenes alcohols, aldehydes, ketons, esters, lactones, coumarins, ethers, and oxides. Monoterpenes compounds are found in nearly all essential oil and have a structure of 10 carbons atoms and at least one double bond. The 10 carbons atoms are derived from two atoms isoprene units. Monoterpenes react readily to air and heat sources. These components have anti-inflammatory, antiseptic, antiviral, and antibacterial therapeutic properties. Sesquiterpenes consist of 15 carbons atoms and have complex pharmacological actions. It has anti-inflammatory and anti-allergy properties. Based on, there are three main aromatic groups which are phenols, terpenes alcohols, and aromatic aldehydes.

2.3 Extraction theory

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method for extraction is steam distillation, but as technological advances are more efficient then the economical methods has being developed which is solvent extraction method. There are several extraction methods for making resins and extracts from plants, and each will be discussed briefly below. Some plants contain alkaloids as part of their chemical composition, and these different alkaloids will extract into different solvents. For example, *Blue Lotus* contains alkaloids that will only extract into alcohol, whereas *Amanita muscaria* contains an alkaloid that will extract into water, but will be destroyed in alcohol. When doing resin extractions from plants, it is important to know what chemical compounds will extract into what solvents.

2.3.1 Hydrodistillation

Hydrodistillation (Figure 2.4) is the gentler process for obtaining essential oils. In this method, Agarwood chips are fully submerged in water, producing a ‘soup’, and the still is brought to boil. The resultant steam of which contains the aromatic plant molecules being captured and condensed. The oil will normally float on top of the ‘hydrosol’ (the distilled water component) and may be separated off. When the condensed material cooled down, the oil and hydrosol is separated and the decanted oil to be used as essential oil. This method protects the oil so extracted to a certain degree, since the surrounding water acts as a barrier to prevent it from overheating. Hydrodistillation can be performed at a reduced pressure (under vacuum) to reduce the temperature to less than 100°C, which is useful in protecting the plant material as well as essential oil. In spite that the hydro-distillation is, the most common method to extract and isolate the essential oils because for perfumery, the high temperatures can destroy the most delicate fragrance molecules, so hydro-distillation is preferred, but it is a time consuming process and needs a large amounts of plant material.

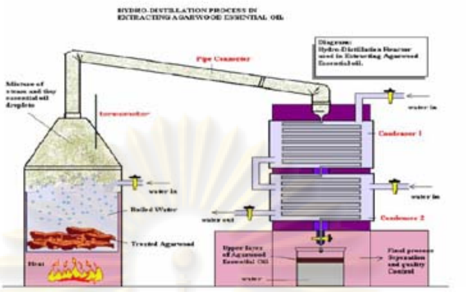


Figure 2.4 Hydrodistillation.

2.3.2 Steam distillation

Steam distillation (Figure 2.5) seems to be the best method for the extraction and isolation of essential oils from the plant materials. The desired plant material is placed onto a still. A still is a specialized piece of equipment that is used in the distillation process. It consists of a vessel into which heat is added and a device that is used for cooling. The plant is first placed into the vessel. Next steam is added and passed through the plant. The heat from the steam helps to open the pockets of the plant that contain the plant's aromatic molecules or oils. Once open, the plant releases these aromatic molecules and in this state, the fragrant molecules are able to rise along with the steam. The vapors carrying these molecules travel within a closed system towards the cooling device. Cold water is used to cool the vapors. As they cool, they condense and transform into a liquid state. The liquid is collected in a container and as with any type of oil/water mixture, it separates. The oils float towards the top while the water settles below. From there, it's a simple matter of removing the oils that have been separated. These are the highly condensed, aromatic oils used in aromatherapy.

Figure 2.5 Steam distillation.

2.3.3 Maceration

Maceration is an extracting process to extract important matter from plant by fermenting plant with solvent in closed container such as Erlenmeyer flask or blow. Fermenting time is seven days, during these time try to shake or stir often. When the time ups, pour the extracted matter from marc as much as possible. Collect the extracted matter and distil them. In case extracting of all the mtter is required, repeat the process may times. The advantage of this extracting technique is using a lot of solvent.

2.3.4 Percolation

Percolation is continuously extracting process. The process will be done by "percolation machine" Fermenting plant with solvent. Leave if for an hour to let it extremely swell and then contain plant dust layer by layer into the percolation. Pour solvent into the container until above the plant dust approximately 0.5 cm. And leave it 24 hours. After that separate the extracted matter from marc. Beware drying plant keep pouring solvent above the olant. Keep the extracted matter until the process is completed. Try to take the extracted matter from marc as much as possible and then distil them from marc.

2.3.5 Enfleurage

The enfleurage process is a cold fat extraction process used on a few types of delicate flowers [8], which yield no direct oil on distillation. Enfluerage process is mainly applied to flowers that do not yield appreciable amounts of oils by steam distillation method or which are too delicate to withstand the temperature of boiling water.

In this method, chassis (glass plates in a frame) are covered with highly purified and odorless vegetable or animal fat and the petals of the botanical matter that are being extracted are spread across it and pressed in. The flowers are normally freshly picked before it encased in their fatty bed. The petals remain in this greasy compound for a few days to allow the essence to disperse into the compound, where the then depleted petals are removed and replaced with a fresh harvest of petals.

This process is repeated until the greasy mixture is saturated with the essence. When the mixture has reached saturation point the flowers are removed and the enfleurage pomade. The fat and fragrant oil are then washed with alcohol to separate the extract from the remaining fat, which is then used to make soap. The mixture is then subjected to heating treatment to boil off alcohol and the remains of essential oil are collected. This method is very labor-intensive and subsequently very costly. This method is sometimes used to extract essential oil from tuberoses and jasmine.

2.3.6 Solvent extraction

A hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solution is filtered and concentrated by distillation, a substance containing resin (resinoid), or a combination of wax and essential oil (known as concrete) remains. The most important factor in a success of this practice is the selection of the solvent. The solvent must be selective, which is quickly and completely dissolve the odoriferous components, but have only a minimum of inert matter, have a low boiling point, be chemically inert to the oil, evaporate completely without leaving any odorous residue, low priced and, if possible, non-flammable.

Solvent extraction uses very little heat so it's able to produce essential oils whose fragrance would otherwise be destroyed or altered during steam distillation. Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost. In this process, a chemical solvent such as hexane is used to saturate the plant material and pull out the essential oils. The plant is removed and this renders a solvent. The solvent is then boiled off under a vacuum or in a centrifugal force machine to help separate it from the essential oil. Because the solvent has a lower boiling point than the essential oil, it evaporates and the oil is left.

The solvent is cooled back into liquid and reclaimed. Along with the essential oil, the fats, waxes, and heavier oils can be extracted. This produces a substance called a concrete. The process is continued by dissolving oils into warm alcohol. The alcohol is removed under a vacuum and pure essential oil is left. Although more cost-efficient than enfleurage, solvent extraction is more expensive than steam distillation and least costly compare to supercritical CO_2 extraction, so it is reserved for costly oils which cannot be distilled. A solvent extracted essential oil is called an absolute.

2.3.7 Soxhlet extraction

Soxhlet extraction (Figure 2.6) is continuous extracting process using a low boiling solvent. The extraction is evaporation solvent in flask and then distil it in thimble which containing plant dust. When solvent extracting chamber is as high as appropriate level, siphon will be happen. The flask is heated by heating metal, so the solvent evaporates and the extracting matter is leave in flask. When the stem solvent contact condenser it will be distilled and become a new extraction. The process will be repeated until the extraction is completed. The extraction by heat may cause some chemical decomposition.

Figure 2.6 Soxhlet extraction.

2.3.8 Supercritical fluid extraction

This process is another method of extraction using carbon dioxide gas which is kept under high pressure at a constant temperature. Plants are placed in a stainless steel tank and, as carbon dioxide is injected into the tank, pressure inside the tank builds up. Under high pressure, the carbon dioxide turns into a liquid and acts as a solvent to extract the essential oils from the plants. When the pressure is decreased, the carbon dioxide returns to a gaseous state, leaving no residues behind. The equipment for this process is very expensive and so are the resulting oils. Carbon dioxide extractions have fresher, cleaner, and crisper aromas than steam-distilled essential oils, and they smell more similar to the living plants because high heat is not used. This extraction method produces higher yields and makes some materials easier to handle. Many essential oils that cannot be extracted by steam distillation can be obtainable with supercritical carbon dioxide extraction.

There are many positive aspects of the supercritical CO_2 extraction process and the resultant supercritical CO_2 essential oils. The CO_2supercritical extraction process eliminates the need for potentially harmful solvents like hexane, avoiding unnecessary environmental pollution and potential human bodily harm. Another very important consideration is that the supercritical CO_2 extraction process avoids heat degradation to the plant matter, producing an essential oil that is a more authentic version of the original plant matter.

Another positive aspect to the CO_2 distillation process is the aroma of the essential oil. The CO_2supercritical extract offers a more genuine aroma of the actual herb, spice or plant. The aroma of the CO_2extracts of ginger, cardamom and other spices are more active, spirited and warm in nature than the rather flat and lifeless aroma of the same plants that have been steam distilled.

Finally, many people are concerned with the higher unit for unit price of the CO_2 extracted essential oil. Although the supercritical extracts often cost more initially, they are typically more concentrated and thus less of these oils is needed in the production of formulas. Because can generally use less of the CO_2 essential oil than the hydro or steam distilled oil, the higher price is sometimes offset.

2.4 Supercritical fluid extraction (SCF) [10]

2.4.1 Supercritical fluid properties

A supercritical fluid (SCF) is a medium in a state at a pressure and a temperature above the critical point (above critical pressure and critical temperature). The critical point defines the end of the existence of a phase equilibrium between a boiling liquid and a saturated vapour (end of boiling curve). Above this end point a differentiation between liquid and vapour is impossible; the temperature and the pressure defining the critical point are named critical temperature and critical pressure and are characterised by component specific values. Are temperature and pressure above the critical point ($p>P_c$; $T>T_c$) the state is called supercritical. Figure 2.8 are show phase diagram of supercritical fluid region.

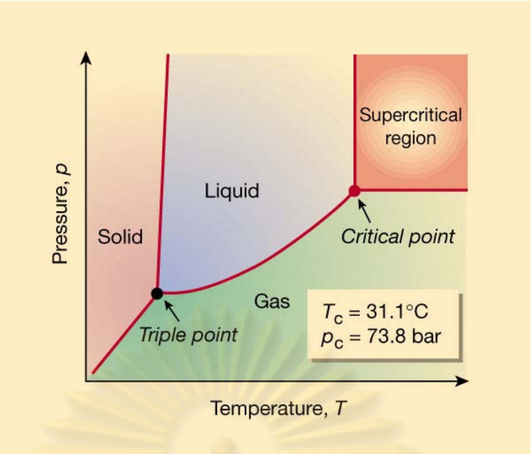


Figure 2.7 Phase diagram of supercritical fluid region. [11]

Supercritical fluids combine properties of liquids (high solubility, high density) and gases (low viscosity). This combination of advantageous properties brings the benefits of the broad field of process applications.

The materials to be used as SCF must therefore have critical temperatures. The most suitable fluids meeting such a criterion are given in Table 2.1.

Table 2.1 Critical properties of various solvents [12]

Chemical	Critical temperature (C)	Critical pressure (bar)
Ammonia	132.5	112.8
Benzene	289	48.9
Carbon dioxide	32.1	73.8
Cyclohexane	280.3	40.7
Ethane	32.2	48.8
Ethylene	9.3	50.4
Isopropanol	235.2	47.6
Propane	96.7	42.5
Propylene	91.9	46.2
Toluene	318.6	41.1
Water	374.2	220.5

Table 2.2 Comparison of average properties of gases, liquids and SCF [12]

State of fluid	**Density** (g/cm3)	**Diffusivity** (cm2/sec)	**Vicosity** (g/cm sec)
Gas			
P=1 atm, T= 15-30 °C	$(0.62\text{-}2)\times10^{-3}$	0.1-0.4	$(1\text{-}3)\times10^{-4}$
Liquid			
P=1 atm, T= 15-30 °C	0.6-1.6	$(0.2\text{-}2)\times10^{-5}$	$(0.2\text{-}3)\times10^{-2}$
Supercritical			
P=Pc, T=Tc	0.2-0.5	0.7×10^{-3}	$(1\text{-}3)\times10^{-4}$
P=4Pc, T=Tc	0.4-0.9	0.2×10^{-3}	$(3\text{-}9)\times10^{-4}$

The SCF transport properties are very attractive as they are dense as liquids but “mobile” like gas (very low viscosity, intermediate diffusivity), as shown on Table 2.2. So, mass transfer (and similarly heat transfer) is fast in SCF in comparison with liquid solvents or water. Moreover, SCF rapidly diffuse in porous media, easing either extraction from solid materials or impregnation of solutes into porous media.

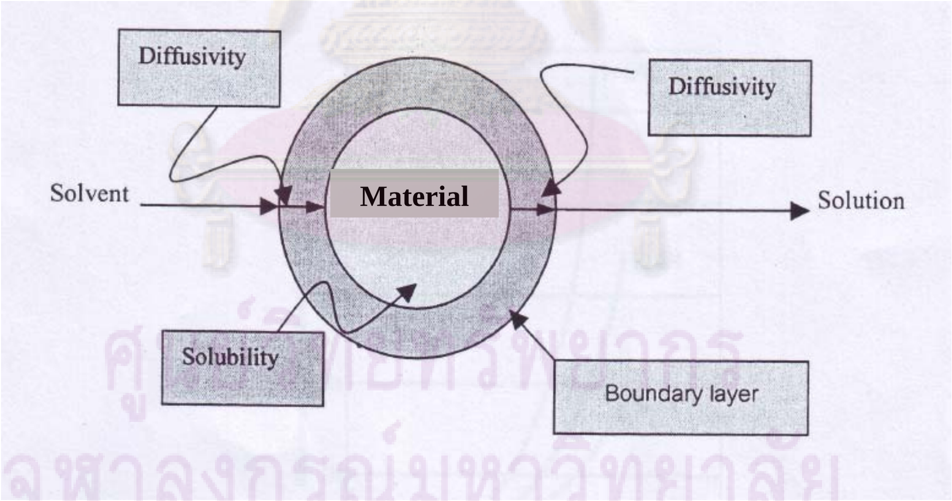


Figure 2.8 Natural product extraction model. [10]

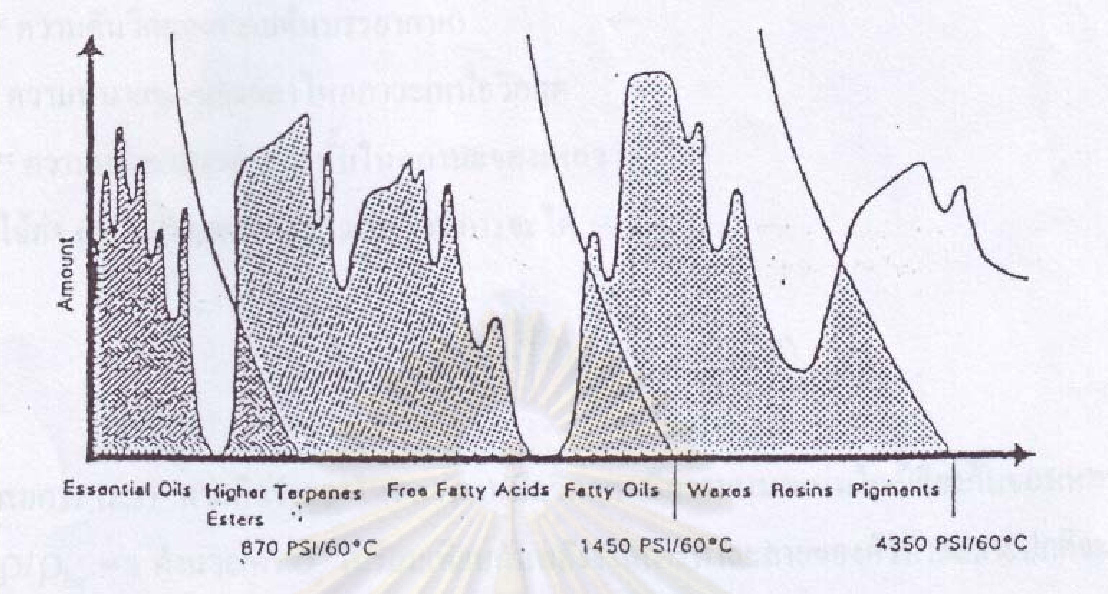


Figure 2.9 Natural product chromatogram obtained by supercritical CO_2 at various extraction conditions. [10]

2.4.2 Effect of parameter on extractability

2.4.2.1 Solvating power

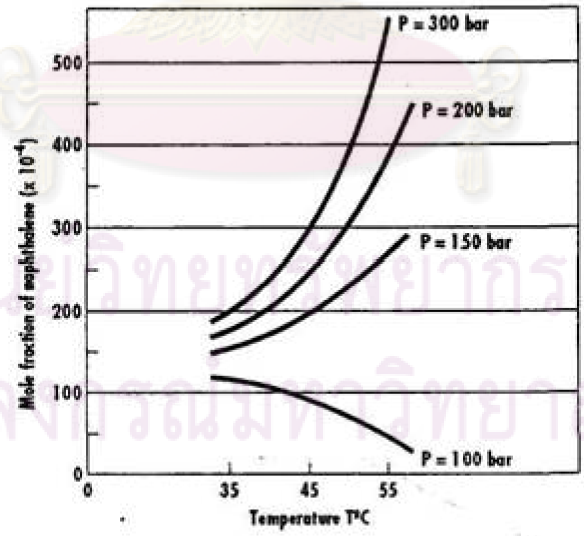


Fig. 2.10 Solubility (mole fraction) of naphthalene in CO_2 as a function of temperature at various pressures. [10]

Figure 2.10 illustrates that at low pressure the solvent power of CO_2 surprisingly decreases with rising temperature; whereas at high pressures it increases in a straightforward fashion as measured by naphthalene solubility. If we replace the parameter "pressure" by the parameter "density," the solubility-temperature relationship becomes much simpler, as shown in Figure 2.10. This anomaly comes about because density decreases dramatically with an increase in temperature at low pressure; whereas at higher pressure, changes in temperature have much less effect on density. Thus density, not pressure, to a first approximation is proportional to the solvent power of the SF. (Supercritical fluid technology would be better served if all scientists discussed experiments in terms of density rather than pressure.) The following trends are based upon many solubility measurements in the region from ambient conditions up to 1000 bar and 100 °C.

2.4.2.2 Density

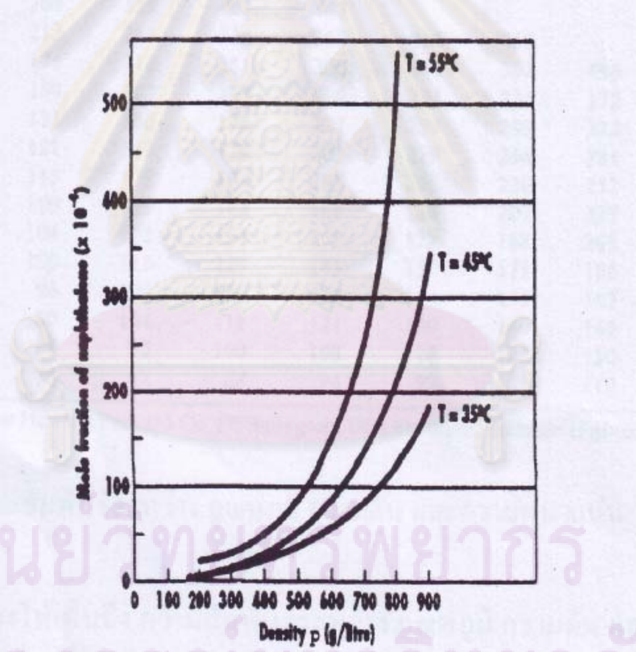
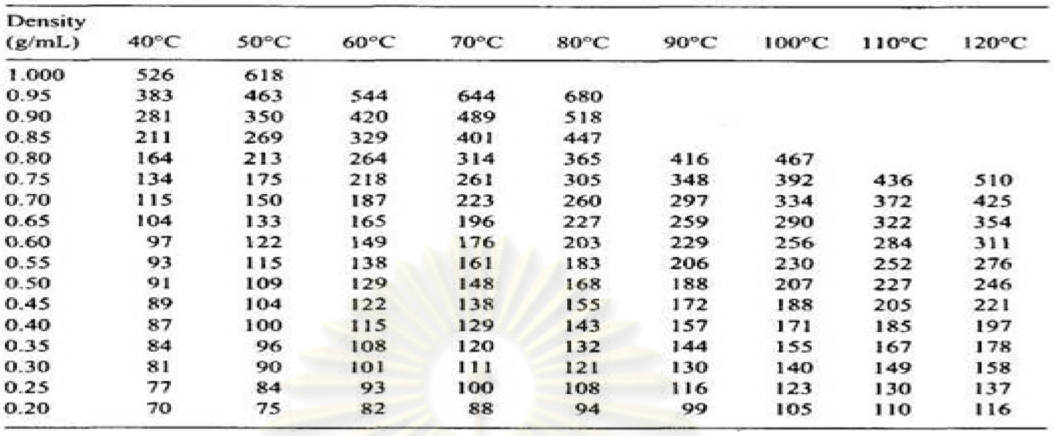


Figure 2.11 Solubility (mole fraction) of naphthalene in CO_2 as a function of density at various temperatures. [10]

- Solvent power of a supercritical fluid increases with density at a given temperature
- Solvent power of a supercritical fluid increases with temperature at a given density

Table 2.3 Density-temperature-pressure relationship for CO_2 [10]



Density (g/mL)	40°C	50°C	60°C	70°C	80°C	90°C	100°C	110°C	120°C
1.000	526	618							
0.95	383	463	544	644	680				
0.90	281	350	420	489	518				
0.85	211	269	329	401	447				
0.80	164	213	264	314	365	416	467		
0.75	134	175	218	261	305	348	392	436	510
0.70	115	150	187	223	260	297	334	372	425
0.65	104	133	165	196	227	259	290	322	354
0.60	97	122	149	176	203	229	256	284	311
0.55	93	115	138	161	183	206	230	252	276
0.50	91	109	129	148	168	188	207	227	246
0.45	89	104	122	138	155	172	188	205	221
0.40	87	100	115	129	143	157	171	185	197
0.35	84	96	108	120	132	144	155	167	178
0.30	81	90	101	111	121	130	140	149	158
0.25	77	84	93	100	108	116	123	130	137
0.20	70	75	82	88	94	99	105	110	116

Pressure is given in bar.

Table 2.3 gives a more detailed listing of the pressure (in bar) requirements necessary to achieve specific CO_2 densities at various temperatures (Fig. 2.11). The listing once again emphasizes the need for higher pressures at higher temperatures to achieve a specified density.

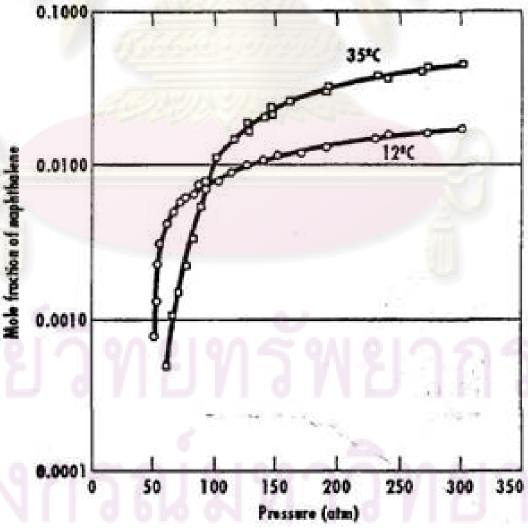


Figure 2.12 Solubility (mole fraction) of naphthalene in ethylene versus pressure at 12 °C and 35 °C. Tt for ethylene = 9.3 °C. [10]

The previous points are illustrated by the solubility behavior of solid naphthalene in supercritical ethylene (Tc = 9.3°C, Pc = 49.8 atm) at 12°C and 35°C versus pressure (Figure. 2.12). The 35°C isotherm (TK = 1.09) is less sensitive to pressure changes in the region near 50 atm than is the 12°C isotherm (TR = 1.01). In other words, changes in density (or solvent power) are more pronounced with changes in pressure at 12°C in the vicinity of the critical point than at 35°C. This results in naphthalene solubility being greater at lower temperatures up to 100 atm. This behavior is referred to as retrograde vaporization. The much lower solubility at 35°C, at pressures less than 100 atm, reflects the large decrease in ethylene density relative to its density at 12°C (100 atm). At pressures greater than 150 atm, the difference in the densities of SF ethylene at 12 and 35°C is not very large. The higher naphthalene solubility at 35°C and at elevated pressure can be explained only by the increase in sublimation pressure of naphthalene on heating from 12 to 35°C. Thus two competing factors affect the solubility of solids in SCF: SF density and solid sublimation pressure.

2.4.1.3 Diffusivity and viscosity

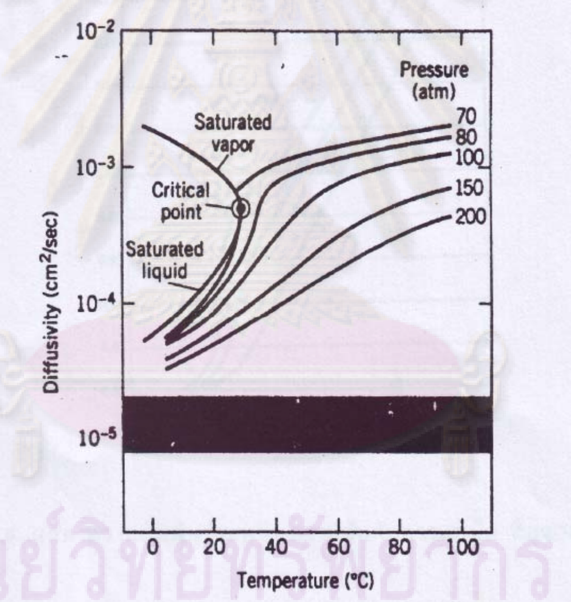


Figure 2.13 Diffusivity of CO_2 versus temperature at various pressures. [10]

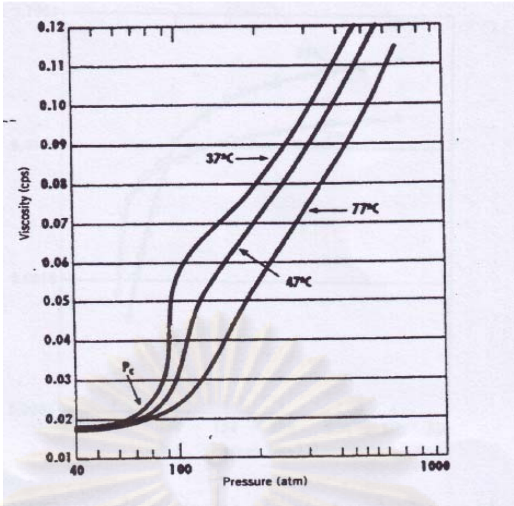


Figure 2.14 Viscosity behavior of CO_2 at various temperatures and pressures. [10]

As was the case for density, values for viscosity and diffusivity are dependent on temperature and pressure. The viscosity and diffusivity of the SF approach those of a liquid as pressure is increased. Whereas an increase in temperature.

At 200 atm, 5 °C. leads to an increase in viscosity of a gas, the opposite is true in the case of SCF. Diffusivity, on the other hand, will increase with an increase in temperature. As evidenced by Figures 2.13 and 2.14, 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 SFs differ by 1-2 orders of magnitude from normal liquids. A review of these important points follows:

- Fixed density, high temperature, high diffusivity, low viscosity
- High Density, fixed temperature, low diffusivity, high viscosity

The properties of gas-like diffusivity and viscosity, coupled with liquid-like density, combined with the pressure-dependent solvating power of SCF have provided the impetus for applying SF technology to analytical separation problems. Finally, the low (essentially zero) value of surface tension of SFs allows better penetration into the sample matrix relative to liquid solvents.

2.5 Literature survey

In 2008, Yadollah Yamini *et al.* [13] studied the extraction of essential oil obtained by hydrodistillation and supercritical (carbon dioxide) extraction methods. The oil was analysed by capillary gas chromatography using flame ionization and mass spectrometric detections. The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). The effects of different parameters such as pressure, temperature, modifier volume and extraction times (dynamic and static) on the supercritical fluid extraction (SFE) of *S. mirzayanii* oil were investigated. The results showed that, under a pressure of 35.5 MPa, temperature of 35 °C, 6% methanol, dynamic extraction time of 50 min and static extraction time of 30 min, extraction was more selective for the linalyl acetate. Thirty four compounds were identified in the hydrodistilled oil. The major components of *S. mirzayanii* were linalyl acetate (7.6%), 1,8-cineole (8.0%), linalool (9.0%) and 8-acetoxy linalool (11.0%). However, by using supercritical carbon dioxide in optimum conditions, only three components contain more than 63% of the oil. The yield of the obtained oil based on hydrodistillation was 2.20% (v/w). Extraction yield based on the SFE varied in the range of 1.50–9.67% (w/w) under different conditions.

In 2008, Tatiana de S. Weinhold *et al.* [14] studied the extraction of *polygala cyparissias* oleoresin obtained by CO_2 and classical organic solvent extractions. Composition of plant extracts and process efficiency are mainly defined by the extraction method used. Plant materials are rich source of valuable substances and the *Polygala cyparissias*, a bush that grows at dune regions in South America, presents important active compounds. Therefore, the present study deals with the assessment of the composition profile of *P. cyparissias* extracts, from root and from stems and leaves, obtained by CO_2 at various conditions of temperature and pressure and by classical organic solvent extractions. Supercritical CO_2 was used at temperatures of 20 and 40 °C, pressures varying from 15 to 20 MPa and solvent flow rate from 1.49 to 4.07 ± 0.02 g CO_2 min^{-1}. The extracts were analyzed by GC and GC–MS techniques, in order to quantify and identify the components presents at the plant material. Target components found at the *P. cyparissias* extracts from different plant sources were triterpenes, phytosterols and methyl salicylate, with notorious biological actions. The CO_2 extraction yields were up to 0.35% (w/w), comparable to low-pressure extraction yields obtained by *n*-hexane and dichloromethane. For optimization purpose, experimental data were correlated by mass transfer models, with low deviation between experimental and modeled data.

In 2007, Seied Mahdi Pourmortazavi and Seiedeh Somayyeh Hajimirsadegh *et al.* [15] studied the use of supercritical fluids, especially carbon dioxide, in the extraction of plant volatile components has increased during two last decades due to the expected advantages of the supercritical extraction process. Supercritical fluid extraction (SFE) is a rapid, selective and convenient method for sample preparation prior to the analysis of compounds in the volatile product of plant matrices. Also, SFE is a simple, inexpensive, fast, effective and virtually solvent-free sample pretreatment technique. This review provides a detailed and updated discussion of the developments, modes and applications of SFE in the isolation of essential oils from plant matrices. SFE is usually performed with pure or modified carbon dioxide, which facilitates off-line collection of extracts and on-line coupling with other analytical methods such as gas, liquid and supercritical fluid chromatography. In this review, we showed that a number of factors influence extraction yields, these being solubility of the solute in the fluid, diffusion through the matrix and collection process. Finally, SFE has been compared with conventional extraction methods in terms of selectivity, rapidity, cleanliness and possibility of manipulating the composition of the extract.

In 2007, M. Bocevska and H. Sovová *et al.* [16] studied the essential oil was extracted from yarrow flowers (*Achillea millefolium*) with supercritical CO_2 at pressure of 10 MPa and temperatures of 40–60 °C, and its composition and yield were compared with those of hydrodistillate. The yield of total extract, measured in dependence on extraction time, was affected by extraction temperature but not by particle size of ground flowers. CO_2-extraction of cuticular waxes was lowest at 60 °C. Major essential oil components were camphor (26.4% in extract, 38.4% in distillate), 1,8-cineole (9.6% in extract, 16.2% in distillate), bornyl acetate (16.7% in extract, 4.3% in distillate), γ-terpinene (9.0% in extract, 9.4% in distillate), and terpinolene (7.6% in extract, 3.9% in distillate). Compared to hydrodistillation, the yield of monoterpenes was lower due to their incomplete separation from gaseous CO_2 in trap but the yield of less volatile components like monoterpene acetates and sesquiterpenes was higher. Hydrolysis of γ-terpinene and terpinolene, occuring in hydrodistillation, was suppressed in supercritical extraction, particularly at extraction temperature of 40 °C.

In 2006, Li Zhiyi *et al.* [17] studied the experimental equipment for separating pepper oil by supercritical fluid extraction. The effects of the extraction pressure, extraction temperature, CO_2 flow rate and the particle size of the row material on the extraction rate were investigated, and the optimum process condition of the supercritical fluid extraction for pepper oil was determined. Based on the mass conservation principle for differential units of extraction bed, the numerical simulation model was established. The total of mass transfer driving force and the equilibrium absorption constant were fitted by the linear driving force approximate theory. The simulation results agreed pretty well with the experimental ones. (Aroma and its pungency food processing as well as in pharmaceutical and cosmetic products).

In 2004, Mostafa Khajeh *et al.* [18] studied the extraction of essential oil of *Carum copticum* cultivated in Iran was obtained by hydrodistillation and supercritical (CO_2) extraction (SFE) methods. The oils were analysed by capillary gas chromatography, using flame ionization and mass spectrometric detection. The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). The effects of different parameters, such as pressure, temperature, modifier volume and extraction time, on the supercritical fluid extraction of *C. copticum* oil were investigated. The results showed that, under pressure of 30.4 MPa, temperature 35 °C, methanol 0% and dynamic extraction time of 30 min, the method was most selective for the extraction of thymol. Eight compounds were identified in the hydrodistilled oil. The major components of *C. copticum* were thymol (49.0%), γ-terpinene (30.8%), *p*-cymene (15.7), β-pinene (2.1%), myrcene (0.8%) and limonene (0.7%). However, by using supercritical carbon dioxide under optimum conditions, only three components constituted more than 99% of the oil. The extraction yield, based on hydrodistillation was 2.8% (v/w). Extraction yield based on the SFE varied in the range of 1.0–5.8% (w/w) under different conditions. The results show that, in Iranian *C. copticum* oil, thymol is a major component.

In 2004, Nasrin Aghel *et al.* [19] studied the dependence of *Mentha pulegium* L. (pennyroyal) essential oil composition, obtained by supercritical carbon dioxide (SC-CO_2), with the following parameters: pressure, temperature, extraction time (dynamic), and modifier (methanol) was studied. The results were also compared with those obtained by conventional hydrodistillation method in laboratory conditions. Regarding the percentages of menthone (30.3%) and pulegone (52.0%), the optimum SFE results were obtained at the following experimental conditions: pressure=100 atm, T=35 °C, dynamic time=10 min, and V_{modifier}=0 μl. The results of hydrodistillation showed that the major components of *M. pulegium* L. were pulegone (37.8%), menthone (20.3%), and piperitenone (6.8%). The evaluation of the composition of each extract was performed by gas chromatography–mass spectrometry.

In 2007, Paula B. Gomes *et al.* [20] studied the extraction of the natural essential oil from a Portuguese-grown rose geranium (*Pelargonium* sp.) using by supercritical fluid extraction (SFE) with carbon dioxide. Geranium oil is an important ingredient in perfumery as middle note and is a natural resource with potential to be explored in Portugal, since this plant is well adapted to our climate and soil. A new SFE equipment was designed and constructed and it was used for the extraction of geranium. The obtained extracts were characterised by GC/MS and their organoleptic quality (colour and odour) was evaluated and compared with the materials obtained by the traditional techniques—hydrodistillation and organic solvent extraction. The best extraction conditions were: extraction time of 15–30 min, temperature of 40 °C, pressure of 90–100 bar, cut fresh plant and dynamic mode. The SFE yields varied between 0.019 and 0.22%. The CO_2 geranium extract had a superior organoleptic quality, with very fresh natural floral–fruity character and a pale yellow colour, most suitable for the use in perfumery.

Paramita Bhattacharjee *et al.* [21] studied the extraction of cottonsseed oil using by supercritical carbon dioxide. Supercritical fluid extraction (SFE) using carbon dioxide is a popular technique for oil extraction due to its high extraction efficiency, short extracting time, lower refining requirement and absence of chemical residues or contamination in the extracted oils. In this work, an attempt has been made to extract oil from a local variety of cottonseed using this technique. The effects of the extraction parameters of pressure, temperature and time of extraction of oil were optimized by using statistical techniques such as central composite rotate design (CCRD) and response surface methodology (RSM), with a view to maximize the oil yield with minimum gossypol extraction. From this study, it could be concluded that the yield of cottonseed oil can be improved at pressure higher than 550 bar, in the temperature zone of 70–80 °C and extracting time of 2–3 h.

CHAPTER III

EXPERIMENTAL

3.1 Chemicals and materials

1. Agarwood (*A. subintegra*) from Trad and Chonburi Provinces
2. Liquid Carbondioxide (99.5%, PraxAir)
3. Methanol (CH_3OH, 99.5%)
4. Heptane (C_7H_{16}) (MERCK)
5. *Anh*. Sodium sulfate (Na_2SO_4, 99%) (Riedel-deHaën)
6. Ethanol (C_2H_5OH, 99.5%)

7 Ethanol (commercial grade)

8. Ethyl acetate ($CH_3COOC_2H_5$, 99.5%)
9. Ethyl acetate (commercial grade)
10. Diethyl ether ($C_2H_5OC_2H_5$, 99.5%)
11. Hexane (C_6H_{14}) (MERCK)
12. Acetone

3.2 Instruments and equipments

1. Stainless steel reactor ¾″
2. High pressure pump (Thar model P-100)
3. High pressure pump (Thar model P-50)
4. Cooling bath (Heto)
5. Water bath (Heto)
6. Automatic back pressure regulator (Jasco)
7. Grinder
8. Sieve 35 mesh
9. Chiller

3.2 Instruments and equipments (cont.)

10. Oil bath
11. Rotary evaporator
12. Soxhlet extractor
13. Heating metal
14. Magnetic stirrer and Magnetic bar
15. Oven
16. Refrigerator
17. Round Bottom 1000 and 2000 mL
18. Erlenmeyer flask 500 mL
19. Beaker 50, 100, 150 and 600 mL
20. Thermometer
21. Condenser
22. Gas Chromatography
23. Gas Chromatography with MS
24. Microsyringe

3.3 Essential oil analysis

The chemical composition of essential oil from agarwood oil and the relative contents of the compounds were analyzed by a Shimadzu GC-14B gas chromatograph equipped with a 30-m DB-1 capillary column and a flame ionization detector (FID).

Table 3.1 GC conditions for determination of essential oil

Condition	Value
Carrier gas (N_2) flow rate	1.0 mL/min
Make up gas (N_2) pressure	80 kPa
Hydrogen pressure (for FID)	60 kPa
Air pressure (for FID)	50 kPa
Detector temperature	250°C
Split ratio	50 : 1
Injection port temperature	250°C
Inject volume	1 μL
Initial column temperature	50 °C
Oven program	4 °C /min to 165 °C 1 °C /min to 175 °C 4 °C /min to 230 °C
Final column temperature	230°C

Figure 3.1 Gas chromatograph.

Table 3.2 GC-MS conditions for determination of essential oil

Condition	Value
Carrier gas (He) flow rate	1.0 mL/min
Make up gas (He) pressure	80 kPa
Hydrogen pressure (for FID)	60 kPa
Air pressure (for FID)	50 kPa
Detector temperature	250°C
Split ratio	50:1
Injection port temperature	250°C
Inject volume	1 μL
Initial column temperature	50 °C
Oven program	4 °C /min to 165 °C 1 °C /min to 175 °C 4 °C /min to 230 °C
Final column temperature	230°C
Energy of ion source	70 eV, EI mode
Mass range	50-500 amu
Mass filter	Quadrupole

Figure 3.2 Gas chromatograph with MS and FID detector.

3.4 Sample Preparation

Agarwood (*Aquilaria subintegra*) was preliminarily ground in a knife grinding mill and then sieved to appropriate particle size before use, and stored in the dark place.

3.5 Supercritical carbon dioxide extraction of essential oil

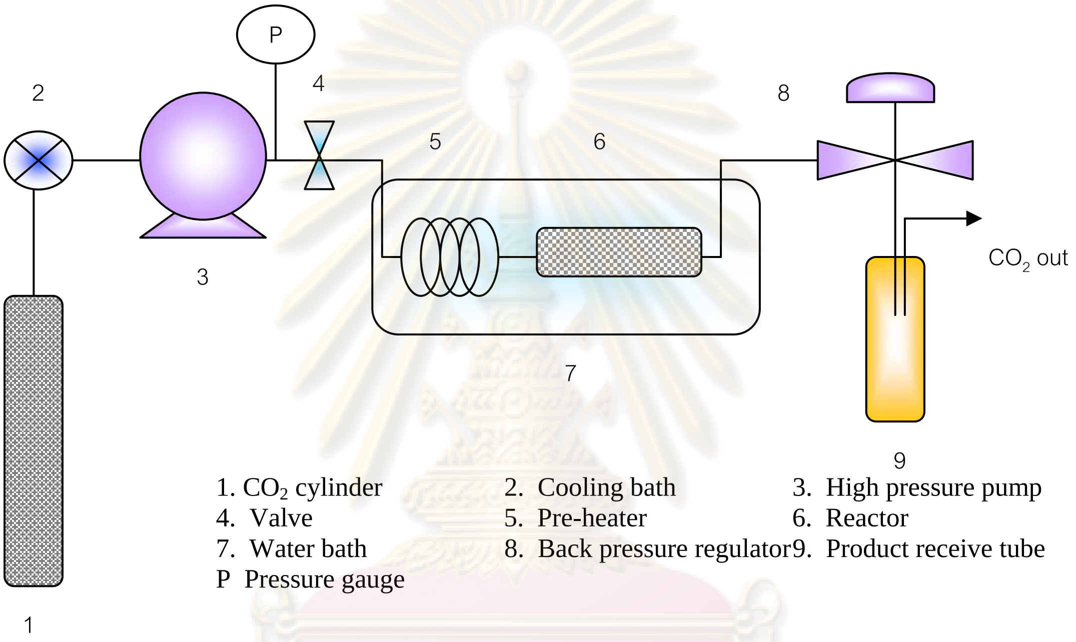


Figure 3.3 Schematic diagram of SC-CO_2 experiments.

Supercritical CO_2 experiment shown in Figure 3.3; a 7 g of the milled agarwood was charged into the reactor. The reactor was placed in the water bath to operating temperature ranges 40 °C to 70 °C. CO_2 was supplied to the reactor by a high pressure pump where a cooling bath was connected to decrease CO_2 temperature down to -9 °C. The pressure was adjusted in the range of 12 to 25 MPa by a back pressure regulator, where CO_2 flow rate ranges 1- 4 g/min. The extracted stream from the reactor was then depressurized through a back pressure regulator and the sample was collected in product receive tube, and then the weight of the sample was measured. Finally, the extraction yield was calculated. The sample was kept in a refrigerator.

3.6 Supercritical carbon dioxide with co-solvent extraction of essential oil

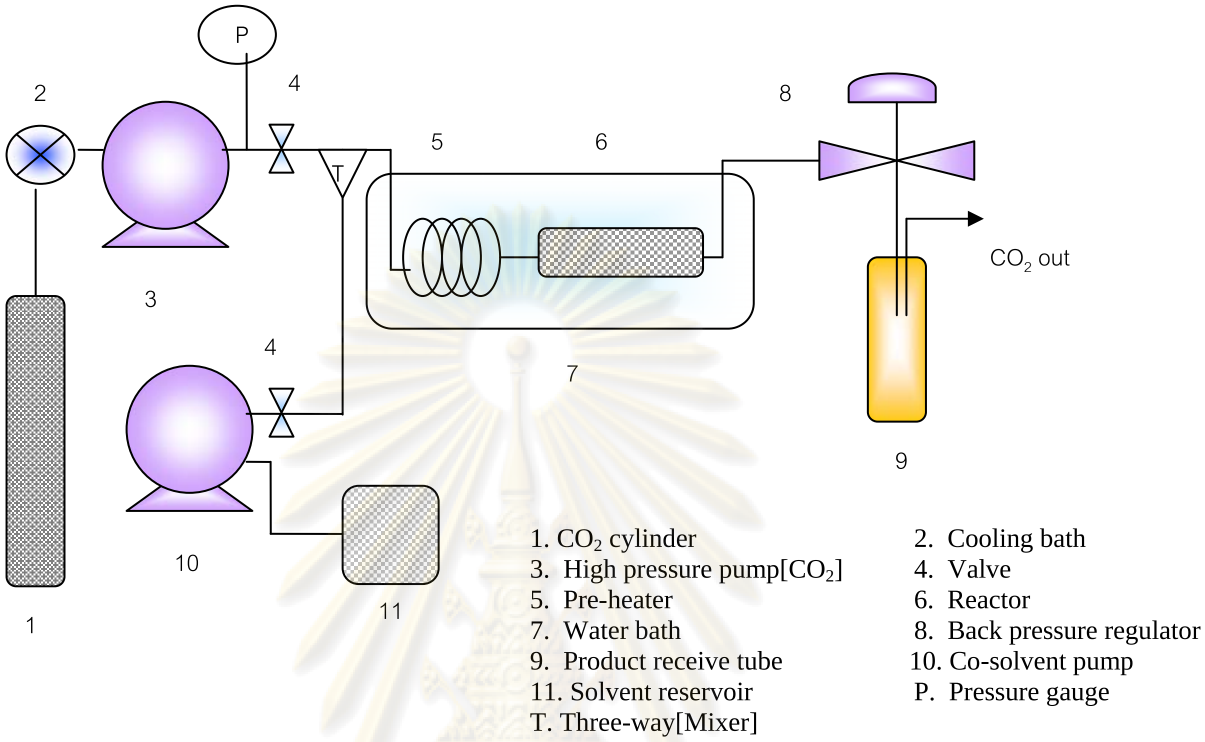


Figure 3.4 Schematic diagram of SC-CO_2 with co-solvent experiments.

Supercritical CO_2 with co-solvent experiment shown in Figure 3.4; a 7g of the milled agarwood mixed with grit was charged into the reactor. The reactor was immersed in a water bath to maintain it at the operation temperature. The liquid CO_2 from cylinder was passed through a cooling bath at – 9 °C. The cooled CO_2 and aqueous co-solvent from solvent reservoir were compressed to desired pressure by high pressure pump and co-solvent pump, respectively. They were then mixed and heated to specified temperature to be turned into the supercritical fluid in three-way, prior to being introduced into the reactor. The extracted stream from the reactor was then depressurized through a black pressure regulator and the sample was collected in product receive tube, the co-solvent was eliminated using rotary evaporator. Then the weight of the sample was measured. Finally, the extraction yield was calculated. The sample was kept in a refrigerator.

3.7 Steam distillation

Figure 3.5 Steam distillation apparatus.

Steam distillation apparatus shown in Figure 3.5, which contained a 2000 mL steam generator flask, a distilling flask, a condenser and a receiving vessel, was used to perform the steam distillation. A 72 g of the milled agarwood was subjected to steam distillation. The steam generator flask was filled with 1000 mL of distilled water and heated with silicone oil bath. As water was vapourized, the steam passed through the distillation flask containing the plant. The vapour passed through the cooled tube, where it condensed. The volatile components were collected into the receiving flask (500 mL) during 2 days of steam distillation and the volatile distillate was collected over anhydrous sodium sulfate and refrigerated. The yield of the sample based on the dry agarwood was weighted and the chemical compositions of the extract were analyzed by GC.

3.8 Soxhlet extraction

Figure 3.6 Soxhlet apparatus.

A 15 g of the milled agarwood was extracted in soxhlet apparatus with 400 mL of ethanol at temperature 65 to 70 °C for 24 h. The ethanol solvent was then replaced with methanol, ethyl acetate and hexane, respectively and the remainder of the procedure and conditions are all the same as before. The yield of the sample based on the dry agarwood was weighed and the chemical compositions of essential oil were analyzed by GC.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Extraction of agarwood *Aquilaria Subintergra* using supercritical CO_2 ($SC\text{-}CO_2$)

In this research, the extraction of agarwood using $SC\text{-}CO_2$ was investigated. Several parameters on the extraction yield were studied, i.e. particle size of dry agarwood; temperature and pressure of extraction condition; CO_2 flow rate; and the type and amount of co-solvent. In addition the extraction of agarwood from different locations was also examined.

4.1.1 Effect of particle size

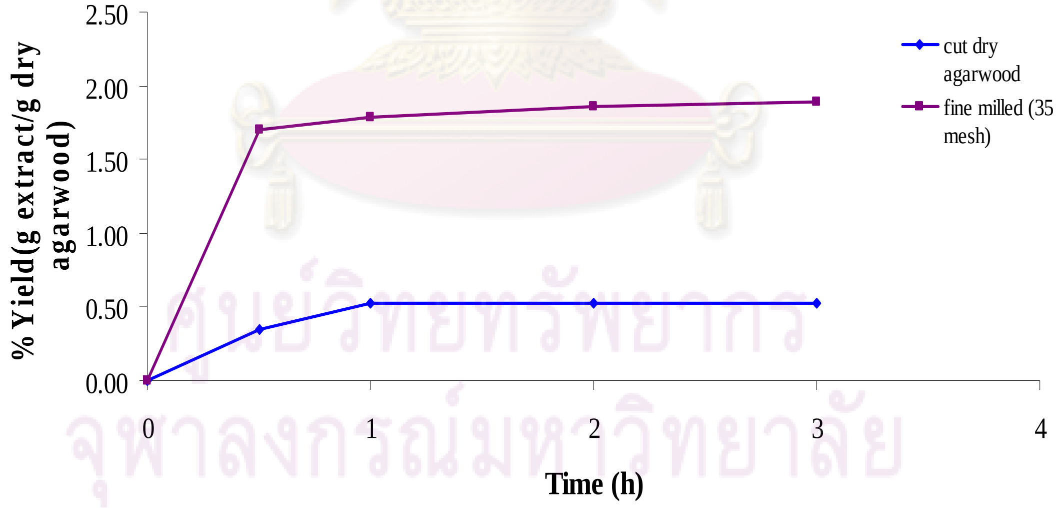


Figure 4.1 Effect of particle size on the extraction yield at T = 50 °C, P = 25 MPa and CO_2 flow rate 2 g/min.

Initially the $SC\text{-}CO_2$ extractions were performed with two sample sizes, cut dry agarwood and fine milled (35 mesh) agarwood, at the temperature of 50 °C, the pressure of 25 MPa and CO_2 flow rate of 2 g/min for 3 h. The effect of particle size on the extraction yield for that condition is shown in Figure. 4.1. As expected, the higher extraction yield was obtained with the smaller particle size. This was because the intraparticle diffusion resistance is smaller for smaller particles due to the shorter diffusion path. Moreover, smaller particles provide greater specific surface area and could also have their cell walls broken during grinding. Thus, the mass transfer resistance is lower and the extract is more accessible to the supercritical solvent [22]. For smaller particle size, the extraction yield could reach about 1.97% at the temperature of 50 °C, the pressure of 25 MPa and CO_2 flow rate 2 g/min for 3 hrs. Thus, the extraction yield obtained by using fine-milled dry agarwood was 4% higher than the yield obtained using cut dry agarwood for 1 hrs. Therefore, dry agarwood with particle size 35 mesh had been used for further study.

4.1.2 Effect of parameters

Optimization of experiment conditions is one of the most important steps in the development of the extraction method. The second step in the SC-CO_2 extraction of agarwood is to optimize the operating conditions (especially pressure and temperature) to obtain an efficient extraction of the extraction yield and the interested compounds (sesquiterpiene compounds that are responsible for the aroma) and to avoid the co-extraction of undesired compounds such as wax, fatty acids, their esters, pigments, etc. Whole optimization procedure was done with the same sample of dry and milled agarwood (35 mesh). Each experiment was performed with 7 g of sample.

The optimization of the method can be carried out step-by-step. Table 4.1 shows SC-CO_2 experimental conditions and the extraction yield: the pressure range of 12-25 MPa, temperature range of 40-70 °C and fixed CO_2 flow rate of 2 g/min for 4 h. The effect of pressure and temperature on essential oil yield (in weight %) were investigated and shown in Figure 4.2-4.3.

Table 4.1 SC-CO_2 experimental conditions and the extraction yields of dry agarwood

No.	**Pressure (Mpa)**	**Temperature (°C)**	**Extraction yield (%w/w)**
1	12	40	0.27
2	12	50	0.20
3	12	55	0.42
4	12	60	0.49
5	12	65	0.24
6	12	70	0.22
7	15	40	0.40
8	15	50	0.41
9	15	55	0.65
10	15	60	0.56
11	15	65	0.62
12	15	70	0.44
13	18	40	0.51
14	18	50	0.93
15	18	55	0.72
16	18	60	0.61
17	18	65	0.83
18	18	70	0.81
19	21	40	0.69
20	21	50	1.14
21	21	55	0.86
22	21	60	0.79
23	21	65	0.91
24	21	70	0.86
25	25	40	0.81
26	25	50	1.93
27	25	55	1.76
28	25	60	0.84
29	25	65	0.92
30	25	70	0.92

4.1.2.1 Effect of temperature

Temperature affects the density of fluid, solvating power and the solubility, diffusion and desorption of the solute extracted from the matrix [23]. The effect of the extraction temperature on the extraction yields for an extraction time of 4 h was studied at constant pressure. Figure 4.2 shows the effect of temperature on the extraction yield at various temperatures (40-70°C). It can be seen that at high pressure when the temperature increased from 40 °C to 50 °C, the amount of the extractionl yield increased. The explanation can be due to the higher diffusivity of solvent (SC-CO_2) at higher temperature. In contrast when the temperature increased from 50 °C to 70 °C, the amount of the extraction yield decreased. Because of increasing temperature, the density of solvent (SC-CO_2) and solubility of solute decreased. While at low pressure (15 MPa and below), the extraction yield increased with increasing temperature.

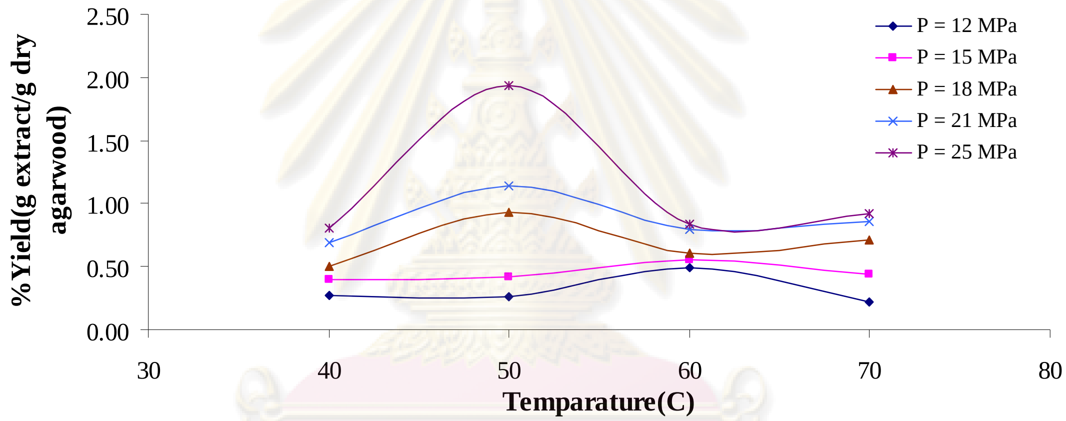


Figure 4.2 Effect of temperature on the extraction yield at P= 12-25 MPa, CO_2 flow rate 2 g/min.

4.1.2.2 Effect of pressure

Pressure affects the density of (SC-CO_2), solvating power and the solubility of the solute extracted from the matrix was studied at a constant temperature. Figure 4.3 shows the effect of pressure on the extraction yield, that yield of the extract increased significantly as the extraction pressure increased from 12 to 25 MPa. It was clear that the extraction yield increased with increasing pressure. It is well known that the density and solvent power of fluid (SC-CO_2) and solubility of solute increased with increasing pressure. Generally speaking, it is desirable to extract the sample just above the point where the desired compound becomes soluble in the fluid (SC-CO_2), so that extraction of other compounds can be minimized. To achieve a good selectivity for an SFE process, careful controlling of the fluid density is essential. By controlling the fluid density, fractionation of the extracts could be achieved.

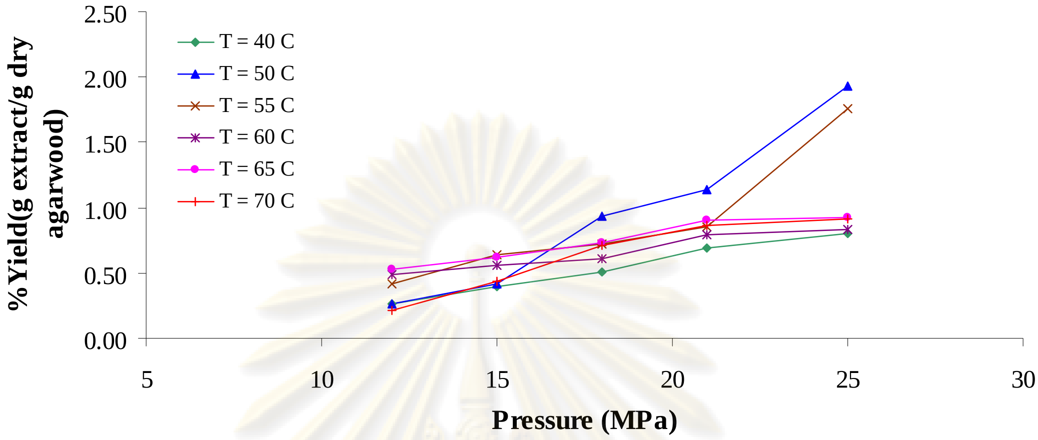


Figure 4.3 Effect of pressure on the extraction yield at T = 40-70 °C, CO_2 flow rate 2 g/min.

From the results, an increase in the pressure of the system can lead to a significant increase in the extraction yield during the extraction, while the temperature does not exert a significant influence. Thus, within the conditions studied, the pressure of 25 MPa and temperature of 50 °C provided the best condition for the extraction in terms of yield.

Although increase in the pressure led to a higher density of the SC-CO_2 inducing a greater solubilization and, consequently, an increase in the extraction yield, however, with a tendency to reduce the selectivity due to the extraction of undesirable components in terms of the quality, such as fatty acids, waxes and pigments. Thus, given that the good quality of the extract is generally evaluated through an analysis of its components. In this research, the quality of agarwood oil was evaluated and compared with that of the agarwood oil obtained by hydrodistillation (commercial).

4.1.2.3 Effect of flow rate

In fact, effect of flow rate was twofold. Firstly, an increase in the flow rate increased the mass transfer, but secondly, it reduced the contact time. Therefore the final extraction efficiency depended on the compensation between the two consequences. In this section was investigated effect of CO_2 flow rate at two different pressures (12 and 25 MPa) and two different temperatures (50 and 65 °C). The effect of CO_2 flow rate on the extraction yield is shown in Figure 4.4.Tthe amount of the extraction yield increased when CO_2 flow rate increasing from 1 g/min to 3 g/min. When the CO_2 flow rate increased, the amount of fresh solvent contacting with the solid matrix also increased. On the other hand, an increase of the CO_2 flow rate to 4 g/min led the amount of the extraction yield to decrease. This is due to the fact that the contact time between solid matrix and solvent was reduced; thus, a more incomplete extraction [24]. Therefore all subsequent experiments were conducted at CO_2 flow rate of 3 g/min.

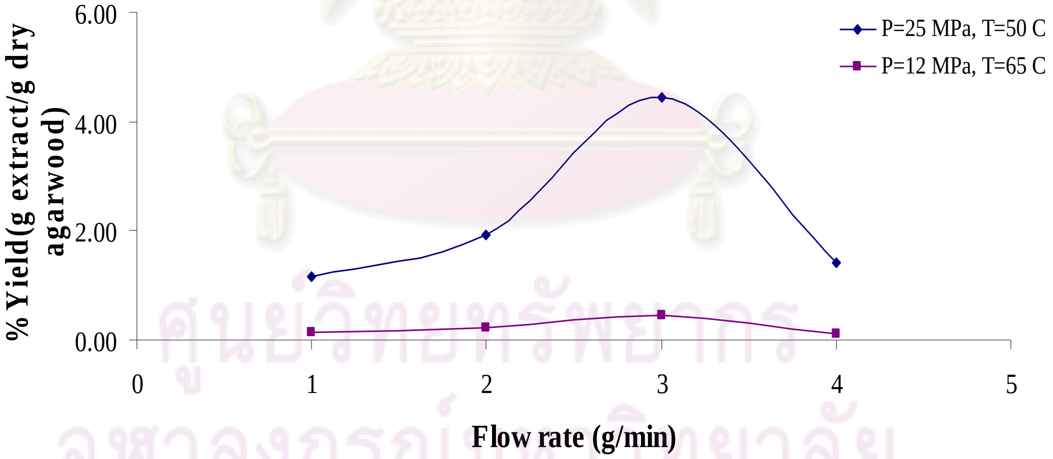


Figure 4.4 Effect of CO_2 flow rate on the extraction yield at P = 25 MPa, T = 50 °C and P = 12 MPa, T = 65 °C.

4.1.2.4 Effect of various co-solvents on the extraction yield

The addition of a relatively small amount of co-solvent in the supercritical fluid can improve the solubility of the solvent. Co-solvents are liquids or gaseous material, which increase the solubility of the interested compound in supercritical phase. In most cases, the selectivity of interested compounds are increased, and in some cases, it increases the over all solubility of two or more compounds whereby the selectivity is decreased. The addition of co-solvent will generally increase the mixture density, which may also contribute to the overall solubility enhancement. The co-solvents used for the present studies were methanol, ethanol and ethyl acetate. These solvents are widely used in many industries. The co-solvents are generally used in supercritical fluid extraction processes in order to reduce time of extraction; however, in some cases, the co-solvent extracted other compounds also so that the purity of interested compound decreases [25]. The co-solvent may effect the extraction in three different ways: (1) increase the analyte's solubility in the supercritical fluid as a result of analyte–co-solvent interactions in the fluid phase; (2) facilitate analyte desorption-the molecules of polar co-solvent are able to interact with the matrix and compete efficiently with the analyte for the active sites in the matrix; (3) distort the matrix–analyte diffusion process and favour penetration of the supercritical fluid inside the matrix when the co-solvent swells the matrix [26].

In this section, various polar co-solvent consist of methanol, ethanol and ethyl acetate were tested in order to modify the polarity of CO_2 and the solubility of the extract in the sample matrix. The effect of co-solvent on the extraction yield (in weight %) were investigated and shown in Figure 4.5-4.6.

Effect of the type and amount of co-solvent

This section intends to demonstrate the different extraction power of co-solvents. It should be noted that, in general, SC-CO_2/co-solvent mixtures should be in the supercritical state (completely miscible) at the operating conditions to achieve the desired co-solvent effect. In this study, three other co-solvents were investigated (methanol, ethanol and ethyl acetate). Methanol and ethanol is among one of the most frequently used solvents for the extraction of components from natural products. The effect of co-solvents on the extraction yield is shown in Figure 4.5. The extraction was conducted with 5 to 10%v/v co-solvent at temperatures of 65 °C and pressures of 12 MPa. With the same extraction conditions, ethyl acetate gave inferior essential oil yield to that with ethanol, indicating a possible limited solubility of ethyl acetate in SC-CO_2, compared with ethanol. On the other hand, SC-CO_2 extraction with methanol gave higher extraction yield than ethyl acetate and gave comparable results to that with ethanol; that is, the extraction yield as high as 4.58%w/w was obtained. It could therefore be drawn from these results that the solubility of methanol in SC-CO_2 was presumably higher than ethyl acetate in SC-CO_2. From the Figure 4.5, increasing the co-solvent concentration from 5 to 10%v/v had little effect on the extraction yield. Although, the presence of various co-solvents led to the increase of the extraction yield (when compared with pure CO_2), but it had a tendency to cause the reduction in the selectivity due to the extraction of undesirable components in terms of the quality, such as fatty acids, their esters, waxes and pigments. Thus, given that the good quality of the extract is generally evaluated through an analysis of its components, in this research, the quality of agarwood oil under experiment was evaluated and compared with that of the agarwood oil obtained by hydrodistillation (commercial1, 2).

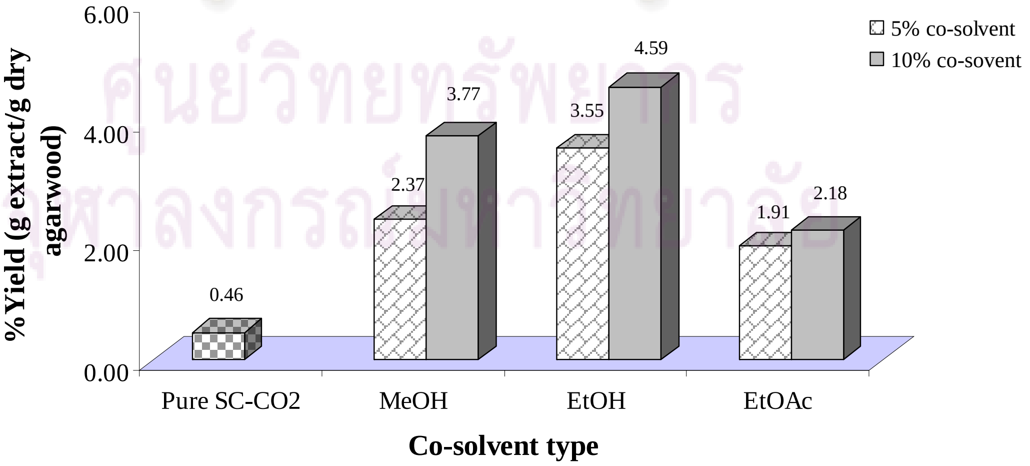


Figure 4.5 Effect of co-solvents on the extraction yield at P = 12 MPa, T = 65 °C and CO_2 flow rate 3g/min.

4.2 Chemical compositions analysis

The chemical compositions were identified by comparison of their mass spectra with those of a computer library. The percentages of compounds were calculated by the area normalization method.

Table 4.2 Chemical compositions of the commercially available essential oil of agarwood

No.	Compound	Peak Area (%)	
		1[a]	**2**[b]
1	Pentanoic acid	–	–
2	Hexanoic acid	0.04	–
3	1-Penten-3-one	0.40	7.47
4	Heptanoic acid	0.10	–
5	Benzyl acetone	0.94	0.71
6	Octanoic acid	0.17	–
7	Nonanoic acid	0.02	0.11
8	Tridecane	–	0.26
9	Cyclohexasiloxane	–	–
10	Decanoic acid	–	–
11	Tetradecane	–	–
12	Aristolene	–	1.88
13	γ-Elemene	3.44	–
14	α-Guaiene	3.68	1.39
15	Epi-ligulyl oxide	0.65	1.25
16	Eudesma-4(14), 11-diene	0.21	–
17	β-Selinene	5.79	1.21
18	(+)-Epi-bicyclosesquiphellandrene	6.63	0.89
19	Valencene	0.14	–
21	α-Agarofuran	0.27	0.11
22	4,5-Dehydro-isolongifolene	0.39	0.17
23	Zierone	1.30	0.61
24	Elemol	0.44	–
25	Germacrene	0.71	3.88
26	Dodecanoic acid	–	0.84
27	1H-Cycloprop[e]azulene	6.81	3.08
28	Guaiol	2.11	–
29	Viridiflorol	2.65	–
30	γ-Eudesmol	–	1.39
31	Agarospirol		1.73

Table 4.2[cont.] Chemical compositions of the commercially available essential oil of agarwood

No.	Compound	Peak Area (%)	
		1[a]	2[b]
32	Guai-1(10)-en-11-ol	1.46	0.59
33	Valerenol	2.04	0.62
34	Hinesol	8.81	–
35	α-Santalol	0.19	0.49
36	Tetradecanoic acid	2.47	1.01
37	Aritolone	3.16	0.45
38	Baimuxinal (Oxo-agarofuran)	0.75	0.14
39	Dehydro-cohumulinic acid	0.71	0.22
40	Pentadecanoic acid	0.53	0.55
41	Palmitic acid	5.63	13.68
42	Dibenzylacetone	1.45	–
43	Heptadecanoic acid	–	6.63
44	8-Octadecanoic aid	–	–
45	Octadecanoic acid	2.20	1.91
46	9-Octadecanoic aid	0.20	1.55
47	Octadec-9-enoic acid	–	0.37
48	9,12-Octadecadienoic aid	–	–
49	8-Naphthol,2-(2-Phenylethyl)chromen	0.07	0.91
50	Estra-1,3,5(10)-trien-17-one	0.18	–
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	0.20	–
52	8-Methoxyflindersiachromone	0.40	0.40

1[a], 2[b]: agarwood oil obtained by hydrodistillation from Chonburi and Trad provinces, respectively

According to qualitative and quantitative analyses, agarwood essential oil is in fact a complex mixture of sesquiterpenes derivatives, chromone derivatives, aliphatic hydrocarbons, and non-sesquiterpenes. The list of chemical compositions found in agarwood essential oil (commercial 1, 2) obtained from Chonburi and Trad is shown in Table 4.2, and their GC-MS chromatograms are shown in Figure 4.6-4.7. The results indicate that there are some similarities and variations among the major compositions. 1H-cycloprop[e]azulene (6.81, 3.08%), β-selinene (5.79, 1.21%), and α-guaiene (3.68, 1.39%) were some of the compositions found in both commercial essential oils. Whereas hinesol (8.81%), γ-elemene (3.44%), viridiflorol (2.65%), guaiol (2.11%), and valerenol (2.04%) were the major sesquieterpenes found in agarwood essential oil from Chonburi (commercial 1), germacrene (3.88%), and agarospirol (1.73%) found in agarwood essential oil from Trad (commercial 2). The variation of chemical compositions is actually caused by different harvesting time, growing location, part of the plant to be extracted, the geographical origin and extraction process.

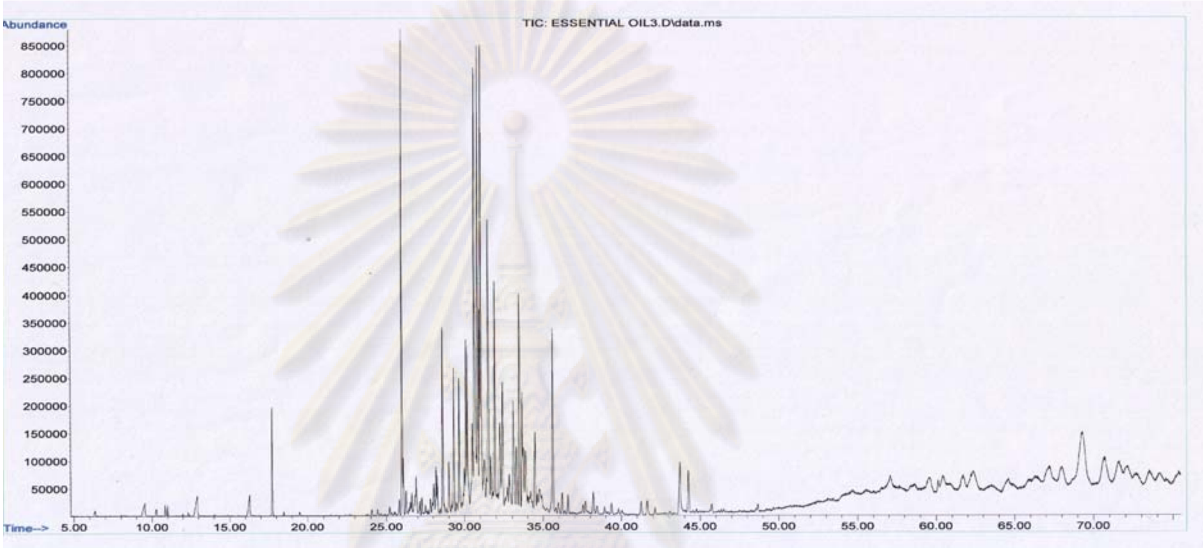


Figure 4.6 GC-MS chromatogram of essential oil of agarwood [commercial 1].

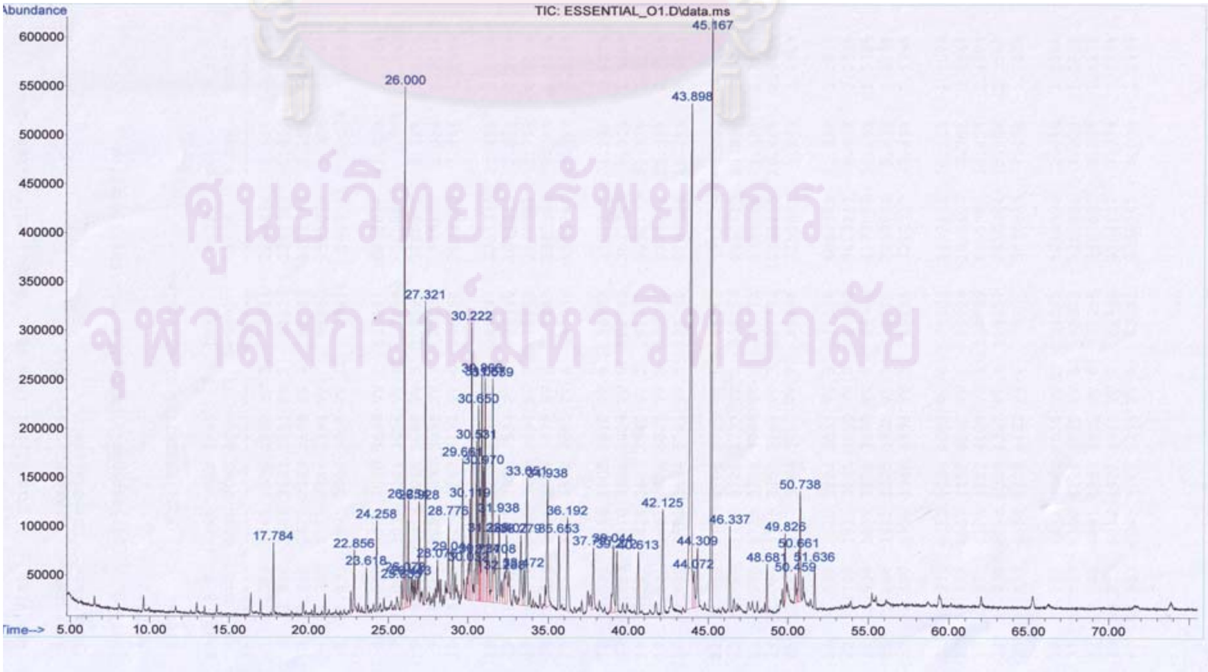


Figure 4.7 GC-MS chromatogram of essential oil of agarwood [commercial 2].

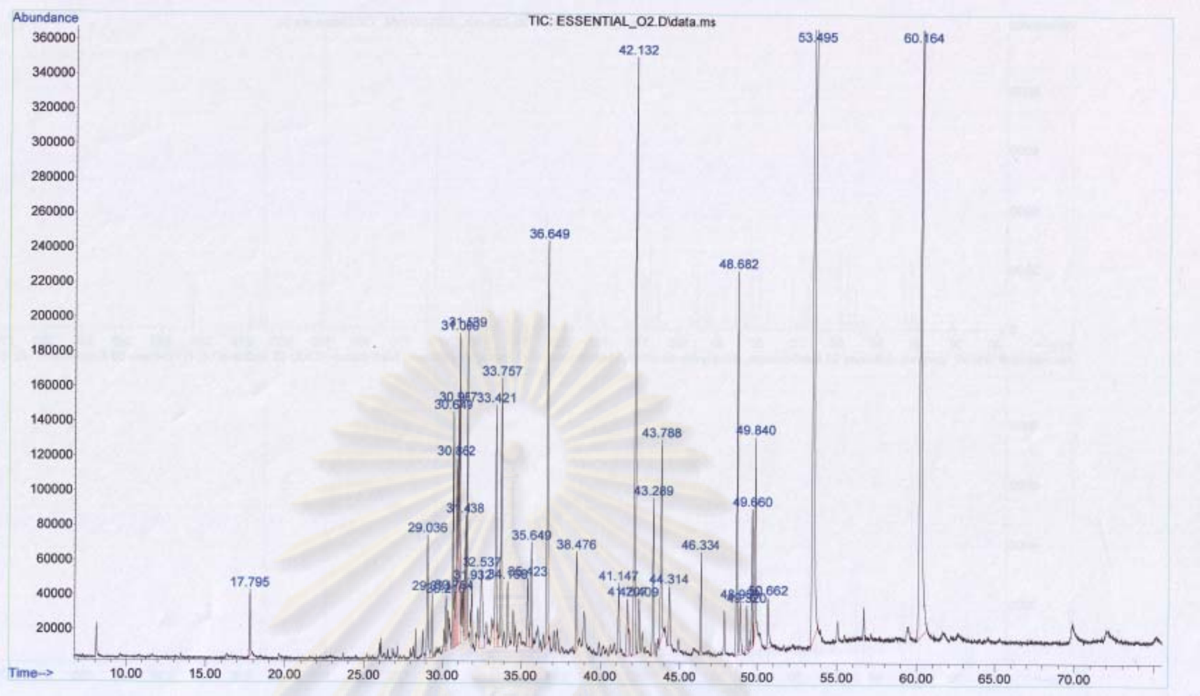


Figure 4.8 GC-MS chromatogram of the extract from SC-CO_2 extraction.

4.2.1 The chemical compositions obtained by SC-CO_2 and SC-CO_2 with co-solvent

The principal parameter for determining the solubility of an organic molecule in CO_2 is the density of CO_2. The density determines the number of interactions between CO_2 and molecules of the organic compounds. If sufficient interactions occur, the cohesive forces between individual molecules of the organic compound are broken and solubilization will occur. Therefore, the solubilization of organic molecules in CO_2 will be a function of the molecular weight of the organic compound and the level of interaction between CO_2 and organic molecules [27].

Based on dry agarwood, agarwood extracted by SC-CO_2 was varied from 0.20 % to 1.93 % (w/w). The results of GC-MS chromatogram of the extract from SC-CO_2 extraction is shown in Figure 4.11, and the composition percentage corresponding to each constituent along with the methods of identification were given in Table D-1 to D-3 (in appendix), wherein the components were listed in order of their elution on the DB-1 column.

On the basis of these extractions, the optimal experimental conditions were chosen according to a) the presence of higher contents of sesquiterpenes derivative compounds (these compounds play a key role in the overall aroma of essential oil), and b) the similarity of the components compared with agarwood oil obtained by hydrodistillation (commercial 1, 2).

As it is concluded from the results, the best extraction condition obtained by supercritical CO_2 extracted was the temperature of 65 °C, the pressure of 12 MPa and flow rate 3 g/min for 3 h.

The attractive result was the extraction of the interested compounds (sesquiterpenes derivative compounds) when compared with agarwood oil from hydrodistillation (commercial 1, 2). The chemical composition of the extract obtained by supercritical CO_2 extraction at the temperature of 65 °C, the pressure of 12 MPa were elemol(4.65%), α-santalol (4.47%), viridiflorol (3.83%), (+)-epi-bicyclosesquiphellandrene (3.48%), germacrene(3.20%), guai-1(10)-en-11-ol (2.55%), epi-ligulyl oxide (2.19%), and agarospirol (1.87%) as the major sesquiterpenes; and 2-(2- phenylethyl)chromen (18.60%) as the major chromone. Therefore, this condition offers a very selective extraction. The pressure and temperature conditions of this condition have been recommended for the extraction of essential oil with SC-CO_2, because their solubility is sufficient at these conditions. While the solubilities of fatty oils, pigment, and waxes presented in the plant material were negligible in this condition.

Li Mei *et al.* [28] reported the composition of hydrodistilled essential oil of Chinese eaglewood. The major component consists of β-agarofuran, kusunol, (-)-jinkoh-eremol, agarospirol, and baimuxifuranic acid. It was found that there were some compositions (jinkoh-eremol and baimuxifuranic acid) that were different from this work caused by different harvesting time, growing location, part of the plant to be extracted, the geographical origin, and extraction process.

In this section, the effect of pressure on the chemical composition of the extract at three different levels (12, 18, and 25 MPa) at constant temperature (65 °C) was investigated. The results showed that the concentrations of elemol, viridiflorol, valerenol, guai-1(10)-en-11-ol, and α-santalol in the extract decreased when higher pressures were used (Table D-1 to D-3, in appendix). However, increasing pressure enhanced the contents of 2-(2-phenylethyl)chromen (18.60 to 51.41%), and 8-methoxyflindersiachromone (0 to 14.22%) in the extract. Due to the fact that higher pressure can be extracted high molecular weigh compounds (waxes, resins and pigments)[10].

The effect of four different temperatures (40, 50, 55, and 65 °C) at constant pressure (12 MPa) on the chemical composition of the extract using pure SC-CO_2 was investigated. Increasing the temperature from 40 to 65 °C at constant pressure drastically increased the concentrations of (+)-Epi-bicyclosesquiphellandrene (0 to 3.48%), elemol (0 to 4.65%), agarospirol (0 to 1.87%), hinesol (0 to 1.77%), viridiflorol (0 to 3.83%), and valerenol (0 to 1.34%) in oil.

And due to the limited solubility of polar organic compounds in SC-CO_2, quantitative extraction of these compounds with pure SC-CO_2 is not possible. The addition of a polar co-solvent to SC-CO_2 has been shown to offer tremendous increases in the extraction efficiency of polar organic compounds, but due to different interactions of the modifier with sample matrices, a specific modifier for a class of compounds must be chosen [13]. In this research, the effect of different co-solvents and co-solvents concentration at the temperature of 50 and 65 °C, and the pressure of 12 and 25 MPa was also evaluated (shown in Table E-4, in appendix).

As it is concluded that the temperature of 65 °C, the pressure of 12 MPa, and 10% ethyl acetate was the better condition in extraction for the interested compounds more than the other co-solvent. The major compositions of the extract obtained by supercritical CO_2 extraction with 10% ethyl acetate at temperature of 65 °C, pressure of 12 MPa were elemol (4.14%), oxo-agarofuran (2.83%), viridiflorol (2.15%), and (+)-epi-bicyclosesquiphellandrene (1.55%) as the major sesquiterpenes; and 2-(2-

phenylethyl)chromen (42.81%) and 8-methoxyflindersiachromone (11.63%) as the major chromone.

This section shows the effect of co-solvent type and co-solvent concentration on the major compositions. Table D-4 (in appendixs) shows the effect of co-solvent at the temperature of 65 °C and the pressure of 12 MPa was investigated: the effect of co-solvent (5% and 10%) on the composition of the extract. In the presence of co-solvents (methanol, ethanol, and ethyl acetate), the percentages of oxo-agarofuran, 2-(2-phenylethyl)chromen and 8-methoxyflindersiachromone increased and the percentages of elemol, germacrene, viridiflorol, (+)-epibicyclosesquiphellandrene, valerenol, agarospirol, and guai-1(10)-en-11-ol decreased in comparison with extraction by pure carbon dioxide. In other word, the co-solvent increased the percentages of co-extracted compounds in the extract composition. Under this condition ethyl acetate was the most efficient co-solvent for the interested compounds. These results showed that the addition of 10% ethyl acetate as co-solvent was more effective than the addition of 5 % ethyl acetate.

It should be noted that the percentages of oxo-agarofuran, 2-(2-phenylethyl)chromen, and 8-methoxyflindersiachromone increased in the presence of co-solvent. It is well known that the addition of a polar co-solvent to SC-CO_2 can greatly improve the extraction by increasing solubility of the polar organic compounds and high molecular weight compounds, by reducing their interaction with sample matrix or by inducing matrix modification, in this way; release of the polar organic compounds from the matrix can be advantageously enhanced [13].

From the results, two extraction methods (SC-CO_2 extraction and SC-CO_2 extraction with 10%ethyl acetate) were compared to those obtained by hydrodistillation (commercail1, 2) (table 4.3). It can be concluded that SC-CO_2 extraction at temperature of 65 °C, pressure of 12 MPa is more selective for the interested compounds (sesquiterpenes derivative compounds) than SC-CO_2 extraction with 10%ethyl acetate.

Table 4.3 The comparative the chemical compositions of the extract from agarwood obtained by SC-CO_2, SC-CO_2 with 10% ethyl acetate and hydrodistillation (commercial)

No.	Compound	Peak Area (%)			
		1^a	2^b	P=12 MPa T = 65 °C	
				pure CO_2	10% EtOAc
1	Pentanoic acid	–	–	–	–
2	Hexanoic acid	0.04	–	0.41	–
3	1-Penten-3-one	0.40	7.47	0.31	3.27
4	Heptanoic acid	0.10	–	–	–
5	Benzyl acetone	0.94	0.71	0.64	0.14
6	Octanoic acid	0.17	–	0.52	–
7	Nonanoic acid	0.02	0.11	–	–
8	Tridecane	–	0.26	–	–
9	Cyclohexasiloxane	–	–	–	–
10	Decanoic acid	–	–	–	–
11	Tetradecane	–	–	–	–
12	Aristolene	–	1.88	–	–
13	γ-Elemene	3.44	–	0.35	–
14	α-Guaiene	3.68	1.39	0.96	–
15	Epi-ligulyl oxide	0.65	1.25	2.19	0.50
16	Eudesma-4(14), 11-diene	0.21	–	–	–
17	β-Selinene	5.79	1.21	0.61	–
18	(+)-Epi-bicyclosesquiphellandrene	6.63	0.89	3.48	1.55
19	Valencene	0.14	–	0.67	–
21	α-Agarofuran	0.27	0.11	0.29	–
22	4,5-Dehydro-isolongifolene	0.39	0.17	0.35	–
23	Zierone	1.30	0.61	0.46	–
24	Elemol	0.44	–	4.65	4.14
25	Germacrene	0.71	3.88	3.20	–
26	Dodecanoic acid	–	0.84	–	–
27	1H-Cycloprop[e]azulene	6.81	3.08	–	–
28	Guaiol	2.11	–	–	–
29	Viridiflorol	2.65	–	3.83	2.15
30	γ-Eudesmol	–	1.39	–	–
31	Agarospirol		1.73	1.87	1.00
32	Guai-1(10)-en-11-ol	1.46	0.59	2.55	–
33	Valerenol	2.04	0.62	1.34	0.42
34	Hinesol	8.81	–	1.77	1.00
35	α-Santalol	0.19	0.49	4.47	–
36	Tetradecanoic acid	2.47	1.01	0.18	1.24
37	Aritolone	3.16	0.45	0.99	–
38	Baimuxinal (Oxo-agarofuran)	0.75	0.14	1.69	2.83
39	Dehydro-cohumulinic acid	0.71	0.22	0.52	–
40	Pentadecanoic acid	0.53	0.55	0.33	0.57
41	Palmitic acid	5.63	13.68	1.74	0.98
42	Dibenzylacetone	1.45	–	0.32	2.30
43	Heptadecanoic acid	–	6.63	0.52	2.58
44	8-Octadecanoic aid	–	–	0.92	0.64
45	Octadecanoic acid	2.20	1.91	1.05	1.79
46	9-Octadecanoic aid	0.20	1.55	1.39	6.27
47	Octadec-9-enoic acid	–	0.37	1.05	1.58
48	9,12-Octadecadienoic aid	–	–	–	–
49	2-(2-Phenylethyl)chromen	0.07	0.91	18.60	42.81
50	Estra-1,3,5(10)-trien-17-one	0.18	–	–	–
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	0.20	–	0.79	2.77
52	8-Methoxyflindersiachromone	0.40	0.40	–	11.63

1^a, 2^b: agarwood oil obtained by hydrodistillation (commercial) from Chonburi and Trad provinces, respectively.

4.3 Steam distillation and soxhlet extraction

The steam distillation and soxhlet extraction methods have been traditionally used in the extraction of oils in commercial. In this study, the efficiency of their methods were compared with SC-CO_2 extraction and SC-CO_2 extraction with ethyl acetate, by the extraction yield (% weight) and the chemical composition (% peak area) analysis by GC. For the soxhlet extraction method, methanol, ethanol, ethyl acetate, and hexane were used and the extraction was performed at the temperature of 65-70 °C for 24 h.

4.3.1 The extraction yield

Many organic compounds tend to decompose at high sustained temperatures. Separation by normal distillation would then not be an option, so water or steam is introduced into the distillation apparatus. By adding water or steam, the boiling points of the compounds are depressed, allowing them to evaporate at lower temperatures, preferably below the temperatures at which the deterioration of the material becomes appreciable. If the substances to be distilled are very sensitive to heat, steam distillation can also be combined with vacuum distillation. After distillation the vapors are condensed as usual, usually yielding a two-phase system of water and the organic compounds, allowing for simple separation.

The extract obtained by hydrodistillation (Li Mei *et al*. [28]) and steam distillation were pale yellow. The extraction yield of steam distillation and hydrodistillation[28] were similar (0.28% and 0.21%w/w, respectively) (Figure 4.9) but the yield was lower than SC-CO_2 and SC-CO_2 with ethyl acetate. The chemical compositions of the extract obtained by steam distillation analysis by GC analysis are shown in Table 4.4.

In the case of soxhlet extraction, in many cases, soxhlet extraction is not selective, because interfering compounds may also be extracted by the heated solvent of choice which may further complicate the assay of the analytes of interest. The choice of solvent obviously controls the solvating power as well as the temperature of the extraction. Under these conditions the integrity of the analytes may not be maintained in that thermal decomposition or reaction with the solvent may occur. Perhaps the greatest disadvantage of using the soxhlet method for extractions is its utilization of expensive, high purity organic solvents. While automation is seldom attempted, it does have the advantage that numerous soxhlet extraction can be simultaneously performed. Furthermore, hardware for soxhlet extraction is fairly simple; however, for quantitative work, extreme care must be taken to avoid introduction of contaminants and to minimize losses in sample transfer and solvent exchange [15].

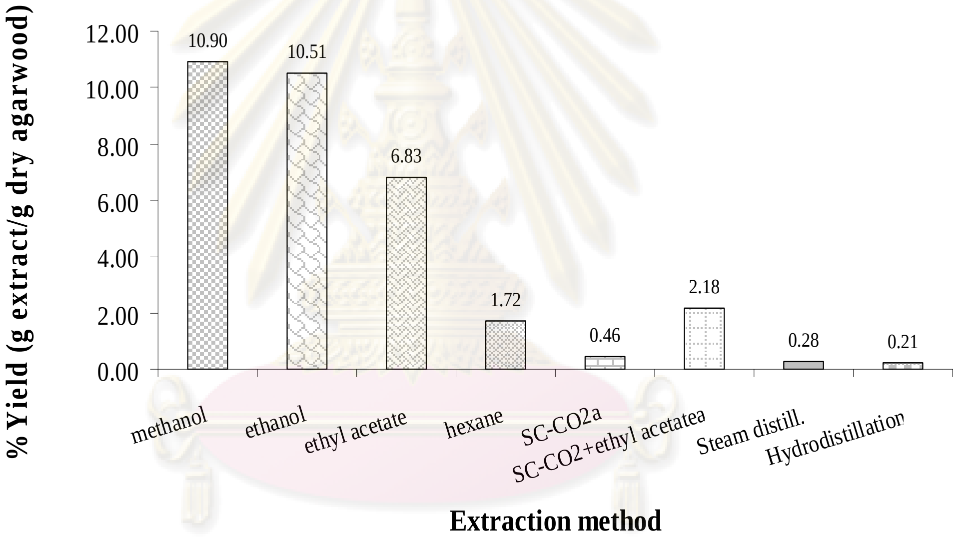


Figure 4.9 Effect of solvent type in soxhlet extraction on the extraction yield when compared with SC-CO_2 extraction with and without co-solvent, steam distillation and hydrodistillation [28].

a: T= 65 °C, P=12 MPa and CO_2 flow rate 3 g/min

In this section, the effect of solvent type in soxhlet extraction on the essential oil yield were compared with SC-CO_2 extraction with and without co-solvent (ethyl acetate), steam distillation and hydrodistillation [28] are shown in Figure 4.9. The extractionl yields of absolute from methanol, ethanol, ethyl acetate, hexane in soxhlet extraction and four extraction methods (SC-CO_2 extraction with and without co-solvent (ethyl acetate), steam distillation and hydrodistillation [28]) were 10.89 %

w/w, 10.51 % w/w, 6.82 % w/w, 1.72% w/w, 0.46 %w/w, 2.18 %w/w, 0.28% w/w, and 0.21% w/w (dry basis), respectively. For the characteristics of absolute from four solvents in soxhlet extraction, as shown in Figure 4.10, while methanol and ethanol absolutes were brown and had strong scent; ethyl acetate absolute was dark yellow and had strong scent; hexane absolute was pale yellow and had mild scent. The results show that the quantity of the extract using soxhlet extraction was higher than that achieved using SC-CO_2 with and without co-solvent, steam distillation, and hydrodistillation [28]. This was because the solvent used in soxhlet extraction can be evaporated and condensed in the same way as the steam distillation. While the hydrodistillation [28] and steam distillation gave the lowest yield because these two methods delivered only the volatile fraction constituted of the extract. However, soxhlet extraction has a tendency to reduce the extractibility selectivity due to the extraction of undesirable components in terms of the quality, such as fatty acid, waxes, and pigments. The results are shown in Table 4.4.

Figure 4.10 Characteristics of absolutes from four solvents: methanol, ethanol, ethyl acetate, and hexane in soxhlet extraction, respectively.

4.3.2 The chemical composition obtained by steam distillation and soxhlet extraction.

Based on dry agarwood, agarwood extracted by soxhlet extraction was varied from 1.72-10.89 % (w/w), whereas by steam distillation was 0.28 % (w/w). The composition percentages corresponding to each constituent along with the methods of identification are given in Table 4.4.

For the major compositions obtained by soxhlet extraction, oxo-agarofuran (2.64%), viridiflorol (1.85%), and elemol (1.16%) were the major sesquiterpenes and 2-(2-phenylethyl)chromen(55.98%), and 8-methoxyflindersiachromone (18.41%) were the major chromone. For the major compositions of the extract obtained by steam distillation, viridiflorol (3.93%), agarospirol (2.32%), oxo-agarofuran (2.12%), and aristolene (1.68%) were the major sesquiterpenes.

Thus, in order to evaluate the quality of the extract obtained by soxhlet extraction and steam distillation, Table 4.4 shows the comparative composition of the essential oil obtained by two extraction methods compared with agarwood oil obtained by hydrodistillation (commercial grade1,2). On the basis of these extractions, the optimal experimental conditions were chosen according to a) the presence of higher contents of sesquiterpenes compounds (these compounds play a key role in the overall aroma of essential oil) and b) the similarity of the components compared with commercial agarwood oil obtained by hydrodistillation. Table 4.4 indicated that the soxhlet extraction using ethanol as solvent and steam distillation are more selective for the interested sesquiterpenes compounds when compared with agarwood oil (commercial grade) than the essential oil obtained by the soxhlet extraction using other organic solvents, but it was less effective than pure carbon dioxide (at temperature of 65 °C, pressure of 12 MPa). Moreover, the extraction using by pure carbon dioxide has a shorter extraction time.

Table 4.4 The comparative the chemical compositions of the extract from agarwood obtained by soxhlet extraction and steam distillation

No.	Compound	1[a]	2[b]	Peak Area (%)				
				Soxhlet				Steam distillation
				EtOH	MeOH	EtOAc	Hexane	
1	Pentanoic acid	–	–	0.82	1.02	0.70	–	–
2	Hexanoic acid	0.04	–	0.49	–	0.12	–	–
3	1-Penten-3-one	0.40	7.47	0.70	1.89	0.38	0.38	13.90
4	Heptanoic acid	0.10	–	0.92	–	0.10	–	0.89
5	Benzyl acetone	0.94	0.71	3.19	2.38	1.28	0.17	8.02
6	Octanoic acid	0.17	–	0.43	0.12	0.14	0.19	1.13
7	Nonanoic acid	0.02	0.11	–	0.16	0.09	–	–
8	Tridecane	–	0.26	0.12	–	–	–	–
9	Cyclohexasiloxane	–	–	–	–	–	–	–
10	Decanoic acid	–	–	0.30	0.32	0.18	–	–
11	Tetradecane	–	–	–	–	0.08	–	1.13
12	Aristolene	–	1.88	–	0.49	0.39	–	1.68
13	γ-Elemene	3.44	–	0.10	0.09	0.12	–	–
14	α-Guaiene	3.68	1.39	0.53	0.35	0.36	–	1.44
15	Epi-ligulyl oxide	0.65	1.25	0.58	0.11	0.06	–	–
16	Eudesma-4(14), 11-diene	0.21	–	–	–	0.20	–	–
17	β-Selinene	5.79	1.21	–	0.11	0.12	–	–
18	(+)-Epi-bicyclosesquiphellandrene	6.63	0.89	0.31	0.21	0.13	–	0.86
19	Valencene	0.14	–	0.17	–	–	–	0.25
21	α-Agarofuran	0.27	0.11	–	–	0.07	–	0.14
22	4,5-Dehydro-isolongifolene	0.39	0.17	–	0.12	0.36	–	–
23	Zierone	1.30	0.61	0.13	–	0.07	–	0.09
24	Elemol	0.44	–	1.16	0.71	0.57	–	1.22
25	Germacrene	0.71	3.88	1.16	0.11	0.44	–	–
26	Dodecanoic acid	–	0.84	0.17	–	0.06	–	–
27	1H-Cycloprop[e]azulene	6.81	3.08	–	–	0.23	–	–
28	Guaiol	2.11	–	–	0.06	–	–	–
29	Viridiflorol	2.65	–	1.85	1.09	0.59	–	3.93
30	γ-Eudesmol	–	1.39	–	–	–	–	0.25
31	Agarospirol		1.73	0.80	0.51	0.40	–	2.32
32	Guai-1(10)-en-11-ol	1.46	0.59	0.80	–	0.28	–	–
33	Valerenol	2.04	0.62	–	0.08	0.06	–	0.49
34	Hinesol	8.81	–	0.86	–	–	–	–
35	α-Santalol	0.19	0.49	0.72	0.64	0.58	–	–
36	Tetradecanoic acid	2.47	1.01	0.19	0.47	0.42	–	8.21
37	Aritolone	3.16	0.45	0.65	0.10	0.54	–	1.19
38	Baimuxinal (Oxo-agarofuran)	0.75	0.14	2.64	1.67	1.79	0.37	2.12
39	Dehydro-cohumulinic acid	0.71	0.22	0.38	0.09	0.13	–	–
40	Pentadecanoic acid	0.53	0.55	0.21	0.27	0.16	–	–
41	Palmitic acid	5.63	13.68	0.58	1.76	1.00	0.30	1.14
42	Dibenzylacetone	1.45	–	1.19	0.53	0.15	–	0.29
43	Heptadecanoic acid	–	6.63	1.14	1.61	0.97	0.12	1.24
44	8-Octadecanoic aid	–	–	1.14	1.32	1.06	0.12	1.95
45	Octadecanoic acid	2.20	1.91	1.37	1.93	1.09	0.33	0.68
46	9-Octadecanoic aid	0.20	1.55	2.77	5.31	4.52	1.48	0.87
47	Octadec-9-enoic acid	–	0.37	1.88	1.99	1.66	0.32	1.28
48	9,12-Octadecadienoic aid	–	–	–	2.76	–	–	0.53
49	2-(2-Phenylethyl)chromen	0.07	0.91	40.85	35.54	55.98	44.92	0.57
50	Estra-1,3,5(10)-trien-17-one	0.18	–	0.39	1.37	0.07	2.82	0.56
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	0.20	–	3.16	1.44	1.03	1.12	–
52	8-Methoxyflindersiachromone	0.40	0.40	13.55	11.70	18.41	17.71	–

1[a], 2[b]: agarwood oil obtained by hydrodistillation (commercial) from Chonburi and Trad provinces.

4.4 Source of agarwood affects the extraction yield and the chemical composition obtained by SC-CO_2 extraction with and without co-solvent

The comparison of the extraction yield percentages between agarwood from Chonburi and Trad province obtained by SC-CO_2 extraction with and without co-solvent at temperature of 65 °C, pressure of 12 MPa are shown in Table 4.5. The extract was light yellow (in pure SC-CO_2) and yellow (in SC-CO_2 with ethyl acetate). The extraction yield of agarwood from Chonburi province was similar to that from Trad province.

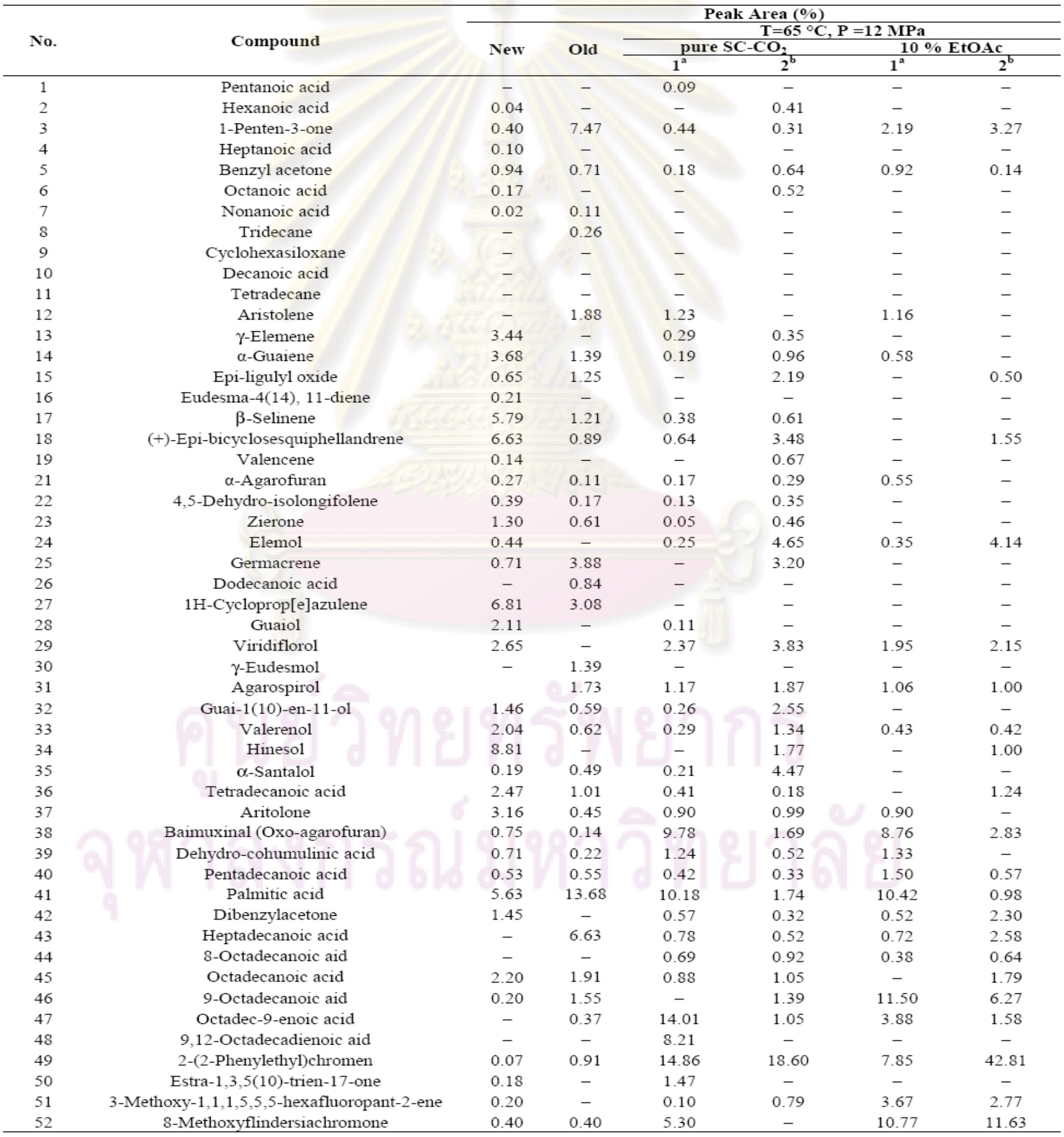
Table 4.5 Comparison of the extraction yield percentages between agarwood from Chonburi and Trad provinces

Condition	**% The extraction yield** (g oil/g dry agarwood)	
	1^a	2^b
T = 65 °C and P = 12 MPa	0.26	0.24
T = 65 °C, P = 12 MPa and 10%EtOAc	2.88	2.18

1^a: Chonburi province.
2^b: Trad province.

The chemical compositions obtained by pure SC-CO_2 extracts from different locations are shown in Table 4.6. The major compositions of the extract from Chonburi province were oxo-agarofuran (9.78%), viridiflorol (2.37%), and agarospirol (1.17%) as sesquiterpenes, 2-(2-phenylethyl)chromen (14.86%) and 8-methoxyflindersiachromone (5.30%) as chromones. Whereas the major composition of the extract from Trad province were elemol(4.65%), α-santalol (4.47%), viridiflorol (3.83%), germacrene (3.20%), guai-1(10)-en-11-ol(2.55%), and oxo-agarofuran (1.69%) as sesquiterpenes, 2-(2- phenylethyl)chromen (18.60%) as chromones. While the oil compositions obtained by SC-CO_2 with co-solvents (10% EtOAc) extracts from different locations are shown in Table 4.6. The major compositions of the extract from Chonburi province were oxo-agarofuran (8.76%), viridiflorol (1.95%), and agarospirol (1.06%) as sesquiterpenes; 8-methoxyflindersiachromone (10.77%) and 2-(2-Phenylethyl)chromen (7.85%) as chromones. The major compositions of the extract from Trad province were elemol (4.14%), oxo-agarofuran (2.83%), and viridiflorol (2.15%) as sesquiterpenes; 2-(2-phenylethyl)chromen (42.81%) and 8-methoxyflindersiachromone (11.63%) as chromones. The variation of chemical compositions is actually caused by different harvesting time, growing location, part of the plant to be extracted, the geographical origin.

Table 4.6 The comparative the chemical compositions of the extract from agarwood obtained by SC-CO_2 with and without co-solvent from different locations



No.	Compound	Peak Area (%)					
		T=65 °C, P =12 MPa					
		New	Old	pure SC-CO_2		10 % EtOAc	
				1^{a}	2^{b}	1^{a}	2^{b}
1	Pentanoic acid	–	–	0.09	–	–	–
2	Hexanoic acid	0.04	–	–	0.41	–	–
3	1-Penten-3-one	0.40	7.47	0.44	0.31	2.19	3.27
4	Heptanoic acid	0.10	–	–	–	–	–
5	Benzyl acetone	0.94	0.71	0.18	0.64	0.92	0.14
6	Octanoic acid	0.17	–	–	0.52	–	–
7	Nonanoic acid	0.02	0.11	–	–	–	–
8	Tridecane	–	0.26	–	–	–	–
9	Cyclohexasiloxane	–	–	–	–	–	–
10	Decanoic acid	–	–	–	–	–	–
11	Tetradecane	–	–	–	–	–	–
12	Aristolene	–	1.88	1.23	–	1.16	–
13	γ-Elemene	3.44	–	0.29	0.35	–	–
14	α-Guaiene	3.68	1.39	0.19	0.96	0.58	–
15	Epi-ligulyl oxide	0.65	1.25	–	2.19	–	0.50
16	Eudesma-4(14), 11-diene	0.21	–	–	–	–	–
17	β-Selinene	5.79	1.21	0.38	0.61	–	–
18	(+)-Epi-bicyclosesquiphellandrene	6.63	0.89	0.64	3.48	–	1.55
19	Valencene	0.14	–	–	0.67	–	–
21	α-Agarofuran	0.27	0.11	0.17	0.29	0.55	–
22	4,5-Dehydro-isolongifolene	0.39	0.17	0.13	0.35	–	–
23	Zierone	1.30	0.61	0.05	0.46	–	–
24	Elemol	0.44	–	0.25	4.65	0.35	4.14
25	Germacrene	0.71	3.88	–	3.20	–	–
26	Dodecanoic acid	–	0.84	–	–	–	–
27	1H-Cycloprop[e]azulene	6.81	3.08	–	–	–	–
28	Guaiol	2.11	–	0.11	–	–	–
29	Viridiflorol	2.65	–	2.37	3.83	1.95	2.15
30	γ-Eudesmol	–	1.39	–	–	–	–
31	Agarospirol		1.73	1.17	1.87	1.06	1.00
32	Guai-1(10)-en-11-ol	1.46	0.59	0.26	2.55	–	–
33	Valerenol	2.04	0.62	0.29	1.34	0.43	0.42
34	Hinesol	8.81	–	–	1.77	–	1.00
35	α-Santalol	0.19	0.49	0.21	4.47	–	–
36	Tetradecanoic acid	2.47	1.01	0.41	0.18	–	1.24
37	Aritolone	3.16	0.45	0.90	0.99	0.90	–
38	Baimuxinal (Oxo-agarofuran)	0.75	0.14	9.78	1.69	8.76	2.83
39	Dehydro-cohumulinic acid	0.71	0.22	1.24	0.52	1.33	–
40	Pentadecanoic acid	0.53	0.55	0.42	0.33	1.50	0.57
41	Palmitic acid	5.63	13.68	10.18	1.74	10.42	0.98
42	Dibenzylacetone	1.45	–	0.57	0.32	0.52	2.30
43	Heptadecanoic acid	–	6.63	0.78	0.52	0.72	2.58
44	8-Octadecanoic aid	–	–	0.69	0.92	0.38	0.64
45	Octadecanoic acid	2.20	1.91	0.88	1.05	–	1.79
46	9-Octadecanoic aid	0.20	1.55	–	1.39	11.50	6.27
47	Octadec-9-enoic acid	–	0.37	14.01	1.05	3.88	1.58
48	9,12-Octadecadienoic aid	–	–	8.21	–	–	–
49	2-(2-Phenylethyl)chromen	0.07	0.91	14.86	18.60	7.85	42.81
50	Estra-1,3,5(10)-trien-17-one	0.18	–	1.47	–	–	–
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	0.20	–	0.10	0.79	3.67	2.77
52	8-Methoxyflindersiachromone	0.40	0.40	5.30	–	10.77	11.63

1^{a}, 2^{b}: agarwood oil obtained by SC-CO_2 extraction with and without co-solvent from Chonburi and Trad provinces, respectively.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The parameters under study show different influences on the extracted. The effect of pressure and temperature control both its yield and composition, since a slight variation of these parameters leads to a change in the density and diffusivity of the supercritical CO_2 fluid. As the pressure rises (at the same temperature), the solvent also solubilizes non-volatile components (such as wax, pigment and fatty acid) that contaminate the extract. Taking into account the general variation of the yield with flow rate and particle size, the extraction yields increases with increasing CO_2 flow rate and smaller pieces of dried agarwood. With regard to the temperature, an increase of temperature (at the same pressure) leads to a decrease of the density of solvent (SC-CO_2) but increases the diffusivity of solvent (SC-CO_2), resulting in a lower yield but the chemical composition becomes richer in the interested sesquiterpenes compounds. Therefore, the optimal condition for extraction essential oil by SC-CO_2 extraction is temperature 65 °C and pressure 12 MPa because this condition minimizes the undesirable compounds and it gives high amount of the interested. Moreover, there is an investigation of the effect of co-solvents (methanol, ethanol, and ethyl acetate) which are added at various volumes (5-10%) v/v into SC-CO_2 fluid. The extraction with co-solvents give higher yield than SC-CO_2 without co-solvents but the chemical compositions from this method consist of chromone derivatives and long chain carboxylic acid. The soxhlet extraction (by methanol, ethanol, ethyl acetate, and hexane) gives the highest yield among all methods but the chemical compositions from this method consist of undesirable components in terms of the quality, such as high molecular weigh compounds and long chain carboxylic acid. This method also requires much longer time than SC-CO_2

extraction (3 hrs compared with 24 h). In the case of steam distillation, it gives lower yield than the SC-CO_2 extraction, although some chemical compositions from both extraction methods are similar. Besides, this method requires much longer time than SC-CO_2 extraction (3 h compared with 2 day). From the results, the advantages of SC-CO_2 extraction include no additional step for solvent removal, no thermal degradation of most labile compounds; shorter extraction period and high selectivity in the interested compounds with positive effects on the quality.

5.2 Recommendations

The agarwood extraction by SC-CO_2 with and without co-solvent should be scaled up and reinvestigated.

There should be scent testing by having a group of people smelling the scent of agarwood extracted with different methods.

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APPENDICES

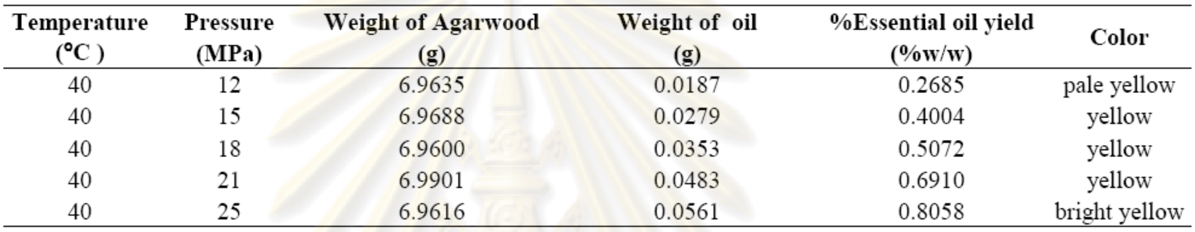
Appendix A

Supercritical CO_2 Extraction

Experimental data

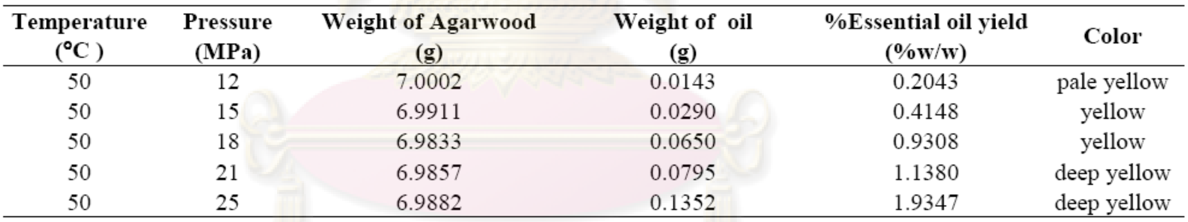
Table A-1 SC-CO_2 experimental conditions and extraction yields for agarwood oil.

Temperature 40 °C Pressure 12-25 MPa
Flow rate 2 g/min Time 4 h.



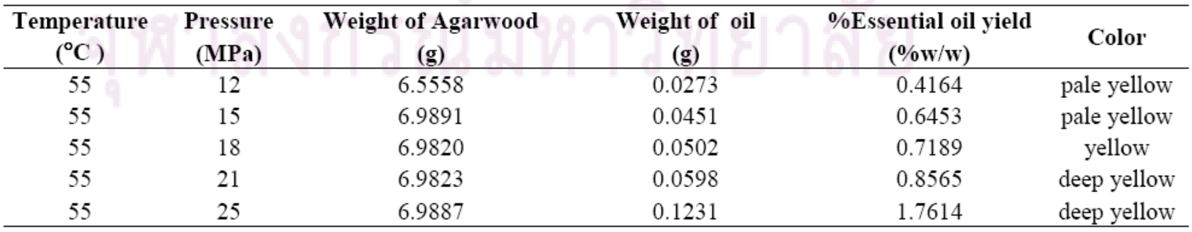
Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
40	12	6.9635	0.0187	0.2685	pale yellow
40	15	6.9688	0.0279	0.4004	yellow
40	18	6.9600	0.0353	0.5072	yellow
40	21	6.9901	0.0483	0.6910	yellow
40	25	6.9616	0.0561	0.8058	bright yellow

Temperature 50 °C Pressure 12-25 MPa
Flow rate 2 g/min Time 4 h.



Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
50	12	7.0002	0.0143	0.2043	pale yellow
50	15	6.9911	0.0290	0.4148	yellow
50	18	6.9833	0.0650	0.9308	yellow
50	21	6.9857	0.0795	1.1380	deep yellow
50	25	6.9882	0.1352	1.9347	deep yellow

Temperature 55 °C Pressure 12-25 MPa
Flow rate 2 g/min Time 4 h.



Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
55	12	6.5558	0.0273	0.4164	pale yellow
55	15	6.9891	0.0451	0.6453	pale yellow
55	18	6.9820	0.0502	0.7189	yellow
55	21	6.9823	0.0598	0.8565	deep yellow
55	25	6.9887	0.1231	1.7614	deep yellow

Temperature 60 °C Pressure 12-25 MPa
Flow rate 2 g/min Time 4 h.

Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
60	12	6.9895	0.0343	0.4907	paleyellow
60	15	6.9947	0.0390	0.5576	yellow
60	18	6.9643	0.0422	0.6059	yellow
60	21	6.9954	0.0554	0.7919	deep yellow
60	25	6.9774	0.0583	0.8355	deep yellow

Temperature 65 °C Pressure 12-25 MPa
Flow rate 2 g/min Time 4 h.

Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
65	12	7.0100	0.0166	0.2368	pale yellow
65	15	6.9878	0.0434	0.6210	yellow
65	18	6.9844	0.0583	0.8347	yellow
65	21	6.9858	0.0633	0.9061	deep yellow
65	25	6.9861	0.0645	0.9233	deep yellow

Temperature 70 °C Pressure 12-25 MPa
Flow rate 2 g/min Time 4 h.

Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
70	12	6.2940	0.0136	0.2161	yellow
70	15	6.9897	0.0305	0.4363	yellow
70	18	6.9795	0.0566	0.8109	yellow
70	21	6.9941	0.0601	0.8593	deep yellow
70	25	6.9822	0.0640	0.9166	deep yellow

Temperature 50 °C Pressure 25 MPa
Flow rate 1-4 g/min Time 3 h

Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Flow rate (g/min)
50	25	6.9977	0.0812	1.1604	1
50	25	6.9882	0.1352	1.9347	2
50	25	6.9934	0.3101	4.4342	3
50	25	6.9903	0.0992	1.4191	4

Temperature 55 °C		Pressure 25 MPa
Flow rate 1-4 g/min		Time 3 h

Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Flow rate (g/min)
55	25	7.0382	0.0388	0.5513	1
55	25	6.9887	0.1231	1.7614	2
55	25	7.0170	0.1625	2.3158	3
55	25	6.9972	0.0449	0.6417	4

Temperature 65 °C		Pressure 12 MPa
Flow rate 1-4 g/min		Time 3 h

Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Flow rate (g/min)
65	12	6.7121	0.0102	0.1520	1
65	12	7.0100	0.0166	0.2368	2
65	12	6.7121	0.0307	0.4574	3
65	12	6.9865	0.0084	0.1202	4

Temperature 50 °C		Pressure 25 MPa
Flow rate 2 g/min		Time 3 h		Particle size no milled

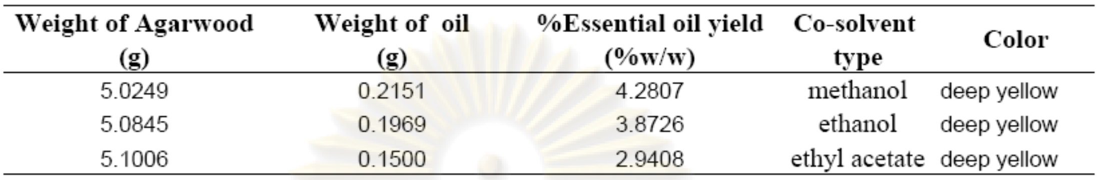
Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Time (h)
50	25	5.3446	0.0186	0.3480	0.5
50	25	5.3446	0.0279	0.5220	1
50	25	5.3446	0.0281	0.5258	2
50	25	5.3446	0.0281	0.5258	3

Temperature 50 °C		Pressure 25 MPa
Flow rate 2 g/min		Time 3 h		Particle size 35 mesh

Temperature (°C)	Pressure (MPa)	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Time (h)
50	25	6.9883	0.1186	1.6971	0.5
50	25	6.9883	0.1246	1.7830	1
50	25	6.9883	0.1299	1.8588	2
50	25	6.9883	0.1320	1.8889	3

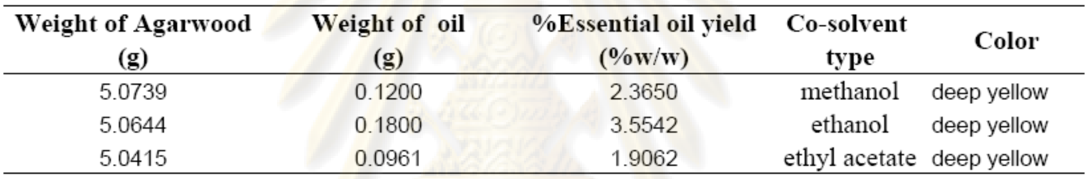
Table A-2 SC-CO_2 with co-solvent experimental conditions and extraction yields for agarwood oil.

Temperature 50 °C	Pressure 25 MPa
Flow rate 3 g/min	Time 3 h
Co-solvent 5%co-solvent	



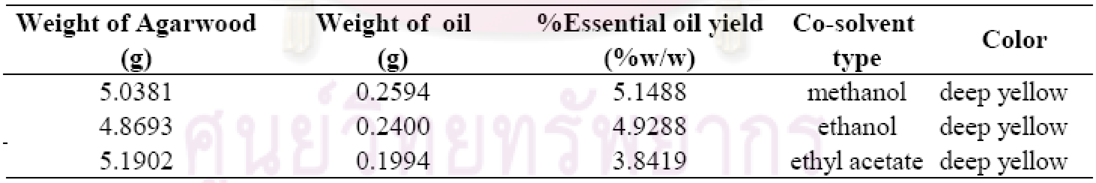
Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Co-solvent type	Color
5.0249	0.2151	4.2807	methanol	deep yellow
5.0845	0.1969	3.8726	ethanol	deep yellow
5.1006	0.1500	2.9408	ethyl acetate	deep yellow

Temperature 65 °C	Pressure 12 MPa
Flow rate 3 g/min	Time 3 h
Co-solvent 5%co-solvent	



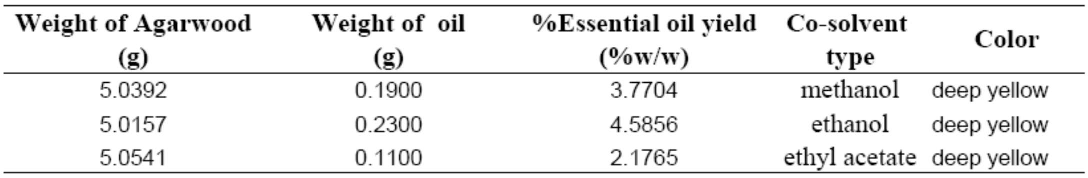
Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Co-solvent type	Color
5.0739	0.1200	2.3650	methanol	deep yellow
5.0644	0.1800	3.5542	ethanol	deep yellow
5.0415	0.0961	1.9062	ethyl acetate	deep yellow

Temperature 50 °C	Pressure 25 MPa
Flow rate 3 g/min	Time 3 h
Co-solvent 10%co-solvent	



Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Co-solvent type	Color
5.0381	0.2594	5.1488	methanol	deep yellow
4.8693	0.2400	4.9288	ethanol	deep yellow
5.1902	0.1994	3.8419	ethyl acetate	deep yellow

Temperature 65 °C	Pressure 12 MPa
Flow rate 3 g/min	Time 3 h
Co-solvent 10%co-solvent	



Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Co-solvent type	Color
5.0392	0.1900	3.7704	methanol	deep yellow
5.0157	0.2300	4.5856	ethanol	deep yellow
5.0541	0.1100	2.1765	ethyl acetate	deep yellow

The essential oil yield (wt.%) was calculate from the formula

%Essential oil yield = [(weight of oil)/(weight of dry agarwood)]×100

Weight of oil (g) = weight of tube$_a$- weight of tube$_b$

Remark: a = weight of tube after extract
b = weight of tube before extract

Appendix B

Soxhlet Extraction and Steam distillation

Experimental data

Soxhlet Extraction

Temperature: 65-70 °C Volume: 400 ml.
Time: 24 h.

Solvent type	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
Methanol	14.9942	1.6340	10.8975	Dark yellow
Hexane	15.0050	0.2578	1.7181	Dark yellow
Ethanol	15.0114	1.5776	10.5093	Pale yellow
Ethyl acetate	15.0843	1.0300	6.8283	Deep yellow

Steam distillation

Temperature: 95 °C Volume: 1200 ml
Time: 2 days

Solvent type	Weight of Agarwood (g)	Weight of oil (g)	%Essential oil yield (%w/w)	Color
DI water	72.91	0.2083	0.2857	Pale yellow

Appendix C

Calculation of Composion of Agarwood oil Content

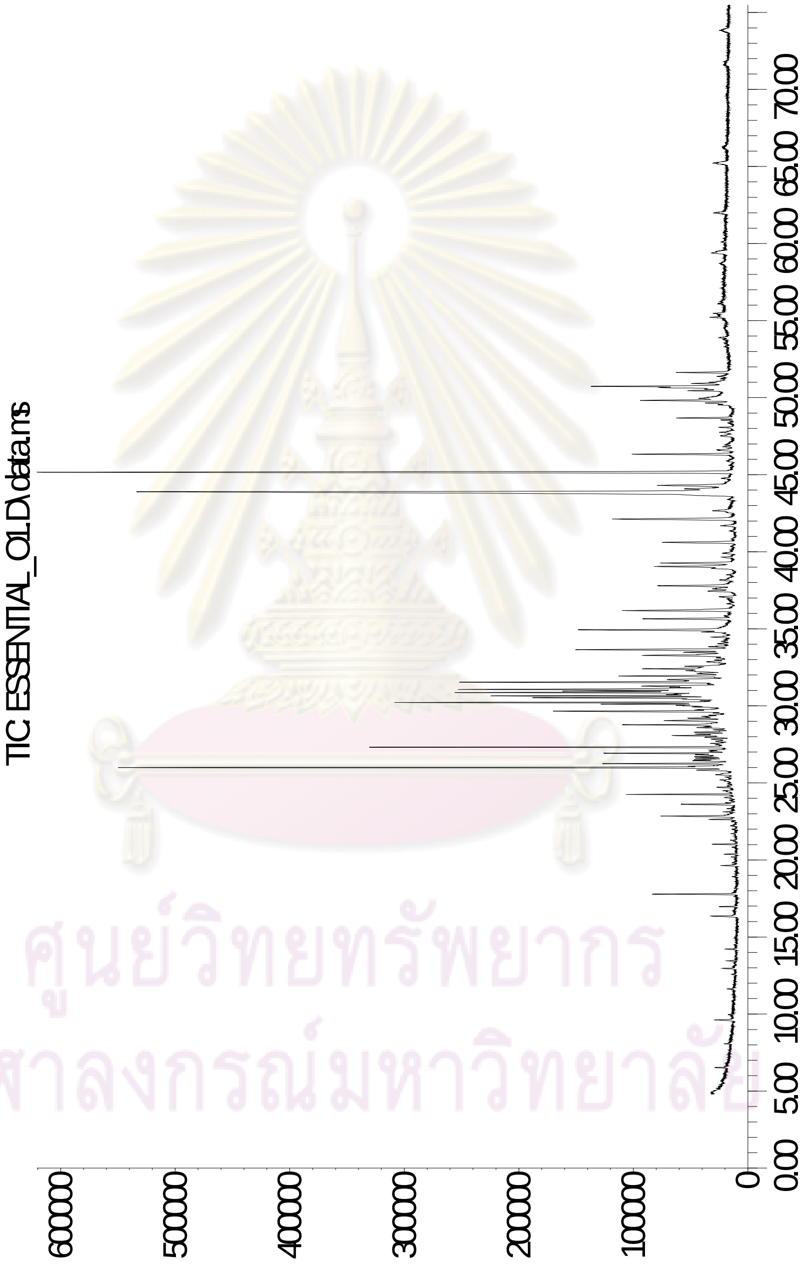


Figure C-1 Gas chromatogram of agarwood oil product

The composition content (wt.%) was calculate from the formula

$$\text{wt.\%} = \% \text{ Area} = (\text{Arae}_i/\text{Area}_{total})*100$$

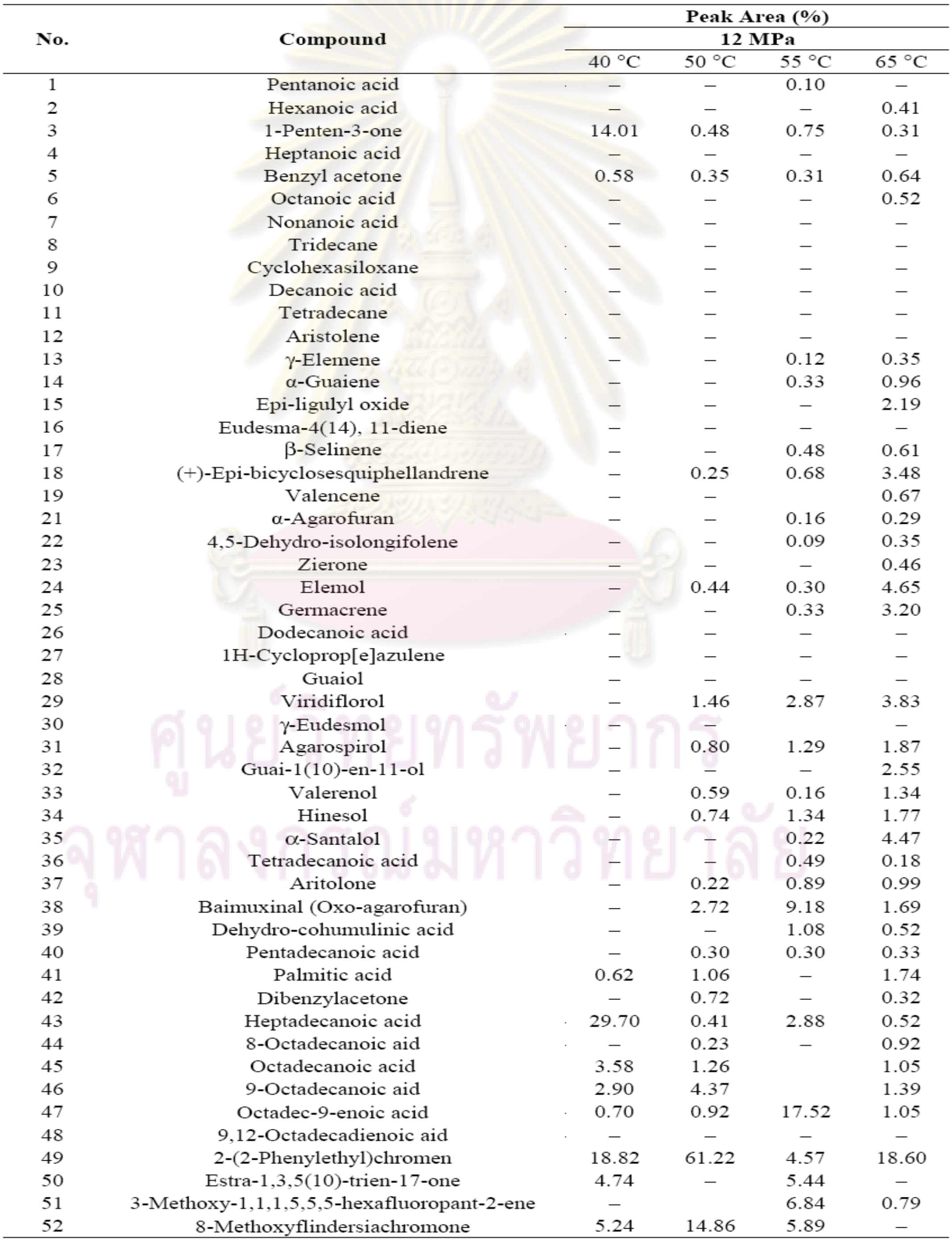
Where Area_{total} = The total area of composition

A_i = The area of composition i

Appendix D

Agarwood oil Composition

Table D-1 The comparative the chemical composition of the extract obtained by supercritical CO_2 extraction (SC-CO_2) under different conditions.



No.	Compound	Peak Area (%)			
		12 MPa			
		40 °C	50 °C	55 °C	65 °C
1	Pentanoic acid	–	–	0.10	–
2	Hexanoic acid	–	–	–	0.41
3	1-Penten-3-one	14.01	0.48	0.75	0.31
4	Heptanoic acid	–	–	–	–
5	Benzyl acetone	0.58	0.35	0.31	0.64
6	Octanoic acid	–	–	–	0.52
7	Nonanoic acid	–	–	–	–
8	Tridecane	–	–	–	–
9	Cyclohexasiloxane	–	–	–	–
10	Decanoic acid	–	–	–	–
11	Tetradecane	–	–	–	–
12	Aristolene	–	–	–	–
13	γ-Elemene	–	–	0.12	0.35
14	α-Guaiene	–	–	0.33	0.96
15	Epi-ligulyl oxide	–	–	–	2.19
16	Eudesma-4(14), 11-diene	–	–	–	–
17	β-Selinene	–	–	0.48	0.61
18	(+)-Epi-bicyclosesquiphellandrene	–	0.25	0.68	3.48
19	Valencene	–	–		0.67
21	α-Agarofuran	–	–	0.16	0.29
22	4,5-Dehydro-isolongifolene	–	–	0.09	0.35
23	Zierone	–	–	–	0.46
24	Elemol	–	0.44	0.30	4.65
25	Germacrene	–	–	0.33	3.20
26	Dodecanoic acid	–	–	–	–
27	1H-Cycloprop[e]azulene	–	–	–	–
28	Guaiol	–	–	–	–
29	Viridiflorol	–	1.46	2.87	3.83
30	γ-Eudesmol	–	–		–
31	Agarospirol	–	0.80	1.29	1.87
32	Guai-1(10)-en-11-ol	–	–	–	2.55
33	Valerenol	–	0.59	0.16	1.34
34	Hinesol	–	0.74	1.34	1.77
35	α-Santalol	–	–	0.22	4.47
36	Tetradecanoic acid	–	–	0.49	0.18
37	Aritolone	–	0.22	0.89	0.99
38	Baimuxinal (Oxo-agarofuran)	–	2.72	9.18	1.69
39	Dehydro-cohumulinic acid	–	–	1.08	0.52
40	Pentadecanoic acid	–	0.30	0.30	0.33
41	Palmitic acid	0.62	1.06	–	1.74
42	Dibenzylacetone	–	0.72	–	0.32
43	Heptadecanoic acid	29.70	0.41	2.88	0.52
44	8-Octadecanoic aid	–	0.23	–	0.92
45	Octadecanoic acid	3.58	1.26		1.05
46	9-Octadecanoic aid	2.90	4.37		1.39
47	Octadec-9-enoic acid	0.70	0.92	17.52	1.05
48	9,12-Octadecadienoic aid	–	–	–	–
49	2-(2-Phenylethyl)chromen	18.82	61.22	4.57	18.60
50	Estra-1,3,5(10)-trien-17-one	4.74	–	5.44	–
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	–		6.84	0.79
52	8-Methoxyflindersiachromone	5.24	14.86	5.89	–

Table D-2 The comparative the chemical composition of the extract obtained by supercritical CO_2 extraction (SC-CO_2) under different conditions.

No.	Compound	Peak Area (%)			
		18 MPa			
		40 °C	50 °C	55 °C	65 °C
1	Pentanoic acid	–	0.26	0.27	0.23
2	Hexanoic acid	–	–	–	–
3	1-Penten-3-one	1.77	0.28	0.36	–
4	Heptanoic acid	–	–	–	–
5	Benzyl acetone	0.26	0.54	0.57	0.45
6	Octanoic acid	–	–	–	–
7	Nonanoic acid	–	–	–	–
8	Tridecane	–	–	–	–
9	Cyclohexasiloxane	–	–	–	–
10	Decanoic acid	–	–	–	–
11	Tetradecane	–	–	–	–
12	Aristolene	0.98	–	–	–
13	γ-Elemene	–	0.06		0.11
14	α-Guaiene	–	–	–	–
15	Epi-ligulyl oxide	–	–	–	–
16	Eudesma-4(14), 11-diene	–	–	–	–
17	β-Selinene	–	0.07	0.28	0.21
18	(+)-Epi-bicyclosesquiphellandrene	0.54	0.22	0.13	0.16
19	Valencene	–	–	–	–
21	α-Agarofuran	–	–	–	–
22	4,5-Dehydro-isolongifolene	–	–	–	0.05
23	Zierone	0.45	–	–	–
24	Elemol	0.39	0.32	0.59	0.31
25	Germacrene	–	–	–	0.05
26	Dodecanoic acid	–	–	–	–
27	1H-Cycloprop[e]azulene	–	–	–	–
28	Guaiol	–	–		–
29	Viridiflorol	0.29	0.89	1.23	1.20
30	γ-Eudesmol	0.81	–		0.07
31	Agarospirol	0.71	0.44	0.60	0.59
32	Guai-1(10)-en-11-ol	–	0.09	0.15	0.19
33	Valerenol	0.37	0.39	0.56	0.66
34	Hinesol	0.50	0.46	0.65	0.60
35	α-Santalol	–	0.06	0.11	0.09
36	Tetradecanoic acid	–	0.07	0.33	0.21
37	Aritolone	–	0.29	0.10	0.53
38	Baimuxinal (Oxo-agarofuran)	1.70	1.82	2.45	2.60
39	Dehydro-cohumulinic acid	–	0.12	0.22	0.36
40	Pentadecanoic acid	–	0.30	0.33	0.54
41	Palmitic acid	0.90	1.63	1.45	1.49
42	Dibenzylacetone	1.67	0.52	0.55	0.49
43	Heptadecanoic acid	0.42	0.71	0.77	0.80
44	8-Octadecanoic aid	0.34	0.28	0.20	0.88
45	Octadecanoic acid	10.36	0.85	0.91	0.89
46	9-Octadecanoic aid	–	4.03	3.82	2.51
47	Octadec-9-enoic acid	0.07	1.41	1.81	0.36
48	9,12-Octadecadienoic aid	–	–	–	–
49	2-(2-Phenylethyl)chromen	49.94	55.48	53.89	43.72
50	Estra-1,3,5(10)-trien-17-one	–	0.77	–	4.64
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	-	0.73	0.38	3.76
52	8-Methoxyflindersiachromone	15.60	16.15	14.83	13.38

Table D-3 The comparative the chemical composition of the extract obtained by supercritical CO_2 extraction (SC-CO_2) under different conditions.

No.	**Compound**	**Peak Area (%)**			
		25 MPa			
		40 °C	50 °C	55 °C	65 °C
1	Pentanoic acid	0.20	0.17	0.24	0.31
2	Hexanoic acid	–	–	–	–
3	1-Penten-3-one	0.34	2.80	0.45	0.49
4	Heptanoic acid	–	0.29	–	–
5	Benzyl acetone	0.61	0.37	0.49	0.65
6	Octanoic acid	–	–	–	–
7	Nonanoic acid	–	0.12	–	–
8	Tridecane	–	–	–	–
9	Cyclohexasiloxane	–	–	–	–
10	Decanoic acid	–	–	–	–
11	Tetradecane	–	–	–	–
12	Aristolene	0.86	–	–	0.42
13	γ-Elemene	0.09	0.14	0.16	0.08
14	α-Guaiene	0.04	0.07	0.08	0.10
15	Epi-ligulyl oxide	0.06	–	0.04	0.04
16	Eudesma-4(14), 11-diene	–	–	–	–
17	β-Selinene	0.08	0.16	0.45	0.17
18	(+)-Epi-bicyclosesquiphellandrene	–	0.15	0.22	0.16
19	Valencene	–	0.37	–	–
21	α-Agarofuran	–	0.29	0.04	0.03
22	4,5-Dehydro-isolongifolene	0.05	0.12	0.09	0.05
23	Zierone	0.07	–	0.07	0.05
24	Elemol	0.54	0.47	0.48	0.38
25	Germacrene	0.09	0.56	0.50	0.06
26	Dodecanoic acid	–	–	–	–
27	1H-Cycloprop[e]azulene	–	–	–	–
28	Guaiol	–	–		–
29	Viridiflorol	2.06	1.52	1.59	1.00
30	γ-Eudesmol	0.12	–	0.12	–
31	Agarospirol	0.85	0.76	0.78	0.66
32	Guai-1(10)-en-11-ol	–	0.09	0.29	0.12
33	Valerenol	0.07	0.32	0.32	0.08
34	Hinesol	–	0.75	0.72	0.68
35	α-Santalol	0.13	0.07	0.19	0.10
36	Tetradecanoic acid	0.18	0.16	0.33	0.16
37	Aritolone	0.54	0.39	0.73	0.52
38	Baimuxinal (Oxo-agarofuran)	2.29	1.47	2.51	2.48
39	Dehydro-cohumulinic acid	0.13	0.09	0.45	0.04
40	Pentadecanoic acid	0.30	0.25	0.50	0.38
41	Palmitic acid	1.22	0.63	1.89	1.64
42	Dibenzylacetone	0.30	0.55	0.68	0.38
43	Heptadecanoic acid	0.51	4.75	0.68	0.41
44	8-Octadecanoic aid	0.26	0.45	0.34	–
45	Octadecanoic acid	1.19	1.23	1.14	0.97
46	9-Octadecanoic aid	4.16	–	3.93	3.91
47	Octadec-9-enoic acid	2.51	7.75	2.28	1.61
48	9,12-Octadecadienoic aid	–	–	–	–
49	2-(2-Phenylethyl)chromen	48.36	36.42	43.15	51.41
50	Estra-1,3,5(10)-trien-17-one	2.08	0.06	3.30	1.20
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	2.34	0.24	2.70	0.82
52	8-Methoxyflindersiachromone	14.81	12.76	12.12	14.22

Table D-4 The comparative the chemical composition of the extract obtained by supercritical CO_2 extraction (SC-CO_2) with co-solvent under different solvent type at pressure 12 MPa, temperature 65.°C, flow rate 3 g/min, 10%wlw of co-solvent and pressure 25 MPa, temperature 50.°C, flow rate 3 g/min.

No.	Compound	Peak Area (%)						
		P= 25 MPa T = 50 °C						
		pure CO_2	5%MeOH	10% MeOH	5%EtOH	10% EtOH	5% EtOAc	10% EtOAc
1	Pentanoic acid	0.17	0.27	–	–	0.34	–	0.26
2	Hexanoic acid	–	–	–	–	0.24	–	–
3	1-Penten-3-one	2.80	0.69	–	0.33	1.72	0.84	2.37
4	Heptanoic acid	0.29	–	–	–	–	–	–
5	Benzyl acetone	0.37	0.53	0.52	0.29	0.63	0.86	0.66
6	Octanoic acid	–	–	–	–	–	–	–
7	Nonanoic acid	0.12	–	–	–	–	–	–
8	Tridecane	–	–	–	–	–	–	–
9	Cyclohexasiloxane	–	–	–	–	–	–	–
10	Decanoic acid	–	–	–	–	–	–	–
11	Tetradecane	–	–	–	–	0.27	–	–
12	Aristolene	–	–	–	0.51	–	–	–
13	γ-Elemene	0.14	0.21	–	0.12	–	–	–
14	α-Guaiene	0.07	0.02	–	–	0.28	–	–
15	Epi-ligulyl oxide	–	0.02	–	–	–	–	–
16	Eudesma-4(14), 11-diene	–	0.02	–	–	–	–	–
17	β-Selinene	0.16	–	–	–	–	–	–
18	(+)-Epi-bicyclosesquiphellandrene	0.15	–	0.85	–	0.26	0.90	0.93
19	Valencene	0.37	–	–	–	–	–	–
21	α-Agarofuran	0.29	0.02	–	–	–	–	–
22	4,5-Dehydro-isolongifolene	0.12	0.03	–	0.10	–	–	–
23	Zierone	–	0.04	–	–	–	–	–
24	Elemol	0.47	0.41	–	0.60	1.00	–	1.44
25	Germacrene	0.56	0.09	–	0.87	0.88	–	–
26	Dodecanoic acid	–	–	1.36	–	0.99	–	–
27	1H-Cycloprop[e]azulene	–	0.48	–	–	–	–	–
28	Guaiol	–	0.05	–	–	–	–	–
29	Viridiflorol	1.52	0.88	0.95	1.04	1.19	1.20	1.25
30	γ-Eudesmol	–	0.06	–	–	–	–	–
31	Agarospirol	0.76	0.58	0.49	0.54	0.68	0.56	0.72
32	Guai-1(10)-en-11-ol	0.09	0.13	–	–	0.20	–	0.22
33	Valerenol	0.32	0.17	–	–	0.55	0.36	0.57
34	Hinesol	0.75	–	0.46	–	0.61	0.63	0.67
35	α-Santalol	0.07	–	–	–	0.20	–	1.14
36	Tetradecanoic acid	0.16	0.25	0.49	0.17	0.39	–	0.76
37	Aritolone	0.39	0.64	0.35	0.86	0.16	–	0.38
38	Baimuxinal (Oxo-agarofuran)	1.47	1.45	1.99	2.47	3.39	1.66	2.37
39	Dehydro-cohumulinic acid	0.09	0.42	–	0.28	0.75	–	0.29
40	Pentadecanoic acid	0.25	0.19	–	0.25	0.19	0.40	0.45
41	Palmitic acid	0.63	4.03	0.98	1.25	1.15	0.82	1.01
42	Dibenzylacetone	0.55	1.35	–	0.39	0.64	–	0.15
43	Heptadecanoic acid	4.75	0.70	–	0.26	0.35	0.53	0.27
44	8-Octadecanoic aid	0.45	0.27	–	1.10	0.39	–	0.36
45	Octadecanoic acid	1.23	–	0.72	0.86	2.34	0.87	1.48
46	9-Octadecanoic aid	–	1.56	2.78	–	7.69	2.99	5.31
47	Octadec-9-enoic acid	7.75	3.83	2.17	6.81	5.31	0.36	0.83
48	9,12-Octadecadienoic aid	–	2.22	–	–	1.54	–	–
49	2-(2-Phenylethyl)chromen	36.42	43.88	45.00	29.85	27.76	60.61	41.49
50	Estra-1,3,5(10)-trien-17-one	0.06	0.07	4.84	2.48	2.67	–	1.93
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	0.24	0.14	1.13	7.52	5.68	–	3.21
52	8-Methoxyflindersiachromone	12.76	11.99	15.49	9.52	9.68	19.29	12.95

Table D-4(cont.) The comparative the chemical composition of the extract obtained by supercritical CO_2 extraction (SC-CO_2) with co-solvent under different solvent type at pressure 12 MPa, temperature 65.°C, flow rate 3 g/min,10%wlw of co-solvent and pressure 25 MPa, temperature 50.°C,flow rate 3 g/min.

No.	Compound	Peak Area (%)						
		P=12 MPa T = 65 °C						
		pure CO_2	5%MeOH	10% MeOH	5%EtOH	10% EtOH	5% EtOAc	10% EtOAc
1	Pentanoic acid	–	0.53	0.66	0.46	0.44	–	–
2	Hexanoic acid	0.41	–	–	0.19	0.44	–	–
3	1-Penten-3-one	0.31	0.44	1.34	0.24	0.39	2.61	3.27
4	Heptanoic acid	–	–	–	–	–	–	–
5	Benzyl acetone	0.64	0.90	0.76	1.06	0.90	0.45	0.14
6	Octanoic acid	0.52	–	–	–	–	–	–
7	Nonanoic acid	–	–	–	–	–	–	–
8	Tridecane	–	–	–	–	–	–	–
9	Cyclohexasiloxane	–	–	–	–	–	–	–
10	Decanoic acid	–	–	–	–	–	–	–
11	Tetradecane	–	–	0.09	0.17	–	–	–
12	Aristolene	–	0.46	0.35	0.60	0.39	0.56	–
13	γ-Elemene	0.35	–	–	0.14	–	–	–
14	α-Guaiene	0.96	–	0.20	0.29	–	–	–
15	Epi-ligulyl oxide	2.19	–	–	–	–	0.45	0.50
16	Eudesma-4(14), 11-diene	–	–	–	–	–	1.41	–
17	β-Selinene	0.61	–	–	0.26	–	–	–
18	(+)-Epi-bicyclosesquiphellandrene	3.48	0.38	0.70	–	0.80	1.00	1.55
19	Valencene	0.67	–	–	–	–	–	–
21	α-Agarofuran	0.29	–	–	–	–	–	–
22	4,5-Dehydro-isolongifolene	0.35	–	–	–	–	–	–
23	Zierone	0.46	–	–	–	–	–	–
24	Elemol	4.65	0.28	0.34	0.50	1.19	2.05	4.14
25	Germacrene	3.20	0.24	0.47	0.42	0.49	0.71	–
26	Dodecanoic acid	–	–	–	–	–	–	–
27	1H-Cycloprop[e]azulene	–	–	–	–	–	–	–
28	Guaiol	–	–	–	–	–	–	–
29	Viridiflorol	3.83	0.81	0.66	1.14	0.71	1.23	2.15
30	γ-Eudesmol	–	–	–	0.13	–	–	–
31	Agarospirol	1.87	0.51	0.38	0.70	0.43	0.64	1.00
32	Guai-1(10)-en-11-ol	2.55	–	0.08	0.15	0.31	0.20	–
33	Valerenol	1.34	–	0.29	–	–	0.29	0.42
34	Hinesol	1.77	–	–	–	–	–	1.00
35	α-Santalol	4.47	–	0.59	–	–	11.74	–
36	Tetradecanoic acid	0.18	–	0.58	0.43	0.58	2.44	1.24
37	Aritolone	0.99	0.39	0.49	0.58	0.37	0.45	–
38	Baimuxinal (Oxo-agarofuran)	1.69	2.58	2.33	2.76	1.86	2.03	2.83
39	Dehydro-cohumulinic acid	0.52	–	0.52	0.14	0.46	0.25	–
40	Pentadecanoic acid	0.33	0.22	0.27	0.28	1.04	0.45	0.57
41	Palmitic acid	1.74	0.71	1.38	0.63	0.55	1.22	0.98
42	Dibenzylacetone	0.32	0.45	0.42	0.34	0.64	–	2.30
43	Heptadecanoic acid	0.52	0.30	0.74	0.37	1.78	0.23	2.58
44	8-Octadecanoic aid	0.92	–	0.48	0.11	–	0.52	0.64
45	Octadecanoic acid	1.05	0.91	0.14	0.89	0.66	–	1.79
46	9-Octadecanoic aid	1.39	2.25	1.23	2.42	2.46	12.06	6.27
47	Octadec-9-enoic acid	1.05	0.70	4.44	0.67	0.83	4.21	1.58
48	9,12-Octadecadienoic aid	–	–	0.46	–	–	1.30	–
49	2-(2-Phenylethyl)chromen	18.60	53.43	44.51	46.44	36.86	28.80	42.81
50	Estra-1,3,5(10)-trien-17-one	–	–	0.77	3.07	3.21	–	–
51	3-Methoxy-1,1,1,5,5,5-hexafluoropant-2-ene	0.79	6.58	1.30	1.35	–	–	2.77
52	8-Methoxyflindersiachromone	–	20.47	14.82	18.46	10.25	8.42	11.63

VITA

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