การหาสภาวะที่เหมาะสมสำหรับการสกัดน้ำมันหอมระเหย จากกระวานด้วยคาร์บอนไดออกไซด์วิกฤติยิ่งยวด

นางสาว วิภาวี โยธีพิทักษ์

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OPTIMIZATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ESSENTIAL OIL FROM *Amomum krevanh Pierre*

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	OPTIMIZATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ESSENTIAL OIL FROM *Amomum krevanh Pierre*
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วิภาวี โยธีพิทักษ์ : การหาสภาวะที่เหมาะสมสำหรับการสกัดน้ำมันหอมระเหยจากกระวานด้วยคาร์บอนไดออกไซด์วิกฤติยิ่งยวด (OPTIMIZATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ESSENTIAL OIL FROM *Amomum krevanh Pierre*) อาจารย์ที่ปรึกษา: ผศ.ดร. อาทิวรรณ โชติพฤกษ์, 61 หน้า.

ในงานวิจัยนี้ได้ศึกษาการสกัดน้ำมันหอมระเหยจากกระวานด้วยคาร์บอนไดออกไซด์วิกฤติยิ่งยวด โดยได้ศึกษาอิทธิพลของขนาดอนุภาคที่นำมาใช้ในการสกัดในช่วงระหว่าง 180-800 ไมโครเมตร พบว่าขนาดอนุภาคมีผลต่อปริมาณสารสกัดน้ำมันหอมระเหย โดยขนาดอนุภาคในช่วง 250-355 ไมโครเมตร ทำให้ได้ปริมาณสารสกัดน้ำมันหอมระเหยมากที่สุด นอกจากนี้งานวิจัยนี้ยังใช้การออกแบบการทดลองและพื้นผิวผลตอบเพื่อศึกษาอิทธิพลของสภาวะที่ใช้ในการปฏิบัติการและพยากรณ์สภาวะที่เหมาะสมที่สุดต่อการสกัดน้ำมันหอมระเหยจากกระวานด้วยคาร์บอนไดออกไซด์วิกฤติยิ่งยวด ซึ่งปัจจัยต่างๆที่ใช้ในการปฏิบัติการ ได้แก่ อุณหภูมิที่ใช้ปฏิบัติการในช่วง 33-67 องศาเซลเซียส ความดันที่ใช้ปฏิบัติการในช่วง 91-259 บาร์ และเวลาที่ใช้ในการสกัดในช่วง 20-70 นาที ซึ่งผลการศึกษาพบว่าผลหลักของความดันที่ใช้ปฏิบัติการ และอันตรกิริยาระหว่างอุณหภูมิที่ใช้ปฏิบัติการกับเวลาที่ใช้ในการสกัด มีผลอย่างมีนัยสำคัญต่อผลการสกัดน้ำมันหอมระเหย จากแบบจำลองพื้นผิวผลตอบพบว่าสภาวะการสกัดที่เหมาะสมสำหรับการสกัดน้ำมันหอมระเหยในการทดลองนี้ คือ ที่อุณหภูมิ 64 องศาเซลเซียส ความดัน 277 บาร์ และเวลาในการสกัด 84 นาที ซึ่งที่สภาวะนี้จะได้ปริมาณสารสกัดน้ำมันหอมระเหยมีค่าเท่ากับ 11.90 มิลลิกรัมต่อน้ำหนักกระวานแห้ง 1 กรัม นอกจากนี้ยังได้เปรียบเทียบประสิทธิภาพการสกัดน้ำมันหอมระเหยกับวิธีการสกัดด้วยตัวทำละลาย พบว่าการสกัดน้ำมันหอมระเหยด้วยคาร์บอนไดออกไซด์วิกฤตยิ่งยวดทำให้ได้ปริมาณสารสกัดมากกว่าการสกัดด้วยตัวทำละลาย โดยการสกัดน้ำมันหอมระเหยด้วยตัวทำละลายจะได้ปริมาณสารสกัดน้ำมันหอมระเหยมีค่าเท่ากับ 9.74 มิลลิกรัมต่อน้ำหนักกระวานแห้ง 1 กรัม แต่คุณภาพของสารที่สกัดได้จากทั้งสองวิธีมีค่าใกล้เคียงกันคือสารที่ได้จากการสกัดน้ำมันหอมระเหยจากกระวานด้วยคาร์บอนไดออกไซด์วิกฤติยิ่งยวดประกอบด้วย 1,8-cineole 71.65% beta-pinene 8.64% และ limonene 4.77% ส่วนสารที่ได้จากการสกัดน้ำมันหอมระเหยจากกระวานด้วยตัวทำละลายประกอบด้วย 1,8-cineole 70.86% beta-pinene 7.91% และ limonene 4.30%

ภาควิชา.....วิศวกรรมเคมี.....ลายมือชื่อนิสิต.....วิภาวี โยธีพิทักษ์.....
สาขาวิชา.....วิศวกรรมเคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....
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WIPAWEE YOTHIPITAK: OPTIMIZATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ESSENTIAL OIL FROM *Amomum krevanh Pierre*. THESIS ADVISOR: ASST. PROF. ARTIWAN SHOTIPRUK, PhD., 61 pp.

Supercritical carbon dioxide extraction of essential oil from *Amomum krevanh Pierre* was studied. The influence of particle sizes of the raw materials on essential oil yields was studied for the particle size range between 180 and 800 μm and the maximum essential oil yield was obtained with the particle size of 250-355 μm. Furthermore, the experimental design and response surface methodology was employed in order to investigate the effects of operating condition and to predict the optimal condition for supercritical carbon dioxide. The factors investigated for essential oil were operating temperature in range of 33-67 °C, the operating pressure in range of 91-259 bar, and the extraction time in range of 20-70 min. The results showed that the main effect of operating pressure and the interaction effect between operating temperature and extraction time were significant factors for the essential oil yields. From the response surface model of the experimental data, an optimal condition for essential oil content was found to be at the temperature of 64 °C, the pressure of 277 bar, and the extraction time of 84 minutes. At this condition, the amount of essential oil yield extracted was 11.90 mg/g dry amomum. The yield obtained by SC-CO_2 extraction was higher than the amount obtained by organic solvent extraction which was 9.74 mg/g dry amomum. The major compounds obtained by SC-CO_2 were 1,8-cineole (71.65%), β-pinene (8.64%), and limonene (4.77%) which were similar to that obtained by organic solvent extraction whose compositions was 1,8-cineole (70.86%), β-pinene (7.91%), and limonene (4.30%).

Department.....Chemical Engineering.....Student's signature.....
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CHAPTER I

INTRODUCTION

1.1 Rationale

Amomum krevanh Pierre, a herb belonging to Zingiberaceae family, is one of the most commonly used spices in South East Asia. In Thailand, it is commonly known as amomum or Krawan. The oil from seeds of amomum, whose major component is 1,8-cineole, is widely used as flavor and fragrance in food such as bakery, sausage, meat, toffee, and coffee. Medically, such oil is used for flatulent indigestion, carminative and to stimulate the appetite (Ruangrungsri et al., 1991).

Generally, essential oils are isolated from plants by solvent extraction, steam distillation, or hydrodistillation (Keita et al., 2000). When organic solvents are used for extractions of essential oils, evaporation of the solvent is required whose conditions may cause product degradation. In stead of organic solvents, steam distillation and hydrodistillation can be used, however the methods have some disadvantages such as requiring long extraction time, and the processes lead to thermal degradation (Doneanu et al., 1998; Ebrahimzadeh et al., 2003; Khajeh et al., 2004).

In recent years, supercritical fluid technology has gained increasing popularity for the applications in food processing industry, and extraction with supercritical fluid has become an interesting alternative to the conventional extraction procedures. The physicochemical properties of supercritical fluid are between those of a liquid and a gas. The temperature and pressure that are above the critical values lead these solvents to possess special properties such as high diffusivity and low viscosity, allowing them to better diffuse through natural solid matrix, and thus better extract the natural compounds than the conventional liquid solvents. The most frequently employed supercritical solvent in food and natural products processing is carbon dioxide (CO_2) due to its low toxicity, low cost, and low critical temperature (31.1 ˚C) and pressure (73.8 bar) (Reverchon et al., 2006).

For essential oil, many studies have been carried out to investigate the extraction with supercritical carbon dioxide (SC-CO_2) and the effect of the operating

conditions on the process (Aleksovski et al., 2007; Ghasemi et al., 2007; Sonsuzer et al., 2004), including the study on extraction of *Elettaria cardamomum L.*, a similar plant belonging to the same family as *Amomum krevanh Pierre* (Gopalakrishnan et al., 1991&1994; Marongiu et al., 2004; Lucchesi et al., 2007). For SC-CO_2 extraction of volatile oil from dried seeds, the highest yield was obtained at 90 bar, and 40˚C and the CO_2-extracted cardamom oil was reported to have higher quality than the distilled oil (Lucchesi et al., 2007). Gopalakrishnan et al. (1991) also reported similar results for the optimum volatile oil yield at 100 bar and 40˚C for SC-CO_2 extraction of freshly ground cardamom *(Elettaria cardamomum L.*) seeds. In their study, the authors also reported the reduction of the yield with increasing moisture content in the ground cardamom seeds

In general, the efficiency of SC-CO_2 extraction involves several process variables. In most of the previous studies, the information regarding the process conditions is obtained by conducting one-variable-at-a-time experiments. In such cases, no interaction was assumed between process variables. This assumption indeed might lead to biased results, and thus the true optimality would not be reached. Statistical experimental design has been demonstrated to be a powerful tool for determining the factors effects and their interactions, which allows process optimization to be conducted effectively. Several statistical techniques, such as factorial design and central composite design were employed. As a particular example on extraction of natural products, the techniques have been applied in order to investigate the effects of extraction parameters on supercritical fluid extraction of astaxanthin from *Haematococcus pluvialis* (Thana et al., 2006) and essential oil from *Thymbra spicata* (Sonsuzer et al., 2003).

The aim of the present work is to employ the experimental design and statistical analysis for the investigation of the effects of operating pressure, temperature and extraction time for the supercritical carbon dioxide extraction of essential oil from *Amomum krevanh Pierre*. The essential oil obtained by solvent extraction was used for comparison.

1.2 Objectives

1.2.1 To investigate the effect of particle size for the SC-CO_2 extraction of essential oil from *Amomum krevanh Pierre*. Base on these results, the suitable particle size was used in the subsequent experiment.

1.2.2 To investigate the effects of operating pressure, temperature, and extraction time for the SC-CO_2 extraction of essential oil from *Amomum krevanh Pierre*, using a central composite orthogonal rotatable design.

1.2.3 To model the factor effects with 2^{nd} order response surface equations and to determine the optimal experimental conditions for the SC-CO_2 extraction of essential oil from *Amomum krevanh Pierre* based on these equations.

1.2.4 To compare the efficiency of supercritical carbon dioxide extraction with that of solvent extraction.

1.3 Working Scope

1.3.1 Use of the experimental design to investigate the effect of the following extraction variables: temperature in range (33-67 °C), pressure in range (91-259 bar), and extraction time in range (20-70 minutes) on supercritical carbon dioxide extraction of essential oil from *Amomum krevanh Pierre*.

1.3.2 Determination of the optimal experimental extraction condition for the SC-CO_2 extraction of the essential oil.

1.4 Expected benefits

1.4.1 This investigation provides a new benign alternative for extraction of high quality essential oil from *Amomum krevanh Pierre*.

1.4.2 This investigation provides useful information for scaling up the industrial extraction process.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 *Amomum krevanh Pierre*

Amomum krevanh Pierre (Figure 2.1) is a plant originated in South East Asia, and is commonly known as “Krawan” in Thai. The plant can be classified as follows.

Kingdom : Plantae
Division : Magnoliophyta
Class : Lilopsida
Order : Zingiberales
Family : Zingiberaceae

Figure 2.1 *Amomum krevanh Pierre*

Amomum is a small tree, 1-3 m tall, which usually takes 2-2.5 years for it to start bearing the pale yellow capsules containing many small black seeds. Fruits are gathered just before they are ripe in order to conserve the seed inside the capsule. Amomum seeds are widely used in flavoring purposes in food such as bakery, sausage, meat, toffee, and coffee. The oil is extracted whose major components are 1,8-cineole, as well as limonene, and β-pinene but to the lower extent, and is used in a large number of beauty products (Ruangrangsri et al., 1991. Medically, the oil is used for flatulent indigestion, carminative, bronchitis and to stimulate the appetite.

2.2 Essential oil

An essential oil can be defined as volatile, odoriferous oil of plant materials. Any or all parts of the plant may contain oil. Essential oils are found in buds, flowers, leaves, bark, stems, fruits, seeds, wood, roots, and rhizomes (Reverchon et al., 2006). Various essential oils have been used medicinally at different periods in history. Interest in essential oils has revived in recent decades, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in carrier oil and used in massage, or burned as incense. Many common essential oils have medicinal properties that have been applied in folk medicine since ancient times and are still widely used today. Furthermore, essential oils are used widely as natural flavor additives for food and fragrances in perfumery. They have a complex composition, containing from a few dozen to several hundred constitutes, especially hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactone, acetals, ethers, and esters). Both hydrocarbon and oxygenated compounds are responsible for the characteristic odors and flavors of essential oils (Pourmortazavi et al., 2007).

2.3 Conventional extraction

Conventionally, essential oils are extracted from natural materials by organic solvent extraction, steam distillation and hydrodistillation. Organic solvent extraction is simple as the compounds are highly soluble in several organics solvents, such as methanol, ethanol, hexane, and acetone. However it is time consuming since it requires multiple extraction steps, and in addition, it leaves toxic solvent residues. Steam distillation and hydrodistillation, on the other hand, do not involve use of toxic organic solvents. However, the disadvantages of such techniques are low yield, loss of volatile compounds, as well as the requirement of elevated temperatures. Moreover, water can cause the degradation or chemical modification of essential oil. Therefore, the quality of the essential oil extracts obtained by these methods is easily impaired. Extraction with supercritical fluid is an alternative method that can preserve the native essential oil components, and in recent years, supercritical fluid extraction has

become an alternative to more conventional extraction procedures (Reverchon et al., 2006).

2.4 Supercritical fluid extraction

Supercritical fluids are defined as fluid at the temperatures and pressures above the critical values. These fluids can no longer be classified as a liquid or a gas. The supercritical fluid region can be drawn on a phase diagram as shown in Figure 2.2.

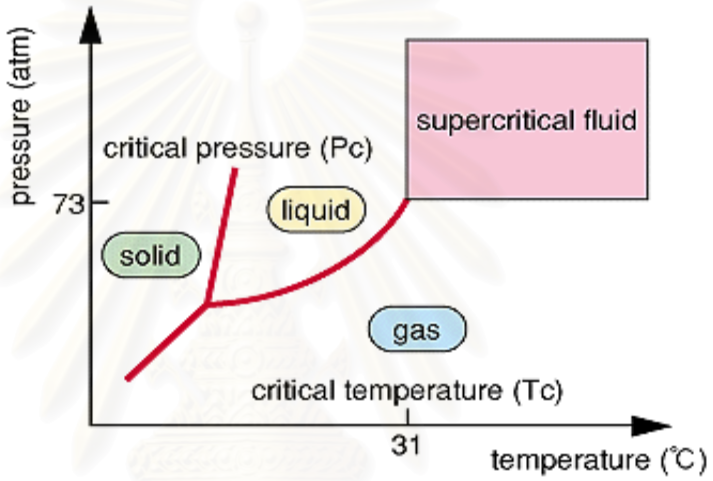


Figure 2.2 Phase diagram of supercritical carbon dioxide

The physicochemical properties of a given fluid, such as density, diffusivity, dielectric constant, and viscosity can be easily controlled by changing the pressure or the temperature without ever crossing phase boundaries (Shivonen et al., 1999). The values for T_c and P_c for selected substances are shown in Table 2.1. The supercritical fluid extraction is a good technique for the production of flavors and fragrances from natural materials and can constitute a valid alternative to the conventional essential oil extraction methods (Caredda et al., 2002). Indeed, this process can be optimally performed operating at mild pressure (from 90 to 100 bar) and temperature (from 40 to 50 °C) since at these process conditions all the essential oil components are largely soluble in supercritical carbon dioxide. Carbon dioxide the most commonly used supercritical fluid in food industry because of its low critical pressure and temperature. Furthermore, the process leaves no toxic residues in the final products and non-flammable properties and its availability in high purity with low cost. It is an

inert gas which dose not reacts with the food constituents. In addition, it is easily removable from the extract following decompression. Pure supercritical carbon dioxide (SC-CO_2) can be used to extract a wide variety of solutes of low-polarity from natural materials, however, in many cases; the polarity of pure CO_2 is too low to quantitatively extract polar solute without the need to add polar organic modifiers or to increase the extraction temperature.

Table 2.1 Features of various solvents at the critical point.

Solvent	**Critical pressure (bar)**	**Critical temperature (°C)**	**Critical density (g/ml)**
Inorganic			
Carbon monoxide	35.5	-139.0	0.311
Carbon dioxide	73.8	31.1	0.460
Nitrous oxide	72.7	36.5	0.450
Ammonia	112.8	132.4	0.235
Water	221.2	374.2	0.323
Hydrocarbon			
Methane	46.4	-82.5	0.162
Ethane	49.4	32.1	0.210
Pentane	33.4	197.2	0.232
Hexane	29.9	234.8	0.234
Alcohol			
Methanol	79.7	240.0	0.272
Ethanol	63.9	243.1	0.275

2.5 Properties of supercritical fluid

2.5.1 Density

The density of a SC-CO_2 can vary from about 0.15 to 1.0 g/cm^3 and is connected to both pressure and temperature. At a constant temperature, the density increases with increasing pressure and at a constant pressure; the density decreases with increasing temperature. The relationship between pressure and density of supercritical fluid at a given temperature can be explained by the ideal gas equation of state as expressed in equation (2.1)

$$PV = zRT \tag{2.1}$$

when

$$\rho = \frac{M}{V} \tag{2.2}$$

the equation becomes

$$\rho = \frac{MP}{zRT} \tag{2.3}$$

where ρ is the density
z is the compressibility factor
R is the gas constant
V is the molar volume
M is the molecular weight.

2.5.2 Diffusivity

Normally, the diffusivity of a solute in supercritical fluid is between those in liquid and gases as shown in Table 2.2. The diffusivities of a solute in supercritical carbon dioxide and in ordinary liquids as a function of temperature and pressure are shown in Figure 2.3 and it can be concluded that

1) The diffusivity of a solute in a supercritical fluid is more than in a liquid solvent.
2) The diffusivity of a solute in a supercritical fluid decreases as pressure increases.

3) The diffusivity of a solute in a supercritical fluid increases with increasing temperature.

This interesting property of supercritical fluid leads to faster mass transfer than that of ordinary liquid solvent.

Table 2.2 Range values of several physicochemical properties of gases, liquids and supercritical fluids

State of fluid	Density (g/cm^3)	Diffusivity (cm^2/s)	Viscosity (g /cm s)
Gas			
P=1.01325 bar; T=21°C	10^{-3}	10^{-1}	10^{-4}
Supercritical			
$P=P_c$; $T=T_c$	0.1-0.8	10^{-3}-10^{-4}	10^{-4}-10^{-3}
Liquid			
P=1.01325 bar; T=15-30°C	1	$<10^{-5}$	10^{-2}

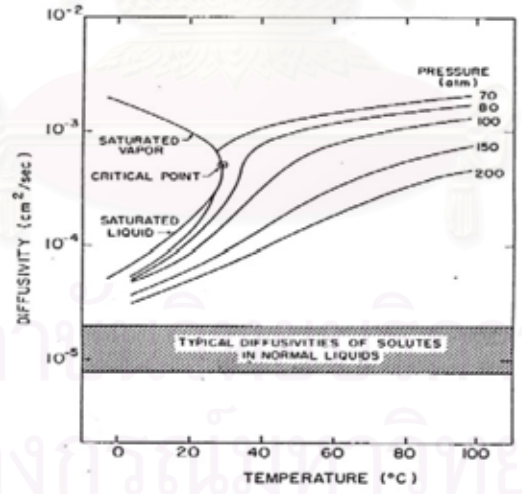


Figure 2.3 Diffusivities of solute in SC-CO_2 as a function of temperature and pressure

2.5.3 Solubility

The solvating power of a supercritical fluid is highly dependent on its temperature and pressure, as can be summarized as follows.

1) The solubility increases with increasing pressure. The increase is very sharp near the critical point as a result of marked changes in solvent density.

2) The solubility increases, remains constant or decreases with increase in the temperature at a constant pressure, depending on the predominant factor, the solute vapor pressure or the solvent density. At low pressures, the solubility decreases somewhat with temperature and at high pressures, it increases markedly with temperature. The maximum solubility increases with increasing temperature at high pressures.

3) The solubility increases with increasing solvent density.

2.5.4 Polarity

A property that influences the solubility of fluid and can be altered to modify the selectivity of an extraction process is polarity. Normally, it occurs when the center of negative charge of a molecule does not coincide with that of its positive charge. This property can be represented by a parameter called a dipole moment. Dipole moments of various substances are shown in Table 2.3

Table 2.3 Permanent dipole moment of some supercritical fluids.

Fluid	Dipole moment (Debye)
CO_2	0.0
SF_6	0.0
Xe	0.0
Ethane	0.0
n-Butane	0.0
N_2O	0.2
Freon-12	0.2
Freon-11	0.5
Freon-22	1.4
NH_3	1.5

CHF_3	1.6
MeOH	1.7

According to the Table 2.3, supercritical fluids can be classified into polar, scarcely polar, and non-polar. For example, CO_2, which have zero dipole moment, is said to be non-polar.

2.6 Experimental design

The traditional optimization procedure varying "one-variable-at-a-time", is a strategy that is said to be based on educated guesswork that does not guarantee the attainment of a true optimum of the extraction conditions (Hockman et al., 1995). The reason is that this method assumes no interactions between the variables, and thus could lead to unbiased results. On the other hand, statistical experimental design is a powerful method for determining the factors effects and their interactions, which allow process optimization to be conducted effectively. Several designs of experiments are used to allow the determination of factor effects and the effect of the factors interaction on the interested response. These include full factorial design, in which all n^k experiments are required for k factors each having n levels. However, the full factorial design has a disadvantage. It requires a large number of experiments, particularly when the number of factors and levels to be investigated is high. Alternatively central composite design (CCD) is probably the most widely used experimental design for fitting a second-order response surface. This design allows the simultaneous variation of all experimental factors and requires fewer numbers of experiments. In this study, central composite orthogonal rotatable design was employed in order to derive optimal supercritical carbon dioxide extraction conditions. This design consists of a 2^k factorial augmented by 2k axial points (±α, 0, 0, …,0), (0, ± α, 0, …, 0), (0, 0, ± α, …, 0), (0, 0, 0, …, ± α) and m repeat observations at the design center points, where k is the number of factor (0, 0, 0, …, 0). The central composite design can be made to be rotatable by choosing $\alpha = F^{1/4}$, where F is the number of factorial points as in Figure 2.4. The number of test runs in a central composite design based on a complete 2^k factorial is $n = 2^k + 2k + m$. The central composite design has the advantage than other designs as it saves time and

materials than the other designs. The experimental matrix for three-variable central composite orthogonal rotatable design is shown in Table 2.4.

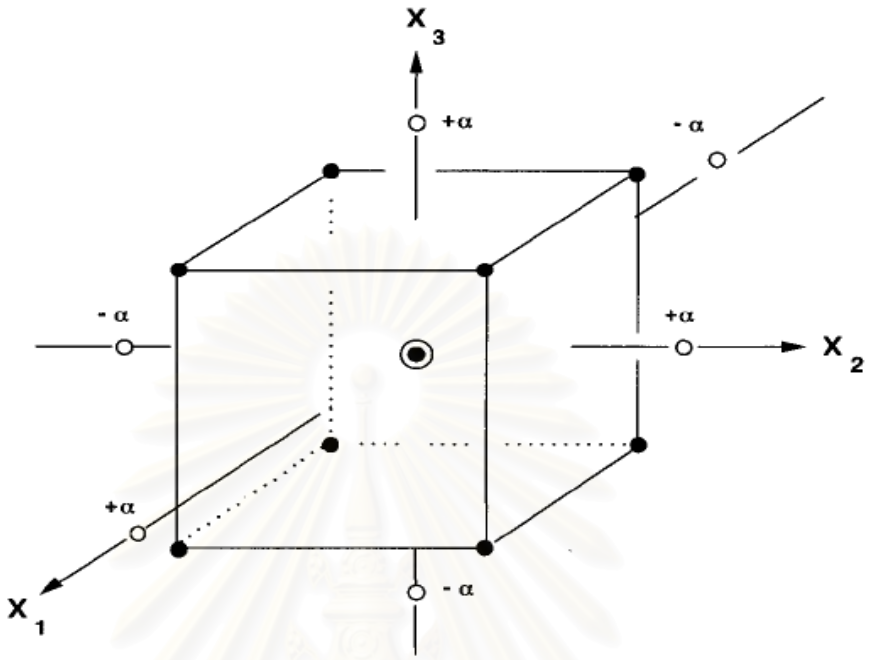


Figure 2.4 Central composite orthogonal rotatable design in three factors.

Table 2.4 Experimental matrix for central composite orthogonal rotatable design for three variables.

Run	X_1	X_2	X_3
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	-1.6818	0	0

10	+1.6818	0	0
11	0	-1.6818	0
12	0	+1.6818	0
13	0	0	-1.6818
14	0	0	+1.6818
15	0	0	0
16	0	0	0
17	0	0	0

To analyze the CCD data, analysis of variance (ANOVA) could be used to determine the factors that have significant effects on the response. In addition, the analysis of central composite designs further proceeds by fitting to the data the general mathematical model (response equation). In this study, a quadratic equation is used which have the following form.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

$$+ \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

where Y is the response, X_1, X_2, X_3 are coded variables of the system, β_0 is y-interception, $\beta_1, \beta_2, \beta_3$ are the partial regression coefficients. In other words, the model is fitted to the observed values of the dependent variable Y, that include (1) main effects for factors (X_1, X_2, X_3), (2) their interactions (X_1X_2, X_1X_3, X_2X_3), and (3) their quadratic components (X_1^2, X_2^2, X_3^2).

In this study, the statistical performing and the model refining were evaluated by using SPSS program, which allows the determination of factor effects and interaction effects, the response equations, from which optimization of extraction conditions could be achieved.

2.7 Literature reviews

Supercritical fluid extraction (SFE) is an interesting technique for the extraction of valuable products from natural materials. This technique has several advantages such as leaving non-toxic residues in the extract, requiring short extraction times, and resulting in minimal thermal degradation. The most frequently employed supercritical solvent in food and natural product processing is carbon dioxide (CO_2) due to its low toxicity, low cost, easy separation from the product, and low critical pressure and temperature (Pourmortazavi et al., 2007). Supercritical carbon dioxide has therefore been applied to extraction of many compounds such as carotenoids, chlorophyll a, and ginsenosides, and etc. Examples of extraction from natural materials derived products extracted by SC-CO_2 are given in Table 2.5.

In the present work, we are interested in SC-CO_2 extraction of essential oil from the seeds of cardamom. Many investigators studied the extraction of essential oils by SC-CO_2 such as from *Thymbra Spicata* (Sonsuzer et al., 2004), from *Artimisia sieberi* using methanol as a co-solvent (Ghasemi et al., 2007), and from *Salvia officinalis L.* (Aleksovski et al., 2007). Reviews on extraction of essential oil from different plant parts by SC-CO_2 are summarized in Table 2.6.

Several investigators studied the extraction of essential oil from *Elettaria cardamomum L.*, which belongs to the same family and has similar characteristics as *Amomum krevanh Pierre*. In 2004, Marongiu et al. extracted the essential oil of cardamom using carbon dioxide at supercritical conditions. Their results show the highest yield of 5.5% was obtained at 90 bar and 40˚C at the carbon dioxide flow rate of 1.2 kg/h. The optimal particles sizes are in the range between 250-425 μm. Lucchesi et al. (2007) studied solvent-free microwave extraction (SFME) of cardamom oil using a central composite design and response surface methodology. Three variables chosen were extraction time, irradiation power, and moisture content and the results showed that all three variables had positive influence on the oil yield. In this study, extraction of essential oil from seeds of *Amomum krevanh Pierre* by supercritical carbon dioxide was be investigated using statistical experimental design. The materials and methods employed in this study are described in chapter 3.

Table2.5 Review of previous investigation on supercritical carbon dioxide extraction of valuable products from natural materials.

Authors	Materials	Compounds	Condition	Analysis	Conclusion
Careri et al., 2001	Algae *Spirulina platensis*	Carotenoids	Temperature 40-80 °C Pressure 150-350 bar Flow rate 2.0 ml/min	HPLC	1) The relationships between variables and responses are polynomial functions. 2) The significant effects for the extraction are pressure, modifier, extraction time, the interaction and curvature effects.
Tonthubthimthong et al., 2001	Neem seeds	Nimbin	Temperature 35-60 °C Pressure 100-260 bar Flow rate 0.24-1.24 ml/min	HPLC	The best extraction condition occurred at 230 bar, 35 °C and a flow rate of 1.24 ml/min for a 2 g sample of neem.

Table2.5 Review of previous investigation on supercritical carbon dioxide extraction of valuable products from natural materials. (continue)

Authors	Materials	Compounds	Condition	Analysis	Conclusion
Wang et al., 2001	Ginseng root hair	Ginseng root hair oil and ginsenosides	Temperature 35-60 °C Pressure 104-312 bar Flow rate 5 ml/min	GC	Crude oil extracted increases with increasing pressure at constant temperature and only increased with temperature when pressure exceeded 242 bar.
Macías-Sánchez et al., 2005	*Nannochloropsis gaditana*	Carotenoids Chlorophyll a	Temperature 40-60 ˚C Pressure 100-500 bar	Spectrophotometer	The extraction of the pigment at a pressure of 400 bar and a temperature of 60 °C can be obtained a significant yield.

Table2.5 Review of previous investigation on supercritical carbon dioxide extraction of valuable products from natural materials. (continue)

Authors	Materials	Compounds	Condition	Analysis	Conclusion
Ziémons et al., 2005	Tithonia diversifolia	Tagitinin C	Temperature 40-80 ˚C Pressure 203- 405 bar	FTIR spectroscopy	1) The best conditions are met for a pressure of 350 bar and A temperature of 68 °C. 2) The extraction time of SC-CO_2 is much shorter than soxhlet extraction.
Sun et al., 2006	Carrots	Carotenoids	Temperature 40-70 ˚C Pressure 276-551 bar Particle size 0.25-2.0 mm Moisture content 0.8-84.6 %	HPLC	The highest carotenoid yields were obtained at 70 °C, 551 bar, 0.25-0.5 mm particle size and 0.8 % moisture content of feed material.

Table2.6 Review of previous investigation on supercritical carbon dioxide extraction of essential oil from plant materials.

Authors	Materials	Compounds	Condition	Analysis	Conclusion
Ebrahimzadeh et al., 2003	*Zataria multiflora Boiss*	Essential oil	Temperature 35-55 ˚C Pressure 101-304 bar Extraction time10-30 min Modifier volume 0-400 µl	GC and GC/MS	1)The optimum conditions extraction of essential oil was more efficient as 304 bar, 55˚C, 20 min and no modifier volume. 2) The compositions of the oils obtained by SFE and steam distillation are not qualitatively different, they differ quantitatively.
Khajeh et al., 2004	*Carum copticum*	Essential oil	Temperature 35-55 ˚C Pressure 101-304 bar Extraction time10-30 min	GC and GC/MS	The SFE was most selective for the extraction of thymol at 304 bar, 35˚C and extraction time of 30 min.

Table2.6 Review of previous investigation on supercritical carbon dioxide extraction of essential oil from plant materials. (continue)

Authors	Materials	Compounds	Condition	Analysis	Conclusion
Sonsuzer et al., 2004	*Thymbra spicata*	Essential oil	Temperature 40-60 ˚C Pressure 80-120 bar Extraction time30-90 min	GC and GC/MS	The optimum conditions minimizing monoterpene hydrocarbons, maximizing yield and oxygenated compound were found as 41˚C, 119 bar and 76.5 min.
Gomes et al., 2007	*Pelargonium sp.*	Rose geranium oil	Temperature 40-100 ˚C Pressure 80-160 bar Extraction time 5-180 min	GC/MS	1) The best conditions were 40 ˚C, 90-100 bar and extraction time of 15-30 min. 2) The SFE yield was significantly influenced by the extraction pressure.

Table2.6 Review of previous investigation on supercritical carbon dioxide extraction of essential oil from plant materials. (continue)

Authors	Materials	Compounds	Condition	Analysis	Conclusion
Ghasemi et al., 2007	*Artemisia sieberi*	Essential oil	Temperature 35-55 ˚C Pressure 101-304 bar Extraction time15-35 min Modifier volume 0-500 µl	GC and GC/MS	1) The highest yields were obtained at a temperature of 45 ˚C, a pressure of 304 bar, a dynamic extraction time of 25 min and in the absence of methanol as modifier. 2) The SFE is more selectively than the hydrodistillation method in the extraction of essential oil and the preservation of its quality.
Aleksovski et al., 2007	*Salvia officinalis L.*	Essential oil	Temperature 25-50 ˚C Pressure 90-128 bar	GC	The highest yields were obtained at 50 ˚C and 128 bar.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Extractions were carried out with high purity carbon dioxide purchased from Thonburiwattana Co. (Bangkok, Thailand). Hexane (purity > 99.5%) was supplied by Sigma-Aldrich. Standards for identification of chromatographic peak were purchased from Fluka. Nitrogen was purchased from Thai industrial gas Co. (Bangkok, Thailand).

3.1.2 Plant material preparation

The seeds of *Amomum krevanh Pierre* used in this experiment were obtained from dried fruits. The seeds were ground in a blender to fine powder. Particle of three different size ranges (180-250, 250-355, and 355-800 μm) were prepared for the determination of the effect of sample size on extraction behavior. The suitable particle size was selected based on these results and used in the subsequent experiment. The ground samples were stored in a dry place until use.

Figure 3.1 Dried fruits of *Amomum krevanh Pierre*

a) b)

Figure 3.2 a) Dried seeds and b) ground seeds of *Amomum krevanh Pierre*

3.2 Methods

3.2.1 Supercritical carbon dioxide extraction

Supercritical carbon dioxide extraction was carried out using SFX-220 extraction system from ISCO. The equipment consisted of an extractor with a maximum capacity of 10 ml, a controller, a restrictor temperature controller and a syringe pump as shown in Figure 3.3. For each experimental run of essential oil extraction, 3.0 g ground seeds of *Amomum krevanh Pierre* was mixed with silica sand and charged into the extraction chamber. The extraction was carried out at a desired temperature and pressured for a specified time period, and the extract was trapped in a tube containing *n*-hexane. After extraction, *n*-hexane was removed with nitrogen stream at room temperature and stored at 5°C until analysis.

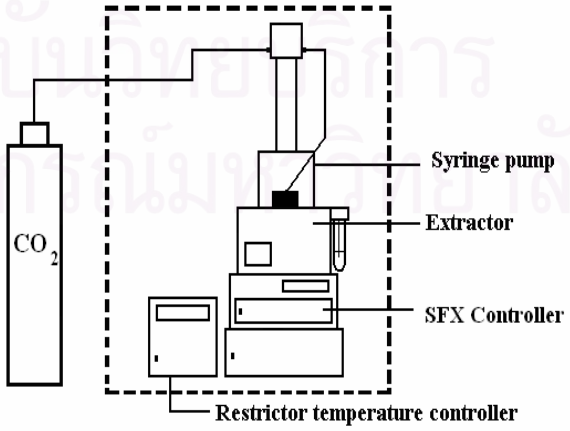


Figure 3.3 SFX-220 extraction system of supercritical carbon dioxide extraction

3.2.2 Solvent extraction

The cardamom seed powder (3.0 g) was extracted with *n*-hexane (30 ml) under sonication for 30 min in an ultrasonic bath, after which the sample were macerated at 25 °C for 24 h. The extract and filtered through filter membrane and the filtrate was concentrated by removing the solvent under nitrogen stream. The concentrated sample was then stored at 5°C until analysis.

3.2.3 GC analysis

GC analyses were performed using a Shimadzu GC-2010 gas chromatography whose system was equipped with a DB-WAX capillary column (30 m $\times$0.25 mm i.d., film thickness 0.25 µm). The SFE samples (1 µl) were injected using the split mode with a split ratio of 1/30. Oven temperature was programmed to increase from 80°C to 130 °C at a rate of 5 °C/min. The injector and detector temperatures were held at 230 and 250 °C, respectively.

3.3 Experimental design and statistical analysis

In this study, the experimental design was used to evaluate the main and interaction effects of the factors: temperature (X_1), pressure (X_2), and extraction time (X_3) on essential oil yield obtained with SC-CO_2 process. Seventeen experiments were performed: eight factorial points for the three factors each at five levels, six axial points, and three repeatability experiments for the measurements at the center of the experimental domain. The ranges and the levels of the factors investigated in this study are summarized in Table 3.1.

Table 3.1 Factors and levels tested for the designed experiment

Variables	Levels				
	-1.68	-1	0	+1	+1.68
X_1:Temperature (°C)	33	40	50	60	67
X_2: Pressure (bar)	91	125	175	225	259
X_3: Extraction time (min)	20	30	45	60	70

The test factors were code according to the following equation:

$$x_i = \frac{X_i - X_i^*}{\Delta X_i} \quad (1)$$

where x_i is coded value of the *i*th factor, X_i the uncoded value of the *i*th factor, X_i^* the uncoded value of the factor at the center point and ΔX_i is the step change value. More specially, coded values of the factors were calculated as follows:

$$\text{Temperature; } x_1 = \frac{X_1 - 50}{10} \quad (2)$$

$$\text{Pressure; } x_2 = \frac{X_2 - 175}{50} \quad (3)$$

$$\text{Extraction time; } x_3 = \frac{X_3 - 45}{15} \quad (4)$$

The statistical analysis of variance (ANOVA) of the experimental results was employed to determine the main effect and the interaction of the factor effects using SPSS program. The response surface equations were then proposed, from which the optimal conditions were determined. Detailed statistical data analysis and experimental design is described in Chapter 2.

CHAPTER IV

RESULTS AND DISCUSSION

In this study, optimization of supercritical carbon dioxide (SC-CO_2) extraction of essential oil from *Amomum krevanh Pierre* was carried out using an experimental design. The experiments were conducted to determine the effects of the extraction conditions on essential oil yields. Experimental design and analysis was employed to investigate the main effects and the interaction effects of the three most important factors on the extraction efficiency of SC-CO_2, which were the operating temperature (X_1), the operating pressure (X_2), and the extraction time (X_3). Then the experimental results were fitted with a response surface equation, from which the optimal conditions for extraction were obtained.

4.1 Effect of particle size on supercritical carbon dioxide extraction

As the first preliminary study, the influence of particle sizes of the raw materials on essential oil yields was evaluated. Three particle sizes: 180-250 μm, 250-355 μm, and 355-800 μm average diameters were extracted at 40°C, 225 bar, for 30 min and the results are shown in Figure 4.1. The maximum essential oil yield (34.98 mg/g) was obtained for the particle whose size range was 250-355 μm. Usually, the smaller particle size would give larger surface area for mass transfer. However, in this study the extraction yields from the sample of smallest particle size (180-250 μm) was lower, possibly because the volatile essential oil was more readily lost from the small size sample and too small particles may cause high pressure drop inside the extraction chamber, plugging of fine particles, as well as channeling, which lessens extraction efficiency. Based on these results, the particle size range of 250-355 μm were used in the subsequent experiment.

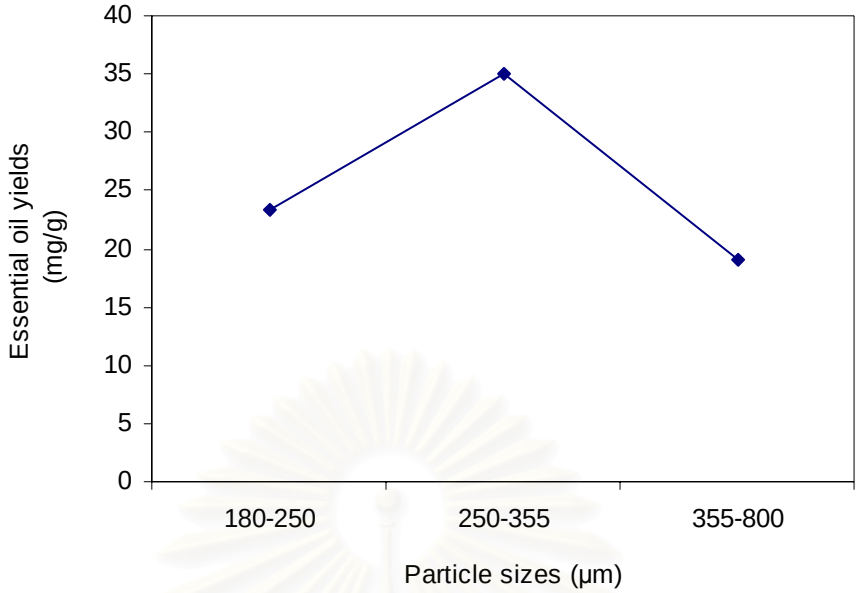


Figure 4.1 Effect of particle sizes on essential oil yields.

4.2 Design of experiment (DOE) for supercritical carbon dioxide (SC-CO_2) extraction

In this study, the experimental design was used to evaluate the main effects and the interaction effects on the performance of SC-CO_2, measured in terms of essential oil yield. The investigated factors were the operating temperature (33-67°C), operating pressure (91-259 bar), and extraction time (20-70 min). Table 4.1 shows the factors and the factor levels under which SC-CO_2 extraction of *Amomum krevanh Pierre* was carried out, according to the central composite orthogonal rotatable design. The experimental matrix and the results for all 17 extraction experiments are shown in Table 4.2. It should be noted that essential oil yields obtained from all 17 experiments were less than obtained from investigation of particle sizes effect. This was due to the fact that these experiments were conducted later and certain amount of essential oil was lost from the ground sample during the storage. Nevertheless, the factor and interaction effect of extraction conditions could still be drawn from the experimental data and the statistical analysis. The result in Table 4.2 shows that the maximum essential oil yield (15.00 mg/g) was obtained at 67°C, 175 bar, and after 45 minutes of extraction.

Table 4.1 Factors and levels tested in the design experiment

Factors	Levels				
	-1.68	-1	0	+1	+1.68
X_1:Temperature (°C)	33	40	50	60	67
X_2: Pressure (bar)	91	125	175	225	259
X_3: Extraction time (min)	20	30	45	60	70

Table 4.2 Experimental matrix and experimental results for the design of experiment.

Run	Temperature	Pressure	Time	Essential oil yield (mg/g)
1	-1	-1	-1	14.08
2	1	-1	-1	12.25
3	-1	1	-1	11.30
4	1	1	-1	13.58
5	-1	-1	1	13.49
6	1	-1	1	10.97
7	-1	1	1	14.96
8	1	1	1	12.17
9	-1.68	0	0	14.03
10	1.68	0	0	15.00
11	0	-1.68	0	9.74
12	0	1.68	0	12.51
13	0	0	-1.68	11.04
14	0	0	1.68	14.62
15	0	0	0	13.91
16	0	0	0	13.77
17	0	0	0	12.92

4.2.1 Statistical analysis of essential oil yields

From the experimental results, the analysis of variance (ANOVA) was used to determine the factors that have important effects on the essential oil yields. The analysis results obtained using a statistical program SPSS 15.0 are shown in Table 4.3.

Table 4.3 ANOVA table for essential oil yields

Dependent variable: Yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37.028(a)	14	2.645	9.214	.102
Intercept	1675.982	1	1675.982	5838.980	.000
X1	4.569	3	1.523	5.306	.163
X2	10.989	3	3.663	12.762	.074
X3	7.020	3	2.340	8.153	.111
X1 * X2	1.842	1	1.842	6.418	.127
X1 * X3	4.129	1	4.129	14.383	.063
X2 * X3	2.121	1	2.121	7.389	.113
X1 * X2 * X3	2.384	1	2.384	8.305	.102
Error	.574	2	.287		
Total	2893.338	17			
Corrected Total	37.602	16			

a R Squared = .985 (Adjusted R Squared = .878)

From the statistical analysis above, it was found that at the confidence interval of 90% ($P<0.1$), the factors that have significant effects on the essential oil yields were the operating pressure, and the interaction between operating temperature and extraction time. Each of these effects is described in more details as follows.

4.2.1.1 Main effect of operating pressure on essential oil yields

The main effect of operating pressure on essential oil yields is shown in Figure 4.2. The results show that the essential oil yields were higher with increasing operating pressure in the range of 91-175 bar. In general, the increase in the supercritical fluid pressure results in an increase in the fluid density, which then increases the solvating power of SC-CO_2. At high fluid density, the interaction between supercritical solvent and the solute molecules increases. However, for the

operating pressures in range of 175-259 bar, the essential oil yields decreased. At such high pressure, it might be possible the sample was densely packed, thus the mass transfer of the SC-CO_2 into the sample matrix was reduced, and the reduction diffusion rates of the extracted materials from the sample matrix to the supercritical fluid environment according to Kazazi et al., (2007).

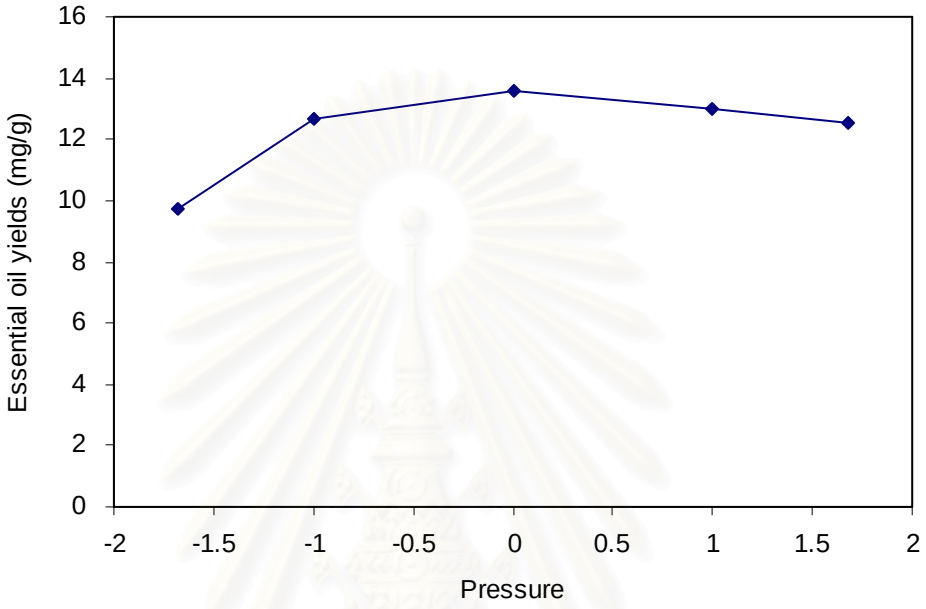


Figure 4.2 Main effect of operating pressure to essential oil yields

4.2.1.2 Interaction effects of operating temperature and extraction time on essential oil yields

The statistical analysis of the experimental results showed that the interaction between operating temperature and extraction time significantly affected the essential oil yields. Figure 4.3 shows the interaction effect between operating temperature and extraction time. It can be seen from this figure that for the extraction time of 30 min, the oil yield slightly increased as the operating temperature increased. However, as the time of extraction increased at 60 min, the oil yield decreased as the operating temperature increased. In typical extraction, longer extraction should generally resulted in higher yield as the time in which the sample and the solvents are in contact would increase. However, when extraction was carried out at higher temperature, the extraction was also subjected to loss of the volatile compound when the process took

place at extended time period, resulted in the decrease in the yield as can be seen in the figure.

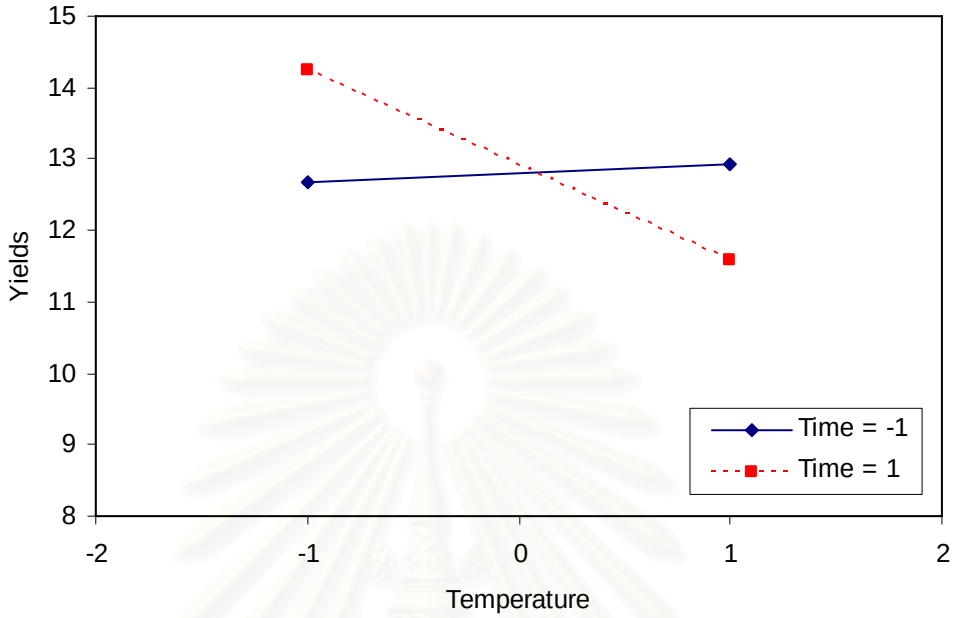


Figure 4.3 Interaction effect between temperature and extraction time for essential oil yields

4.3 Optimal condition for SC-CO_2 extraction of essential oil from *Amomum krevanh Pierre*.

The relations between each factor and the extracts yields were modeled with a response surface using a 2^{nd} order polynomial model. By use of the statistical program SPSS 15.0, the analysis results obtained are summarized in Table 4.4, 4.5 and 4.6.

Table 4.4 Model summary for essential oil yields

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.842(a)	.709	.335	1.25004

a Predictors: (Constant), X2X3, X1X3, X1X2, X3X3, X3, X2, X1, X2X2, X1X1

Table 4.5 ANOVA Table for optimal condition of essential oil yields

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	26.663	9	2.963	1.896	.206(a)
	Residual	10.938	7	1.563		
	Total	37.602	16			

a Predictors: (Constant), X2X3, X1X3, X1X2, X3X3, X3, X2, X1, X2X2, X1X1
b Dependent Variable: Yield

Table 4.6 Coefficients for response surface equation for essential oil yields.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta	B	Std. Error
1	(Constant)	13.355	.630		21.208	.000
	X1	-.237	.338	-.143	-.700	.506
	X2	.429	.338	.259	1.269	.245
	X3	.469	.338	.282	1.385	.208
	X1X1	.810	.510	.325	1.587	.156
	X2X2	-1.208	.510	-.485	-2.367	.050
	X3X3	-.192	.510	-.077	-.377	.718
	X1X2	.480	.442	.221	1.086	.314
	X1X3	-.718	.442	-.331	-1.625	.148
	X2X3	.515	.442	.237	1.165	.282

a Dependent Variable: Yield

Therefore, the response surface equation obtained from the analysis is as follows.

$$Y = 13.355 - 0.237X_1 + 0.429X_2 + 0.469X_3 + 0.810X_1^2 - 1.208X_2^2 - 0.192X_3^2 + 0.480X_1X_2 - 0.718X_1X_3 + 0.515X_2X_3 \quad (4.1)$$

Where Y is the essential oil yields (mg/g dry amomum), X_1, X_2, and X_3 are the operating temperature, the operating pressure, and the extraction time, respectively. The significance of each coefficient was determined by the P-values, that is, if the values of P are less than 0.1, this indicated that the model terms were significant. In this case, the quadratic main effect of operating pressure (X_2^2) was the most significant (P-value=0.05). The response surface for essential oil yields is shown in Figure 4.4-4.6 from which the values of essential oil yields for different levels of the variables can be predicted. Each response plot represents an infinite number of

combination of two test variables with the other maintained at its respective zero level. The plot of the observed and the predicted values are shown in Figure 4.7.

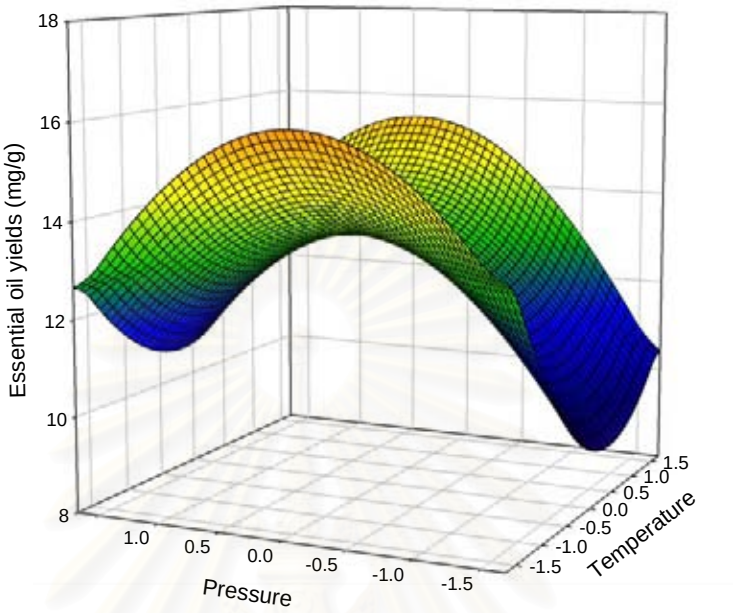


Figure 4.4 Response surface on essential oil yields as a function of temperature and pressure (for code extraction time of 0)

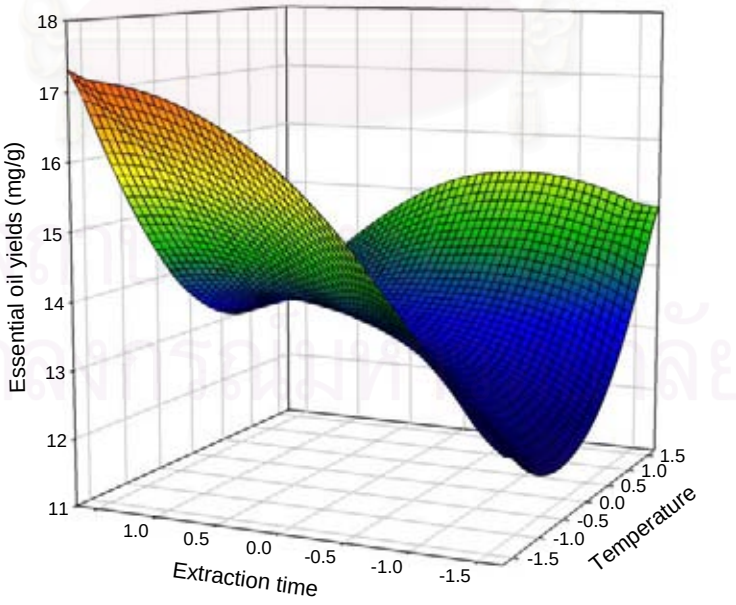


Figure 4.5 Response surface on essential oil yields as a function of temperature and extraction time (for code pressure of 0)

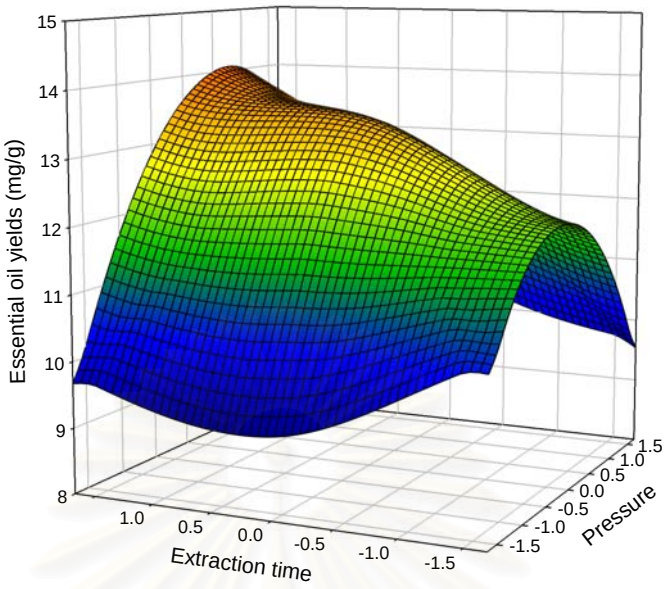


Figure 4.6 Response surface on essential oil yields as a function of pressure and extraction time (for code temperature of 0)

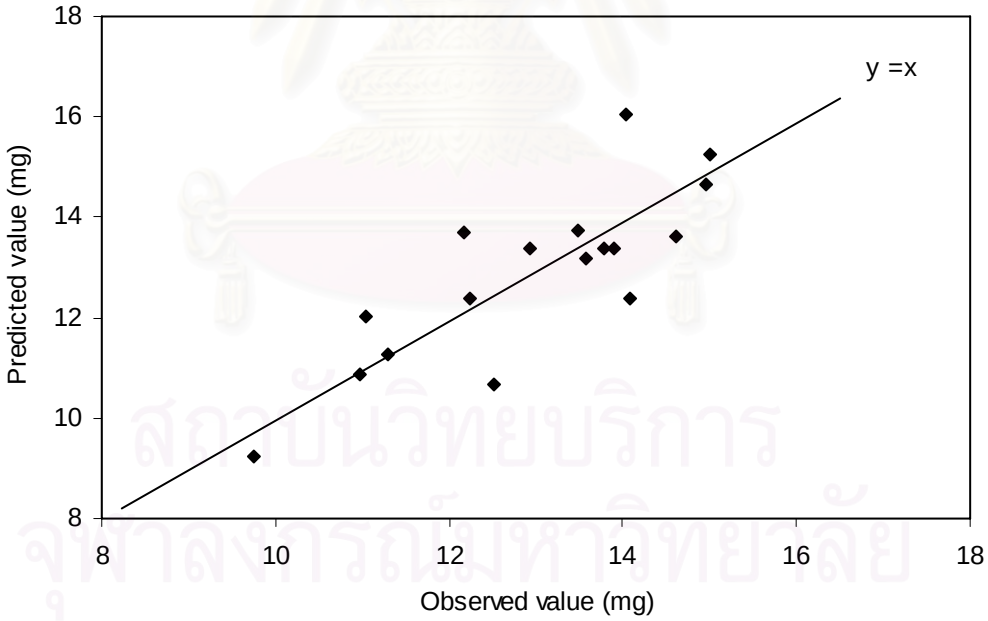


Figure 4.7 Observed value vs. predicted value of essential oil yields model

From the response surface equation for essential oil yields, the optimal condition of this extraction can be determined by taking the partial derivative of the

equation with respect to each of the three factors, and set these partial derivatives equal to 0, and solve the equation. The resulting equations are:

$$dY / dX_1 = 0,\ 0 = -0.237 + 0.810X_1 + 0.480X_2 - 0.718X_3 \quad (4.2)$$

$$dY / dX_2 = 0,\ 0 = 0.429 + 0.480X_1 - 1.208X_2 + 0.515\ X_3 \quad (4.3)$$

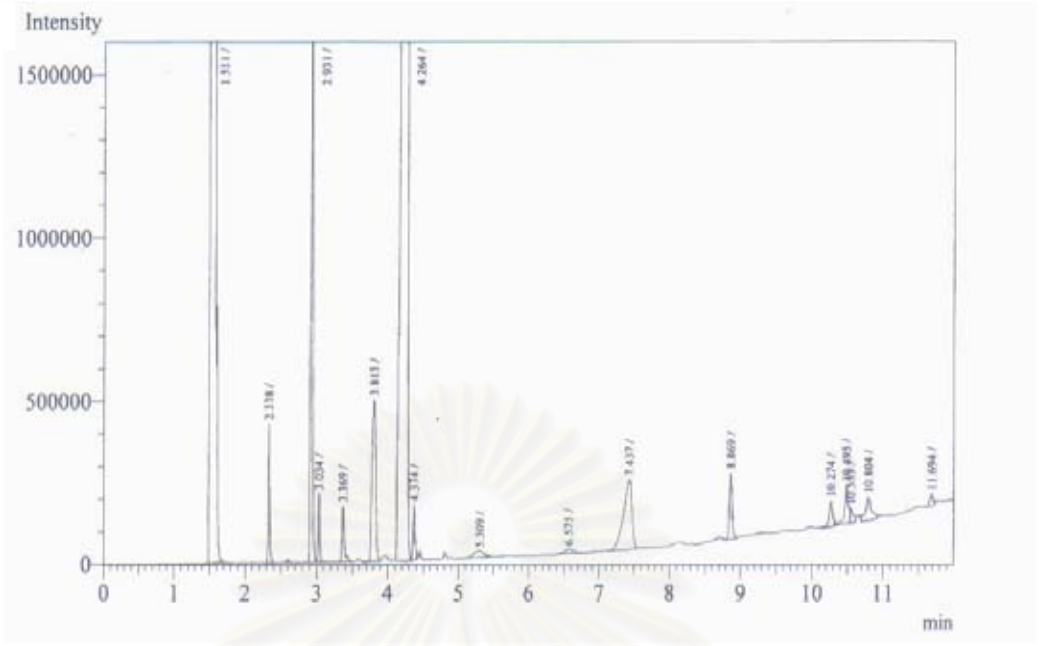
$$dY / dX_3 = 0,\ 0 = 0.469 - 0.718X_1 + 0.515X_2 - 0.192X_3 \quad (4.4)$$

The solutions of these equations yield the optimal extraction condition which was 64°C, 277 bar, and 84 min of extraction. The response values for these conditions were 11.90 mg/g dry amomum. This condition was the extrapolation from the model which therefore would not be considered the most accurate prediction. Thus the optimal condition obtained by response surface in Figure 4.4-4.6 was at 33°C and 175 bar, and the extraction time was 70 min, which gave the highest amount of essential oil of 17.3 mg/g dry amomum.

4.4 Comparison of supercritical carbon dioxide extraction and solvent extraction

Figure 4.8, the supercritical carbon dioxide extraction shows various results in comparison with the solvent extraction using hexane following the method described in Chapter 3. The maximum essential oil yield extracted by SC-CO_2 was approximately 15.0 mg/g dry amomum and was obtained at 67 °C, 175 bar after the extraction of 45 min. The major compounds were: 1,8-cineole (71.65%), β-pinene (8.64%), and limonene (4.77%). The essential oil yield obtained by solvent extraction was 9.74 mg/g dry amomum. The major compounds were: 1,8-cineole (70.86%), β-pinene (7.91%), and limonene (4.30%). The amount of the essential oil yield extracted by SC-CO_2 was higher than those obtained by solvent extraction. But the quality of essential oil obtained by both methods was similar.

a)



b)

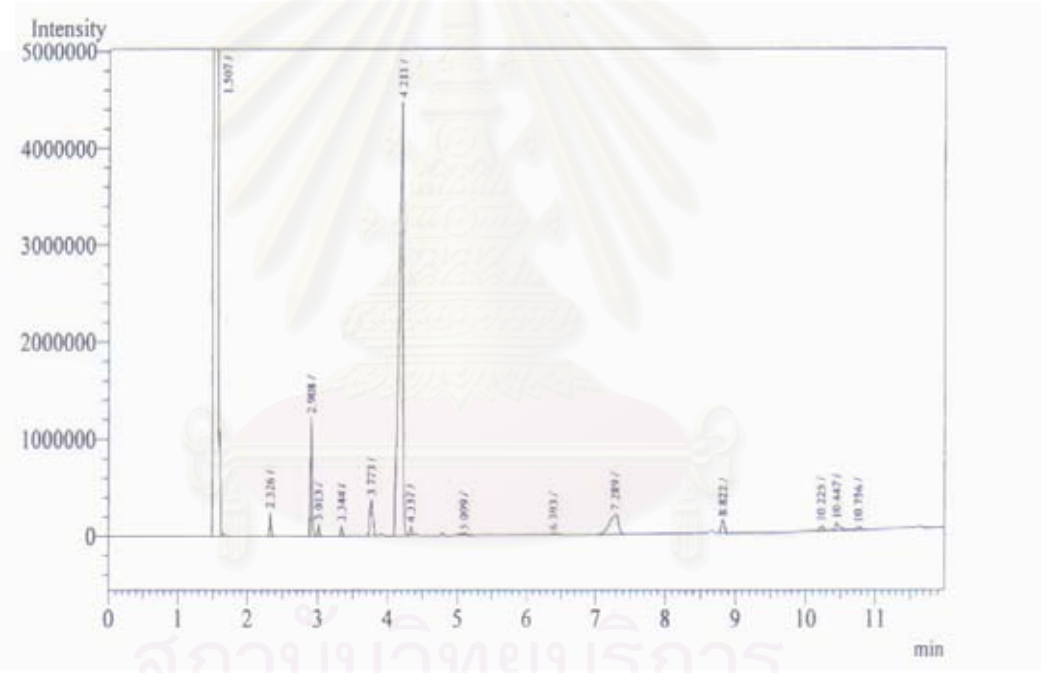


Figure 4.8 GC analysis of essential oil from *Amomum krevanh Pierre* obtained by a) SC-CO_2 extraction (at condition: 67°C, 175 bar, and 45 min) and b) solvent extraction using hexane.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 The particle size in the range of 250-355 μm was suitable for extraction essential oil.

5.1.2 From the analysis of variance (ANOVA), the main effect of pressure (X_2) and the interaction effect between temperature and extraction time (X_1X_3) showed significant effects on essential oil yields.

5.1.3 The following approximate model was proposed for predicting the response surface of essential oil yields.

$$Y = 13.355 - 0.237X_1 + 0.429X_2 + 0.469X_3 + 0.810X_1^2 - 1.208X_2^2 - 0.192X_3^2 + 0.480X_1X_2 - 0.718X_1X_3 + 0.515X_2X_3$$

From the model, the optimal condition for the essential oil yields is at 64°C, 277 bar, and 84 min and the amount of essential oil extract was found as 11.90 mg/g dry amomum. This condition was the extrapolated result and therefore would not be accurately. Thus the optimum condition was proposed within the range of this experiment to be at the temperature of 33°C, the pressure of 175 bar, and the extraction time of 70 min, which gave the highest amount of essential oil of 17.3 mg/g dry amomum.

5.1.4 The extraction of essential oil from *Amomum krevanh Pierre* with SC-CO_2 was successful whose yield was higher than that obtained by solvent extraction. The compositions of essential oils obtained by both methods were similar.

5.1.5 The SC-CO_2 extraction required a shorter extraction time and minimized use of organic solvent.

5.2 Recommendations

5.2.1 For future studies, more detailed analysis of the product should be conducted using gas chromatography mass spectrometry (GC-MS) in order to specify compositions which were found in the extract.

5.2.2 Other parameters that affect the efficiency of extraction; i.e. sample moisture content and modifiers such as methanol, ethanol, and hexane should be considered in the future work.

5.2.3 Central composite orthogonal rotatable design was used in this study to obtain the response surface. However, the optimal point determined from the response equation was outside the experimental domain, particularly the operating pressure and the extraction time. Thus, it is recommended for the future study can be expanded the range of these factors for covered and predicted accurate results.

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APPENDICES

APPENDIX A

DATA ANALYSIS

A-1 Standard calibration curve for GC analysis

Table: A-1 Standard calibration curve data of β-pinene

Concentration of β-pinene (g/ml)	Peak Area
0.0012	2058706
0.0008	1393982
0.0006	1040935
0.0002	689478
0.0004	316269

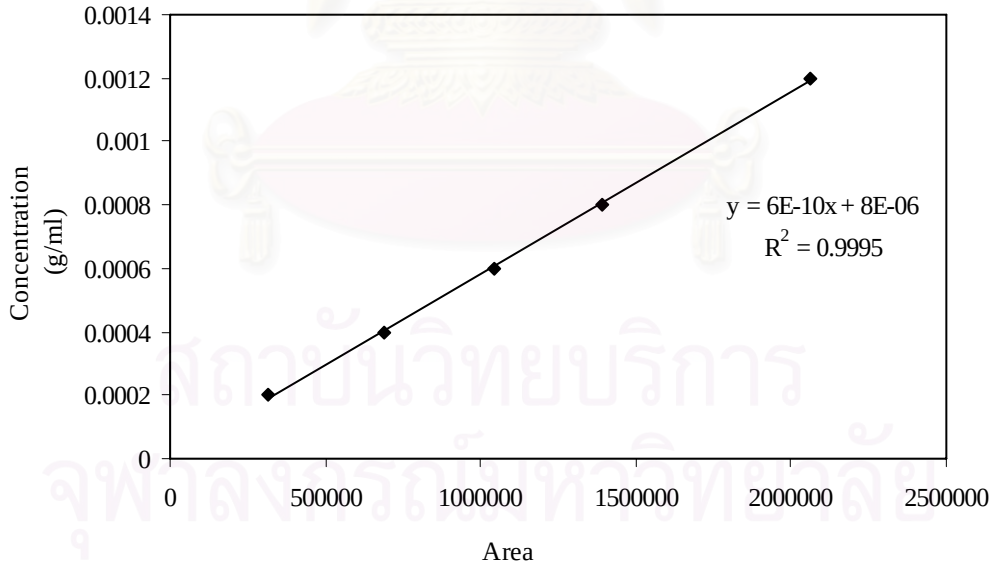

Figure A-1 Standard calibration curve for β-pinene

Table: A-2 Standard calibration curve data of limonene

Concentration of limonene (g/ml)	Peak Area
0.0008	1387134
0.0006	1053559
0.0004	734615
0.0002	361624
0.0001	182033

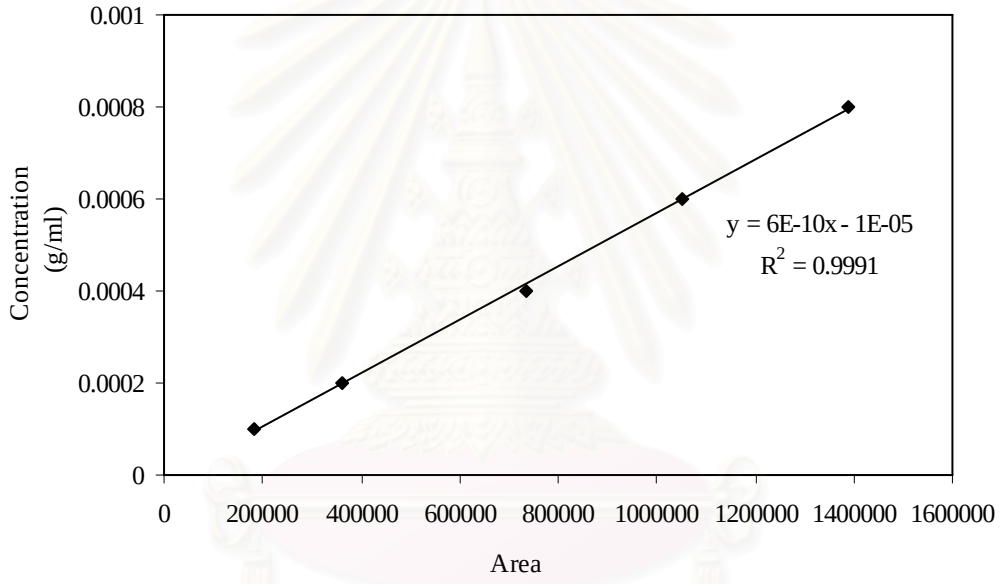

Figure A-2 Standard calibration curve for limonene

Table: A-3 Standard calibration curve data of 1,8-cineole

Concentration of 1,8-cineole (g/ml)	Peak Area
0.020	26903237
0.015	19504169
0.010	12982372
0.005	6764258
0.001	1247189

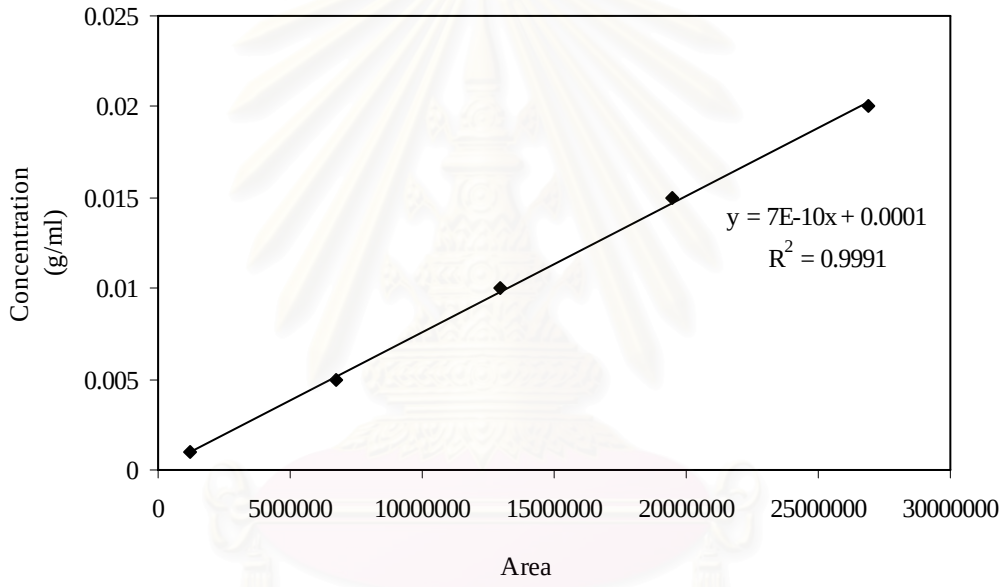

Figure A-3 Standard calibration curve for 1,8-cineole

Table A-4 Experimental data of essential oil yields (n=3)

Run	Temperature (ºC)	Pressure (bar)	Extraction time (min)	Essential oil (mg/g)	% yield
1	40	125	30	14.08	73.03
2	60	125	30	12.25	63.54
3	40	225	30	11.30	58.61
4	60	225	30	13.58	70.44
5	40	125	60	13.49	69.97
6	60	125	60	10.97	56.90
7	40	225	60	14.96	77.59
8	60	225	60	12.17	63.12
9	33	175	45	14.03	72.77
10	67	175	45	15.00	77.80
11	50	91	45	9.74	50.52
12	50	259	45	12.51	64.89
13	50	175	20	11.04	57.26
14	50	175	70	14.62	75.83
15	50	175	45	13.91	72.15
16	50	175	45	13.77	71.42
17	50	175	45	12.92	67.01

APPENDIX B

STATISTICAL ANALYSIS

B-1 Essential oil yields

In this experiment, the operating conditions were varied to finalize the best condition for supercritical carbon dioxide extraction of essential oil from *Amomum krevanh Pierre*. The results analysis can be determined by using the statistical testing program SPSS 15.0 and the results as shown in Table B-1.

Table B-1 ANOVA table for essential oil yields

Dependent Variable: Yields

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37.069(a)	14	2.648	9.225	.102
Intercept	1676.130	1	1676.130	5839.495	.000
X1	4.579	3	1.526	5.318	.162
X2	10.983	3	3.661	12.754	.074
X3	7.020	3	2.340	8.152	.111
X1 * X2	1.843	1	1.843	6.422	.127
X1 * X3	4.147	1	4.147	14.448	.063
X2 * X3	2.122	1	2.122	7.392	.113
X1 * X2 * X3	2.398	1	2.398	8.355	.102
Error	.574	2	.287		
Total	2893.509	17			
Corrected Total	37.643	16			

a R Squared = .985 (Adjusted R Squared = .878)

Consider the hypotheses testing for the essential oil yields as below

1) Testing for the effect of each level of factor X_2 (operating pressure)

   H_0: No difference between each level of factor X_2 or the operating pressure cannot affect the essential oil yields

   H_1: At least one level different from another level of factor X_2 or the operating pressure can affect the essential oil yields

Statistical testing: F = 3.661 / 0.287 = 12.754 or sig. = 0.074

Refuse H_0 if sig. < 0.10. In this case sig. = 0.074 < 0.10, thus refuse H_0 that means the operating pressure can affect the essential oil yields.

2) Testing for the interaction effect of each level of factor X_1 and X_3 (interaction between operating temperature and extraction time)

H_0: No difference between interaction level of factor X_1 and X_3 or the interaction between the operating temperature and pressure cannot affect the essential oil yields

H_1: At least one interaction level different from another interaction level of factor X_1 and X_3 or the interaction between the operating temperature and extraction time can affect the essential oil yields

Statistical testing: F = 4.147 / 0.287 = 14.448 or sig. = 0.063

Refuse H_0 when sig. < 0.10 at confident interval 90%. In this case sig. = 0.063 < 0.10, thus refuse H_0 that means the interaction between the operating temperature and pressure can affect the essential oil yields

B-2 Optimal condition for essential oil yields

Table B-2 Model summary for essential oil yields

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.842(a)	.709	.335	1.25004

a Predictors: (Constant), X2X3, X1X3, X1X2, X3X3, X3, X2, X1, X2X2, X1X1

Table B-3 ANOVA table for essential oil yields

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	26.693	9	2.966	1.896	.206(a)
	Residual	10.950	7	1.564		
	Total	37.643	16			

a Predictors: (Constant), X2X3, X1X3, X1X2, X3X3, X3, X2, X1, X2X2, X1X1

b Dependent Variable: Yields

Table B-4 Coefficients for essential oil yields

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta	B	Std. Error
1	(Constant)	13.356	.630		21.197	.000
	X1	-.237	.339	-.143	-.699	.507
	X2	.430	.339	.259	1.271	.244
	X3	.469	.339	.282	1.384	.209
	X1X1	.810	.511	.325	1.587	.157
	X2X2	-1.208	.511	-.484	-2.366	.050
	X3X3	-.193	.511	-.077	-.378	.717
	X1X2	.480	.442	.221	1.085	.314
	X1X3	-.720	.442	-.332	-1.628	.148
	X2X3	.515	.442	.237	1.165	.282

a Dependent Variable: Yields

From ANOVA table that use for the hypotheses testing which are

H_0: $\beta_1 = \beta_2 = \beta_3 = 0$

H_1: at least one value of $\beta_i \neq 0$; i = 1, 2, 3

Statistical testing: F = 2.966 / 1.564 = 1.896 or sig. = 0.206

At the confidence interval of 79% (P< 0.21), the result shows that sig 0.206 < 0.21, thus refuse H_0 that means at least one factor has relation to the essential oil yields and have to determine whether factor that has relation to the essential oil yields by using t-test. From Table B-4, consider the hypotheses testing for the constant and the regression coefficients as below.

1) Testing for the constant (β_0)

H_0: $\beta_0 = 0$ vs. H_1: $\beta_0 \neq 0$

Statistical testing: t = 21.197 or sig = 0.000

At the confidence interval of 90% (P< 0.1), The result shows that sig = 0.000 < 0.1, thus refuse H_0 that means the equation for this relation is not pass the origin point.

2) Testing for the constant ($\beta_2\beta_2$)

H_0: The quadratic main effect of operating pressure does not has relation to the essential oil yields when another factors are constant

H_1: The quadratic main effect of operating pressure has relation to the essential oil yields when another factors are constant

Statistical testing: t = -2.366 or sig = 0.050

The result shows that sig = 0.050 < 0.1, thus refuse H_0 that means the quadratic main effect of operating pressure has relation to the essential oil yields when another factors are constant.

APPENDIX C

The 17 th Thailand Chemical Engineering and Applied Chemistry Conference

(TiChe 17 th)

29-30 October 2007, The Empress Hotel, Chiang mai, Thailand

Experiments and Statistical Analysis of Supercritical Carbon Dioxide Extraction

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Experiments and Statistical Analysis of Supercritical Carbon Dioxide Extraction

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ABSTRACT

In this study, experimental design was employed in order to investigate the effects of operating condition on supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis* and essential oils from *Amomum krevanh Pierre*. The factors investigated for astaxanthin were operating temperature (40-80 ºC), the operating pressure (300-500 bar), and the extraction time (1-4 hours). The results showed that the main effect of operating pressure, the main effect of extraction time, and the interaction effect between operating temperature and operating pressure were significant factors for the astaxanthin yields. From the response surface model of the experimental data, an optimal condition for astaxanthin content was found to be at the temperature of 90 °C, the pressure of 640 bar, and the extraction time of 2.9 hours. This condition yields the highest amount of astaxanthin extract of 22.66 mg/g dry

algae. The factors investigated for essential oil were operating temperature (30-70 ºC), the operating pressure (90-260 bar), and the extraction time (20-70 min).

Keywords: Supercritical carbon dioxide, *Haematococcus pluvialis, Amomum krevanh Pierre*, Astaxanthin, Essential oil, Central composite design

1. INTRODUCTION

Haematococcus pluvialis is one of the most important microalgae producing many kinds of carotenoids such as beta-carotene, zeaxanthin, lutein and astaxanthin. Of these carotenoids, astaxanthin is found in the largest amount. Astaxanthin is a powerful biological antioxidant as it exhibits strong free radical scavenging activity [1] and protects against lipid peroxidation and oxidative damage of LDL-cholesterol, cells and tissues, and cell membranes.

Amomum (*Amomum krevanh Pierre*) is one of the most commonly used spices and herbs. The oil from seeds of amomum is widely used for flavoring purposes in food. Medicinally, they are used for flatulent indigestion, carminative and to stimulate the appetite.

There has been increasing interest to extract astaxanthin from *H. pluvialis* and essential oil from *A. krevanh*, however the conventional extraction of astaxanthin content and essential oils from natural materials requires toxic organic solvents, such as hexane, acetone, or dichloromethane, and to separate these solvents, evaporation is employed which may cause product degradation. For this reason, in recent years, supercritical fluid extraction (SFE) has become an alternative to more conventional extraction procedures. Supercritical fluid extraction (SFE) is a modern technology with increasing applications in pharmaceutical and food processing industry [2]. The physicochemical properties of supercritical fluid are between those of a liquid and a gas [3]. The temperature and pressure that are above the critical values lead these solvents to possess special properties such as high diffusivity and low viscosity [4], allowing them to better diffuse through natural solid matrix, and thus better extract the natural compounds than the conventional liquid solvents. The most frequently employed supercritical solvent in processing of food and natural products is carbon dioxide (CO_2) due to its low toxicity and low critical temperature and pressure [5]. For extraction of carotenoids from marine materials, many recent studies have been

carried out to investigate the effect of operating conditions and to determine the optimal conditions for the process [6]. In 1991, Narayanan et al. published their results on the extraction of volatile oil from cardamom seeds with Supercritical carbon dioxide (SC-CO_2). It has been reported that when CO_2-extracted cardamom oil has higher quality than the distilled oil [7].

In most of the previous studies, the process conditions have been optimized merely by conducting one-variable-at-a-time experiments. In such case, no interaction between process variables was assumed, and thus causing biased results. Statistical experimental design has been demonstrated to be a powerful tool for determining the factors effects and their interactions, which allow process optimization to be conducted effectively [8]. The central composite design (CCD) is probably the most widely used experimental design for fitting a second-order response surface. This design has the additional capability of intrinsic confirmation of results and estimation of experimental error.

The aim of the present work is to employ statistical analysis for the investigation of the effects of operating pressure, temperature and extraction time for the supercritical carbon dioxide extraction of astaxanthin from *H. pluvialis*. In addition, the comparison was made between the SC-CO_2 extraction and solvent extraction of essential oil from *A. krevanh*.

2. MATERIALS AND METHODS

2.1 Materials

The *Haematococcus pluvialis* strain powder samples were the commercial algae powder (NatuRose®), manufactured by Cyanotech, USA; they were stored in an oxygen free package and kept in a refrigerator at 4°C until use. Astaxanthin standard was obtained from Wako Chemical, USA. Dried fruits of *Amomum krevanh Pierre* were obtained by local market. The seeds of *Amomum krevanh Pierre* used in this experiment were obtained from dried fruits. The seeds were ground in a blender to produce fine powder. The average particle size was 0.3 mm. The ground samples were stored in a dry place until use. Standards for the identification of

chromatographic peaks were from Fluka. SC-CO_2 was carried out with high purity carbon dioxide.

2.2 Supercritical fluid extraction and analysis

Supercritical carbon dioxide extraction was carried out using SFX-220 extraction system from ISCO. The equipment consisted of a 10 ml extractor, a syringe pump, a controller and a restrictor temperature controller.

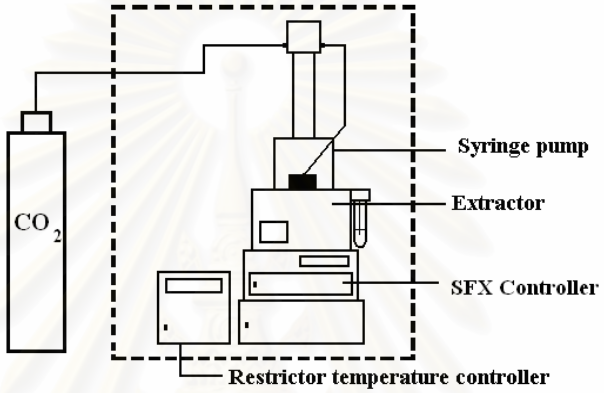

Figure 2.1 SFX-220 extraction system of supercritical carbon dioxide extraction

For each experimental run of astaxanthin extraction, 0.5 g of dried *H. pluvialis* algae was loaded in the extraction chamber. The extract was trapped in acetone and analyzed using a spectrophotometer, Genesys 20 (Thermo spectronic, USA) at the wavelength of 475 nm. For each experimental run of essential oil extraction, 3.0 g dried ground *A. krevanh* were loaded in the extraction chamber. The extract was trapped in a tube containing *n*-hexane. After extraction, *n*-hexane was removed with nitrogen stream at room temperature and analyzed using a gas chromatography. In addition, the solvent extraction was used for comparison.

2.3 GC analysis

GC analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with a DB-WAX capillary column (30 m $\times$0.25 mm i.d., film thickness 0.25 µm). The SFE samples (1 µl) were injected using the split mode with a split ratio of 1/30. Oven temperature was programmed to increase from 80°C to 130°C at a rate of

5 °C/min. Injector and detector temperatures were held at 230°C and 250°C, respectively.

2.4 Experimental design and statistical analysis

In this study the experimental design was used to evaluate the main and interaction effects of the factors: temperature (X_1), pressure (X_2), and extraction time (X_3) on SC-CO_2 process of *H. pluvialis*. Seventeen experiments were performed with three experiments as the repeatability of the measurements at the center of the experimental domain. The three-level face-center central composite design was used to evaluate both the main and the interaction effects of the operating conditions for astaxanthin extraction, denoted as 0 and ±1. The ranges of experimental variables used in the investigation are temperature (40-80°C), pressure (300-500 bar), and extraction time (1-4 hours). All factors and levels tested were reported in Table 2.1.

Table 2.1 Factors and levels tested for the designed experiment of astaxanthin extraction

Variables	Levels		
	-1	0	+1
X_1:Temperature (°C)	40	60	80
X_2: Pressure (bar)	300	400	500
X_3: Extraction time (hour)	1	2.5	4

The statistical analysis of variance (ANOVA) of the experimental results was employed to determine the main effect and interaction of the factor effects using SPSS program. The response surface equations were then proposed, from which the optimal conditions were determined.

3. RESULTS AND DISCUSSION

3.1 Supercritical carbon dioxide extraction of astaxanthin from *H. pluvialis*

The relations between each factor and the extract yields were modeled with a 2^{nd} order polynomial model, using the statistical program, SPSS.
The response surface equation obtained from the analysis is:

$$Y = 10.107 - 0.199X_1 + 1.032X_2 + 0.972X_3 - 0.458X_1^2 - 0.393X_2^2 + 0.127X_3^2 + 0.651X_1X_2 + 0.0288X_1X_3 - 0.451X_2X_3 \quad (3.1)$$

where Y is the astaxanthin yields, X_1, X_2, and X_3 are the operating temperature, the operating pressure, and the extraction time, respectively. The response surface of astaxanthin extract is shown in Figure 3.1.

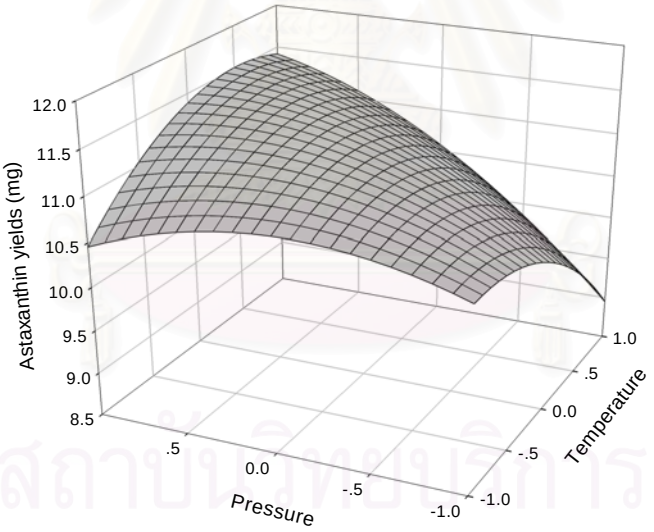

Figure 3.1 Response surface of astaxanthin yields

3.1.1 Main effect of operating pressure to astaxanthin yields

Figure 3.2 shows the main effect of operating pressure to astaxanthin yields and the results show that the astaxanthin yields were higher when the pressure was operated in the range of 300-500 bar. This agrees generally with theory, which relates the compound solubility in SC-CO_2 with the solvent density. Because the

supercritical solvent density increased when the pressure increased, this leads to the increase in the solvent power to dissolve the substances.

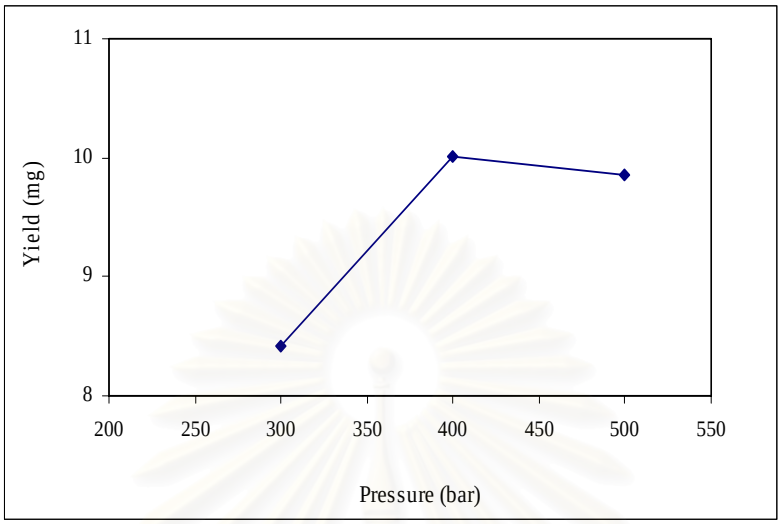

Figure 3.2 Main effect of operating pressure to astaxanthin yields

3.1.2 Main effect of extraction time to astaxanthin yields

The main effect of the extraction time to the astaxanthin yields is shown in Figure 3.3. The results show that the astaxanthin yields were higher with increasing extraction time between 1-4 hours. This is because increasing the time of extraction increases the contact time between the supercritical solvent and the solute.

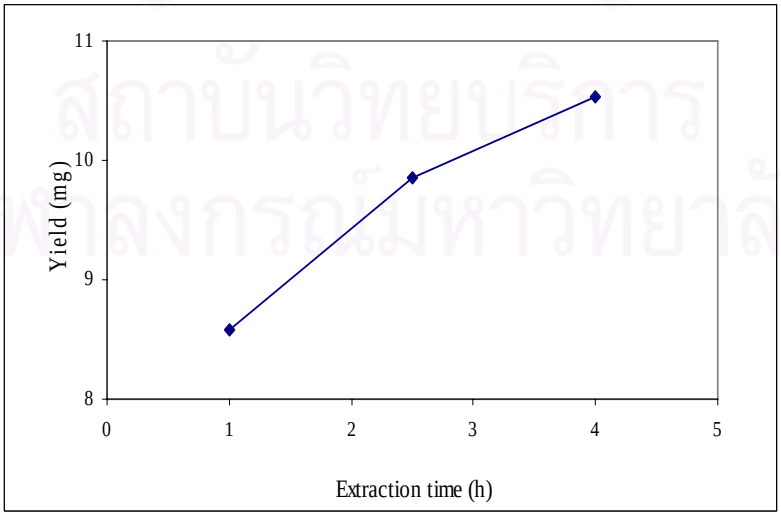

Figure 3.3 Main effect of extraction time to astaxanthin yields

3.1.3 Interaction effects of temperature and pressure on astaxanthin yield

The statistical analysis of the experimental results shows that the interaction between operating temperature and operating pressure affect significantly the astaxanthin yield. The interaction effect between the operating temperature and the operating pressure is plotted in Figure 3.4. The result shows that at pressure 300 bar, the extraction yields were lower when the temperature increased, while at the pressure of 400 and 500 bar, the extraction yields were slightly higher and significantly higher when the temperature increased. The reason of this observation is that the solubility of organic compound depends on a complex balance between supercritical fluid density and solute vapor pressure, which are both controlled by the fluid pressure and temperature.

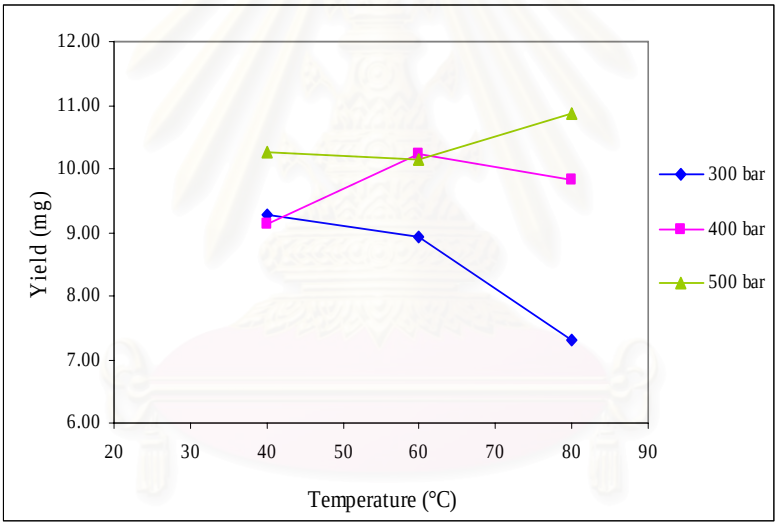

Figure 3.4 Interaction effect between temperature and pressure for astaxanthin yields

3.2 Supercritical carbon dioxide extraction of *A. krevanh*

Two major components of *A. krevanh* extract obtained by SC-CO_2 measured in this study were 1,8-cineole (3.74%) and beta-pinene (0.48%). The chromatogram of the extract is shown in Figure 3.5. Compared with the solvent extraction, the extract was found to have lower yields which were 2.96% for 1,8-cineole and 0.36% for beta-pinene, respectively.

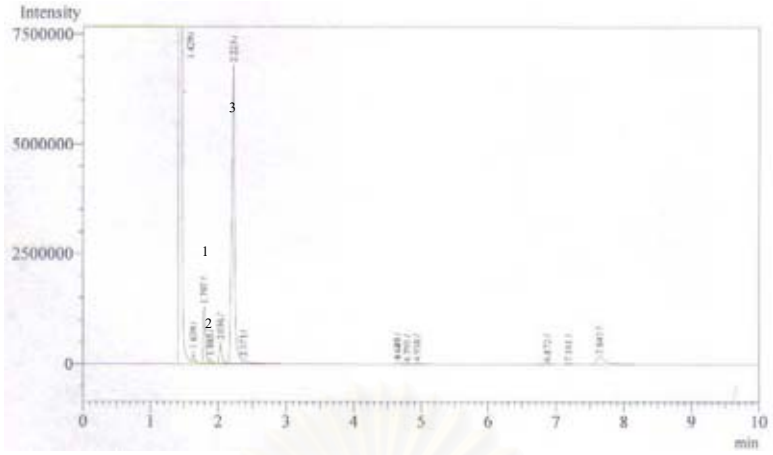

Figure 3.5 Chromatogram of amomum oil by SC-CO_2
(1. beta-pinene, 2. limonene, 3. 1,8-cineole)

4. CONCLUSIONS

In this study, the main effect of pressure (X_2), extraction time (X_3), and the interaction effect between temperature and pressure (X_1X_2) were significant factors affecting astaxanthin yields in SC-CO_2. The optimal condition was proposed within the range of this experiment to be at the temperature of 70 °C, the pressure of 500 bar, and the extraction time of 4 hours. At this condition, the predicted astaxanthin yield was 23.04 mg/g dry algae (or 83.78% recovery). For the extraction of essential oil, the amount of 1,8-cineole and beta-pinene recovered by SC-CO_2 was higher than those obtained with solvent extraction. The detailed statistical analysis will later be presented.

5. REFERENCES

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