EXTRACTION OF PERILLA SEED OIL BY LIQUEFIED DIMETHYL ETHER

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A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE BACHELOR DEGREE IN CHEMICAL ENGINEERING

NARESUAN UNIVERSITY

ACADEMIC YEAR 2016

ใบรับรองปริญญานิพนธ์

ชื่อหัวข้อโครงงาน	การสกัดน้ำมันเมล็ดงาม้อนโดยใช้ไดเมทิลอีเทอร์เหลว			
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คณะวิศวกรรมศาสตร์ มหาวิทยาลัยนเรศวร อนุมัติให้ปริญญานิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรวิศวกรรมศาสตรบัณฑิต สาขาวิชาวิศวกรรมเคมี

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บทคัดย่อ

ในการศึกษานี้ เป็นการศึกษาความเป็นไปได้ของการใช้ไดเมทิลอีเทอร์เหลวสำหรับการสกัดน้ำมันเมล็ดงาม้อน โดยใช้การศึกษาปัจจัยเดียวในการศึกษาผลของตัวแปรในการสกัดที่ส่งผลต่อร้อยละผลได้ของน้ำมัน ได้แก่ ความชื้น อัตราส่วนระหว่างตัวทำละลายกับเมล็ดงาม้อน และอุณหภูมิ ค่าที่เหมาะสมต่อการสกัดคือ ความชื้น 14% อัตราส่วนระหว่างตัวทำละลายกับเมล็ดงาม้อน 5.5:1 และอุณหภูมิ 40 องศาเซลเซียส ซึ่งได้น้ำมัน 78% ต่อมา วิธีผลตอบสนองแบบโครงร่างพื้นผิวถูกนำมาใช้ในการหาสภาวะที่เหมาะสมที่สุด จากผลการทดลองพบว่าสภาวะที่เหมาะสมคือ ความชื้น 20.29% อัตราส่วนระหว่างตัวทำละลายกับเมล็ดงาม้อน 6.23:1 และอุณหภูมิ 31.35 องศาเซลเซียส ภายใต้สภาวะที่เหมาะสมนี้ทำให้ได้น้ำมัน 98.4% ในน้ำมันเมล็ดงาม้อนประกอบด้วยยกรดไขมันอิ่มตัว 11.57% กรดไขมันไม่อิ่มตัวโมเลกุลเดี่ยว 13.75% และกรดไขมันไม่อิ่มตัวโมเลกุลคู่ 70.24% อีกทั้งยังมีกรดไขมันที่จำเป็น เช่น กรดลิโนเลนิก 51% กรดลิโนเลอิก 19.24% และกรดโอเลอิก 13.54% คุณสมบัติทางกายภาพและทางเคมีของน้ำมันเมล็ดงาม้อนสามารถนำไปประยุกต์ใช้ในอุตสาหกรรมเครื่องสำอาง อุตสาหกรรมยา และอุตสาหกรรมอาหาร

Title	Extraction of perilla seed oil by liquefied dimethyl ether		
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Academic Paper	Undergraduate Project B.Eng. (Chemical Engineering Faculty of Engineering, Naresuan University)		
Keywords	Perilla seed oil, Dimethyl ether, Extraction, Response surface methodology		

ABSTRACT

In this study, the possibility of using liquefied dimethyl ether (DME) for perilla seed oil extraction was investigated. The effects of extraction parameters including moisture content, solvent to sample ratio and temperature on oil yields were firstly determined by single parameter study. The suitable conditions determined by single parameter study was at 14% moisture content, 5.5:1 solvent to sample ratio and 40°C which gave approximately 78% recovery. The response surface methodology (RSM) was also used to determine the suitable condition. From RSM results, the optimum conditions were found to be at moisture content of 20.92%, solvent to sample ratio of 6.23:1 and an extraction temperature of 31.35 °C. Under these conditions, the maximum %recovery was 98.4%. The fatty acid profile of perilla oil revealed that it contained 11.57% saturated, 13.75% monounsaturated and 70.24% polyunsaturated fatty acids. Linolenic (51%), linoleic (19.24%), and oleic (13.54%) acids were the main fatty acids in perilla seed oil. Based on its physical and chemical properties, perilla seed oil could be used for applications in the cosmetic, pharmaceutical and food industries.

ACKNOWLEDGMENT

The success of this project can be succeeded by the attentive support from our advisor, Dr.Panatpong Boonnoun. We would like to express our sincere thanks for his invaluable help and good advice, not only advices for this project but also many other suggestions in life to resolve all the problems that arise during working.

In addition, we are also grateful from Assoc. Prof. Dr.Samorn Hirunpraditkoon and Dr.Pamornrat Chantam for their assistance.

Finally, we most gratefully acknowledge my parents and my friends for all their support throughout the period of this project.

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CHAPTER I

INTRODUCTION

1.1 Introduction

Nowadays, most people are interested in taking care of themselves. Supplements are the one of alternative way to improve their health that is easily purchased and there are many in the market. The supplements are the needed nutrients for health which human cannot enough consume in 5 categories of daily food. Omega fatty acids such as omega-3s and 6s are essential for our health and must be consumed as daily diet or supplementation. The best resources for additional human omega-3 polyunsaturated fatty acids (PUFAs) and ALA (Alpha-linolenic acid) are found in some other plant oils such as perilla, flaxseed (linseed), with lower amounts in walnut, canola, soy and animal sources like fish oil. Among those sources of omega-3s and 6s, perilla oil is interested since it is a very rich source of the omega-3, fatty acid, alpha-linolenic acid (ALA). Perilla seed oil consists of ALA about 50 to 60 percent which contains the highest proportion of ALA among plant oils.

Perilla is grown widely in Asia and also in northern of Thailand. The extracts from perilla seed as perilla seed oil suppresses the production of chemical mediator in the allergy and inflammatory responses. These essential fatty acids have been associated with benefits in a wide range of inflammatory conditions, heart diseases, colitis/Crohn's disease, asthma, allergies, antimicrobial, anticancer etc. Perilla is also used for nausea, sunstroke, to induce sweating and as an antispasmodic. In vivo metabolism of polyunsaturated omega-3 fatty acids, it mainly exists in the form of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). The specific omega-3 fatty acids metabolites are inserted in cell membranes throughout the body, where cellular machinery converts them into substances which prevent abnormal clotting, reduce inflammation, and relax blood vessels and improved ventilatory parameters [1].

It is therefore interested by many researchers to study the extraction of perilla seed oil. Generally, perilla seed oil extraction can be achieved by the conventional solvent extraction (normally hexane) and supercritical carbon dioxide (SC-CO_2). However, there are the limitation of toxicity of solvent and high operating pressure that lead to the high operating cost. The alternative solvent for oil extraction;

liquefied dimethyl ether (DME) has been interested in the recent years. Because it is a strong solvent for the extraction of lipid and used in the industry such as food, cosmetic and pharmaceutical. Dimethyl ether is proposed for use as an extraction solvent for the separation of lipids from a range of dairy foods. At ambient pressure (1 atmosphere), dimethyl ether exists as a gas at temperatures above -24° C. It is compressed, under high pressure, for use as a liquid extraction solvent in the course of food processing. Because of its low boiling point, dietary exposure to dimethyl ether will be negligible due to rapid evaporation of any residual dimethyl ether present in food following processing [2].

Since liquefied DME has never been applied for perilla seed oil extraction before. It is therefore lack of extraction conditions by this method. To determine the suitable conditions, design of experiment (DOE) is normally employed for this purpose. DOE can reduce number of experiment which leads to the less chemical and time consumptions. Response surface methodology (RSM) is one of the DOE method for optimization, finding the best set of factor levels to achieve some target. Central Composite Designs (CCD) and Box-Behnken Designs are two major Response Surface Designs which are different in the specified boundary. However, the effect of parameter cannot be determined by RSM since it focuses on the optimization. The single parameter or single factor study which the experiment is carried out by fixing other factors but varying one factor is usually done in order to study the effect of interested parameter. In the research work, both RSM and single factor studies therefore must be determined.

In this study, the extraction of perilla seed oil using liquefied dimethyl ether is therefore investigated. Effects of moisture content in perilla seed, solvent to sample ratio and extraction temperature on oil yield were determined by single parameter study. The optimum extraction condition was determined by Response surface study with Spherical Central Composite Designs (CCD) model. Moreover, the perilla seed oil obtained from this method was characterized by Gas chromatography (GC), Nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FT-IR).

1.2 Objectives

1.2.1 To study the possibility to use liquefied dimethyl ether as solvent for extraction of oil from perilla seed.

1.2.2 To study the effect of extraction parameters including solvent to sample ratio, temperature and moisture on the yield of extracted oil from perilla seed.

1.3 Scope of research

1.3.1 Determine the effect of solvent to sample ratio (w/w), extraction temperature and moisture content on extracted perilla seed oil yield and optimum condition by single parameter study.

- The range of moisture content is 7, 14 and 21%.
- The range of solvent to sample ratio (w/w) is 3:1, 4.5:1, 5.5:1 and 6.23:1.
- The range of extraction temperature is 31, 40 and 49 °C.

1.3.2 Determine the effect of solvent to sample ratio (w/w), extraction temperature and moisture content on extracted perilla seed oil yield and the optimum condition using response surface method.

- The range of moisture content is 7, 10, 14, 18 and 21%.
- The range of solvent to sample ratio (w/w) is 2.77:1, 3.5:1, 4.5:1, 5.5:1 and 6.23:1.
- The range of extraction temperature is 31, 35, 40, 45 and 49 °C.

1.3.3 Extraction time and agitation rate are fixed at 30 minute and 500 rpm, respectively.

CHAPTER II

BACKGROUND & LITERATURE REVIEWS

2.1 Perilla seed [3]

Perilla is an herb of the mint family, Lamiaceae. Perilla, growing in the hills and mountains of East Asia (mainly India, China, Japan, and Korea). The perilla is a traditional food in many Asian cultures. It is also known as shisho and the seed contains a high concentration of fatty acids as much as 40-45 percent oil. The composition of lipids and oil characteristics from perilla seed cultivars were shown in the tables 2.1-2.2 and a picture of perilla seed was shown in figure 2.1.

Table 2.1 The composition of perilla seeds

Component	Percent range
Neutral lipids	91.2–93.9%
- triacylglycerols	88.1–91.0%
- sterol esters	9-11.9%
- hydrocarbons	
- glycerides	
Glycolipids	3.9–5.8%
- esterified sterylglycoside	48.9–53.2%
- steryl glycoside	22.1–25.4%
- monogalactosyldiacylglycerol	21.4-29%
- digalactosyldiacylglycerol	
Phospholipids	2.0–3.0%
- phosphatidylethanolamine	50.4–57.1%
- phosphatidylcholines	17.6–20.6%
- phosphatidic acid	22.3-32%
- lysophosphatidylcholine	
- phosphatidylserine	
- phosphatidylinositol	

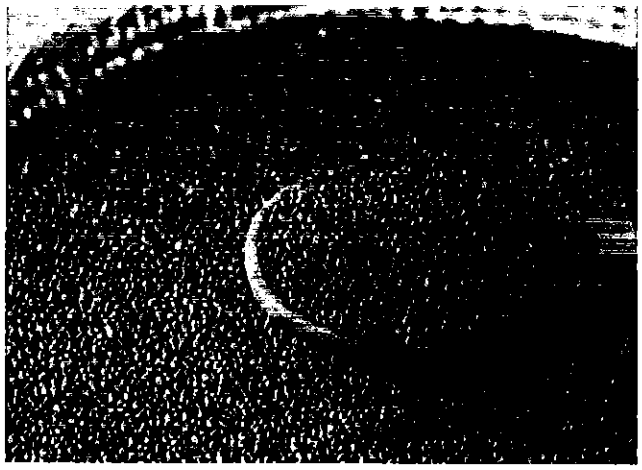

Figure 2.1 Perilla seed [4]

2.2 Perilla seed oil [5-6]

Perilla seed oil contains the highest proportion of ALA among vegetable oils, is widely used in Asian countries for cooking and as a traditional medicine but remains largely unknown in Western societies. Evidence from animal studies suggests that perilla seed oil has ability to prevent atherosclerosis and chemically induced cancer and that it also improves immune.

Table 2.2 The composition of perilla seeds oil

Component	Percent range
Linolenic acid	61.1-64.0%
Linoleic acid	14.3-17.0%
oleic acid	13.2-14.9%

2.3 Oil extraction techniques [7]

2.3.1 Soxhlet extraction

A soxhlet extractor was originally designed for the extraction of a lipid from a solid material. Typically, a soxhlet extraction is used for compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a little amount of solvent to dissolve a larger amount of material.

Figure 2.2 show a soxhlet extractor has three major sections: first section is percolator (boiler and reflux) which circulates the solvent, next section is a thimble (usually made of thick filter paper) which retains the solid and last section is a siphon mechanism which periodically empties the thimble.

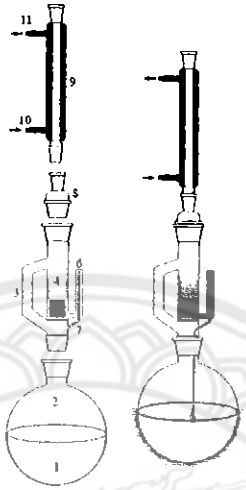

Figure 2.2 Soxhlet extraction [8]

The solvent is heated to reflux. The solvent vapor is transferred to a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor is condensed, and drips back down into the chamber housing the thimble of solid material. The warm solvent slowly fills into the chamber containing the solid material. Some of the desired compound dissolves in the warm solvent. When the chamber is almost full with solvent, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a compound of non-volatile dissolves in the solvent. After each cycles the desired compound is concentrated in the distillation flask. The advantage of this system is used one batch of solvent is recycled.

After extraction the solvent is removed, typically remove solvent by rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble and is usually discarded.

2.3.2 Dimethyl ether extraction

Dimethyl ether is presented for use as an extraction solvent for the extraction of lipids from a range of dairy foods. At ambient dimethyl ether exists as a gas at temperature above −24 °C. It is compressed, with high pressure, for use as a liquid extraction solvent in the food processing.

Animal and human data on inhalational exposure to dimethyl ether show a very low degree of toxicity. Because of its low boiling point, dietary exposure to dimethyl ether will be negligible due to rapid evaporation of any residual dimethyl ether present in food following processing. A maximum permitted level (MPL) for dimethyl ether of 2 mg/kg is proposed which is equivalent to the MPL for the two ether extraction solvents that are currently permitted for use in food manufacture: diethyl ether and di-butyl ether. This MPL is considered to be appropriate.

The overall conclusion of this risk and technical assessment is that the use of dimethyl ether as an extraction aid is technologically justified and raises no public health.

The following information regarding the identity and chemical and physical properties of the extraction solvent dimethyl ether.

2.3.2.1 Identity

Common name:	dimethyl ether
Chemical name (IUPAC):	methoxymethane
Other names:	methyl ether, oxybismethane, dimethyl oxide, wood ether
Molecular formula:	C_2H_6O
Structural formula:	CH_3-O-CH_3
Molecular weight:	46.069 g/mol

2.3.2.2 Chemical and physical properties

Dimethyl ether is a colorless gas at room. It is readily liquefied when compressed to produce a colorless liquid. It is highly flammable but is safe when handled appropriately. Relevant chemical and physical properties for dimethyl ether are provided in table 2.3.

Table 2.3 Chemical and physical properties of dimethyl ether

Characteristic	Property
Boiling point	-24.8 °C at 1 atmosphere
Freezing point (melting point)	-141.5 °C at 1 atmosphere
Flash point	-41 °C
Density of liquid	0.665 g/cm^3 at 25 °C, when liquefied
Density of gas	1.92 g/L at 1 atmosphere and 25 °C
Vapor pressure	4450 mmHg (593 kPa) at 25 °C
Solubility in water	7% by weight at 18 °C and 1 atmosphere

From table 2.3, dimethyl ether is partially soluble in water. It is also soluble in organic solvents that are relatively polar, such as methanol, ethanol, isopropanol, diethyl ether, chloroform, acetone, chlorinated hydrocarbons and toluene.

The main purpose of dimethyl ether is used to extraction lipids from both liquid and dry foods and it has unique properties that make it an effective solvent for extracting both polar and non-polar lipids from food. One of these properties is that it can extract lipids without denaturing the residual proteins in the food, which for some uses is a very important and useful attribute. Dimethyl ether has unique extraction properties compared to other extraction solvents due to the following attributes:

- It is a gas at room temperature and pressure so residues can be easily removed from treated food.
- It is a powerful polar solvent when it is compressed to a liquid and used for extraction near its critical point (40-50 °C).
- It is inert, so there are no by-products produced during extraction.
- Because it is used at relatively mild extraction conditions of temperature and pressure its use does not damage the food it is extracting, which retain most, if not all, of their natural properties such as appearance, flavor, solubility and bioactivity.
- Since it is partially soluble in water it is able to extract lipids from aqueous foods.

2.4 Analytical technique of extracted perilla seed oil [7]

2.4.1 Gas chromatography (GC)

Gas chromatography (GC) used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, separating the different components of a mixture (the relative amounts of such components can also be determined) and may help in identifying a compound. In preparative chromatography, GC can be used for separate the mixture to pure compounds and gas chromatography machine was shown in figure 2.3.

In gas chromatography, inside a piece of glass or metal tubing called a column (an homage to the fractionating column used in distillation) include the stationary phase and mobile phase. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support. The mobile phase or "moving phase" is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen. The gas chromatograph is an instrument used to perform gas chromatography or "aerograph", "gas separator".

The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase causes each compound to elute at a different time and known as the retention time of the compound. GC is useful for analyze by comparison of retention times.

Gas chromatography is in principle similar to column chromatography as well as HPLC and TLC, but has several notable differences. First, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas the stationary phase is a solid and the mobile phase is a liquid. ("Gas-liquid chromatography" is a full name of this procedure which referring to the mobile and stationary phases). Second, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography typically has no such temperature control. Finally, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point or vapor pressure differences. However, fractional distillation is typically used to separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale i.e. microscale.

Gas chromatography is also sometimes known as vapor-phase chromatography (VPC), or gas-liquid partition chromatography (GLPC). These alternative names, as well as their respective abbreviations, are frequently used in scientific literature. Strictly speaking, GLPC is the most correct terminology, and is thus preferred by many authors.

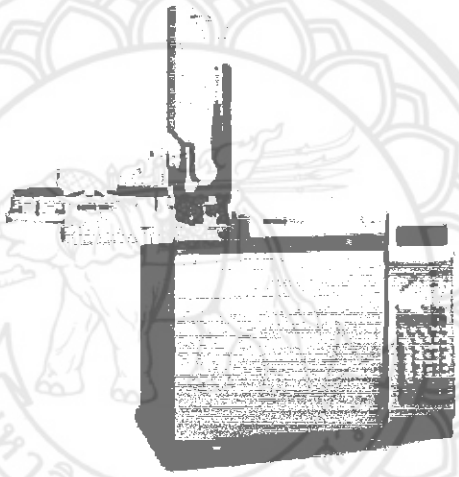

Figure 2.3 Gas Chromatography (GC) [9]

2.4.2 Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FTIR) is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum. For other uses of this kind of technique, Fourier transform spectroscopy was shown in figure 2.4.

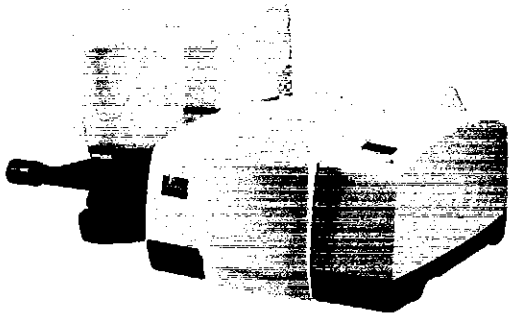

Figure 2.4 Fourier transform infrared spectroscopy (FT-IR) [10]

The goal of any absorption spectroscopy (FTIR, ultraviolet-visible ("UV-Vis") spectroscopy, etc.) is to measure absorbs light of sample at each wavelength. The dispersive spectroscopy technique is to shine a monochromatic light beam at a sample, measure how much of the light is absorbed and repeat for each different wavelength.

Fourier transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many frequencies of light at once and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterwards, a computer takes all these data and work backwards to infer what the absorption is at each wavelength.

The beam described above is generated by starting with a broadband light source one containing the full spectrum of wavelengths to be measured. The light shines into a Michelson interferometer a certain configuration of mirrors, one of which is moved by a motor. As this mirror moves, each wavelength of light in the beam is periodically blocked and transmitted by the interferometer due to wave interference. Different wavelengths are modulated at different rates at each moment and the beam coming out of the interferometer has a different spectrum.

As mentioned, computer processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength). The processing required turns out to be a common algorithm called the Fourier transform (hence the name, "Fourier transform spectroscopy"). Figure 2.5 shows the raw data is sometimes called an "interferogram".

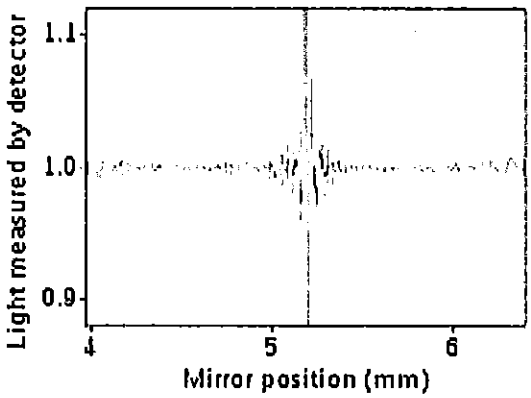

Figure 2.5 An FTIR interferogram [11]

2.4.3 Nuclear magnetic resonance spectroscopy (NMR) [12]

Nuclear magnetic resonance spectroscopy in figure 2.6 is one of the most essential research, powerful and non-invasive tool to obtain molecular level data and microscopic dynamic information, even for opaque systems. The basis of NMR is similar to other spectroscopy, such as UV–visible spectroscopy and infrared – radiowave frequency (RF) of the order of MHz is absorbed by NMR active nuclei under a strong magnetic field.

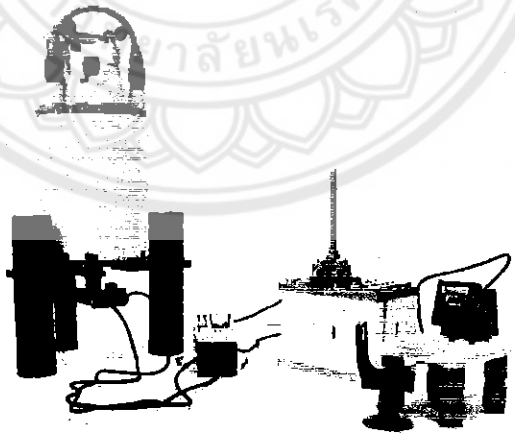

Figure 2.6 NMR spectroscopy [13]

NMR active nuclei are elements with a self-spin parameter of ½, including ^{1}H, ^{11}B, ^{13}C, ^{17}O, ^{19}F and ^{31}P. Each spinning charge creates a small magnetic field. When an external strong magnetic field (B_0) is imposed, these small magnetic fields in equilibrium splits into separate energy states of very small energy difference. The absorption of radio wave frequency that matches the energy difference between two states (i.e. resonance frequency) causes a state transition into a non-equilibrium state.

However, unlike infra-red and UV–vis spectroscopies where absorption occurs at a unique frequency or wavelength, NMR absorption peak is dependent on the strength of imposed magnetic field. The core component in NMR instrumentation includes a radiofrequency generator and powerful magnet (1–20 T). The commonly measured parameters for NMR techniques include chemical shift, spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2).

2.5 Response Surface Methods (RSM) [14]

RSM consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental design. Toward this objective, linear or square polynomial functions are employed to describe the system studied and consequently to explore (modeling and displacing) experimental conditions until its optimization.

Some stages in the application of RSM as an optimization technique are as follows: (1) the selection of independent variables of major effects on the system through screening studies and the delimitation of the experimental region, according to the objective of the study and the experience of the researcher; (2) the choice of the experimental design and carrying out the experiments according to the selected experimental matrix; (3) the mathematic–statistical treatment of the obtained experimental data through the fit of a polynomial function; (4) the evaluation of the model's fitness; (5) the verification of the necessity and possibility of performing a displacement in direction to the optimal region; and (6) obtaining the optimum values for each studied variable.

2.5.1 Screening of variables

Numerous variables may affect the response of the system studied, and it is practically impossible to identify and control the small contributions from each one. Therefore, it is necessary to select those variables with ma jor effects. Screening designs

should be carried out to determine which of the several experimental variables and their interactions present more significant effects. Full or fractional two-level factorial designs may be used for this objective principally because they are efficient and economical

2.5.2 Central composite designs

A central composite design is a 2^k full factorial to which the central point and the star points are added. The star points are the sample points in which all the parameters but one are set at the mean level "m". The value of the remaining parameter is given in terms of distance from the central point. If the distance between the central point and each full factorial sample is normalized to 1, in general, at some value. There are various choices of α. If $\alpha = 1$, the star points would be right on the boundary, and 3^2 design. Thus $\alpha = 1$ is a special case, a case that considered in the 3^k designs. More common choice of α is $\alpha = \sqrt{k}$, which gives a spherical design as shown in figure 2.7 below.

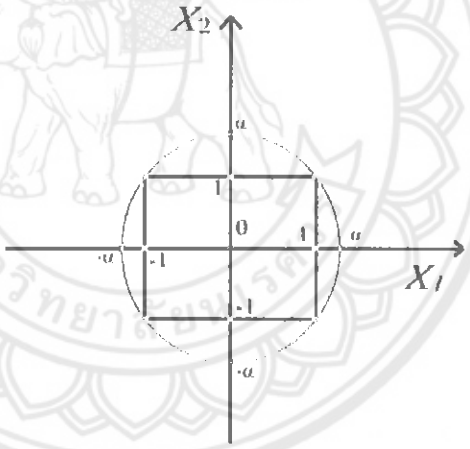

Figure 2.7 spherical design

2^2 design gives the box, and adding the axial points (in green) outside of the box gives a spherical design where $\alpha = \sqrt{k}$. The corner points and the axial points at α are all points on the surface of a ball in three dimensions was shown in figure 2.8.

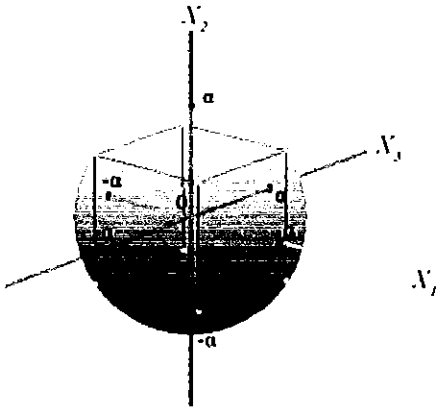

Figure 2.8 central composite design

CCD requires three types of trials, *i.e.*, 2^k factorial trials, $2*k$ axial trials and n_c center point trials, where k is number of factors studied in the experiment. Below is a table that summarizes these designs and compares them to 3^k designs:

Table 2.4 Summarizes these designs and compares them to 3^k designs

		$k = 2$	$k = 3$	$k = 4$	$k = 5$
Central Composite Designs	Factorial points 2^k	4	8	16	32
	Star points 2^k	4	6	8	10
	Center points n_c (varies)	5	5	6	6
	Total	13	19	30	48
3^k Designs		9	27	81	243
Choice of α	Spherical design ($\alpha = \sqrt{k}$)	1.4	1.73	2	2.24
	Rotatable design ($\alpha = (n_F)^{\frac{1}{4}}$)	1.4	1.68	2	2.38

Compare the total number of observations required in the central composite designs versus the 3^k designs.

The spherical designs are rotatable in the sense that the points are all equidistant from the center. Rotatable refers to the variance of the response function.

A rotatable design exists when there is an equal prediction variance for all points a fixed distance from the center, 0. This is a nice property. Pick the center of the design space and analyze the experiments, all points that are equal distance from the center in any direction will have equal variance of prediction.

In order to determine a critical point (maximum, minimum, or saddle), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \qquad (2.1)$$

Where β_{ij} represents the coefficients of the quadratic parameters, X_i and X_j represent the independent variables in the form of coded values and ε is the residual associated to the experiments

To estimate the parameters in Eq. (2.1), the experimental design has to assure that all studied variables are carried out at in at least three factor levels. Thus, two modeling, symmetrical response surface designs are available. Among the more known second-order symmetrical designs are the three-level factorial design, Box–Behnken design and central composite design. These symmetrical designs differ from one another with respect to their selection of experimental points, number of levels for variables, and number of runs and blocks.

2.6 Literature reviews

Table 2.5 Literature reviews

Author	Title	Raw material	Method	Conditions	Results
Liu et al., 2014 [15]	Structural characterization and antioxidant activity evaluation of phenolic compounds from cold-pressed perilla frutescens var. argute seed flour	Perilla seed flour	Cold-press	Extracted with petroleum ether three times at room temperature for 12 hours each. The resulting residue was extracted with 70% ethanol three times at 60 °C for 6 hours each.	A total of 11 phenolic compounds, as well as sucrose and tryptophan, were isolated from cold-pressed perilla seed flour using column chromatography, and their chemical structures were characterized using NMR and LC-MS-IT TOF. Rosmarinic acid and rosmarinic acid-3-o glucoside were the dominant phenolic antioxidants with strong antioxidant activities.
Matsushita et al., 2015 [16]	Compressed n-propane extraction of lipids and bioactive compounds from Perilla (Perilla frutescens)	Perilla seed	Compressed n-propane extraction compare with Soxhlet method	Soxhlet method: using ethyl ether and petroleum ether (1:1 v/v) as solvents, for 16 hours. Compressed n-propane extraction: temperature variation	The higher extraction yield obtained by Soxhlet may be due to the extraction of different compounds soluble in petroleum ether and ethyl ether, overestimating the value of extraction yield. The values of moisture, ash, crude protein, and total fiber were obtained

Author	Title	Raw material	Method	Conditions	Results
				of 40–80°C and pressure 8–16 MPa.	values of 4.73%, 3.8%, 22.6%, and 23.85%, respectively.
Li et al., 2015 [17]	Optimization of ultrasound-assisted hexane extraction of perilla oil using response surface methodology	Perilla seeds	- Extraction of perilla oil using UAE - Single factor experiments - RSM design and statistical analysis	- using n-hexane at 30°C for 15 min using a liquid-to-solid ratio of 5:1 and a power of 400 W. - The effect of ultrasonic power at 300 - 500 W. The effect of extraction temperature at 20 -60°C, The effect of extraction time at 10 - 30 min. The effect of liquid–solid ratio at 3:1 - 11:1 - The complete design consisted of 17 experimental	- UAE were obtain perilla oil 35.2% - Single factor experiments, the optimal UAE power was 400 W., the optimal extraction temperature was 40°C., the optimal extraction time was 15 min., the optimal liquid-to-solid ratio was 7:1 - The optimum conditions for oil yield included a temperature of 41.26°C, an extraction time of 17.11 min and a liquid-to-solid ratio of 7.02:1. The oil yield was 36.30 - approximate 93% of the total fatty acids in perilla seeds oil.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and chemicals

Perilla seeds were obtained from local farm in Chaing rai, Thailand. Dimethyl ether was purchased from Tamiya, Japan. Hexane (99%) obtained from V.S.Chem house.

3.2 Methodology

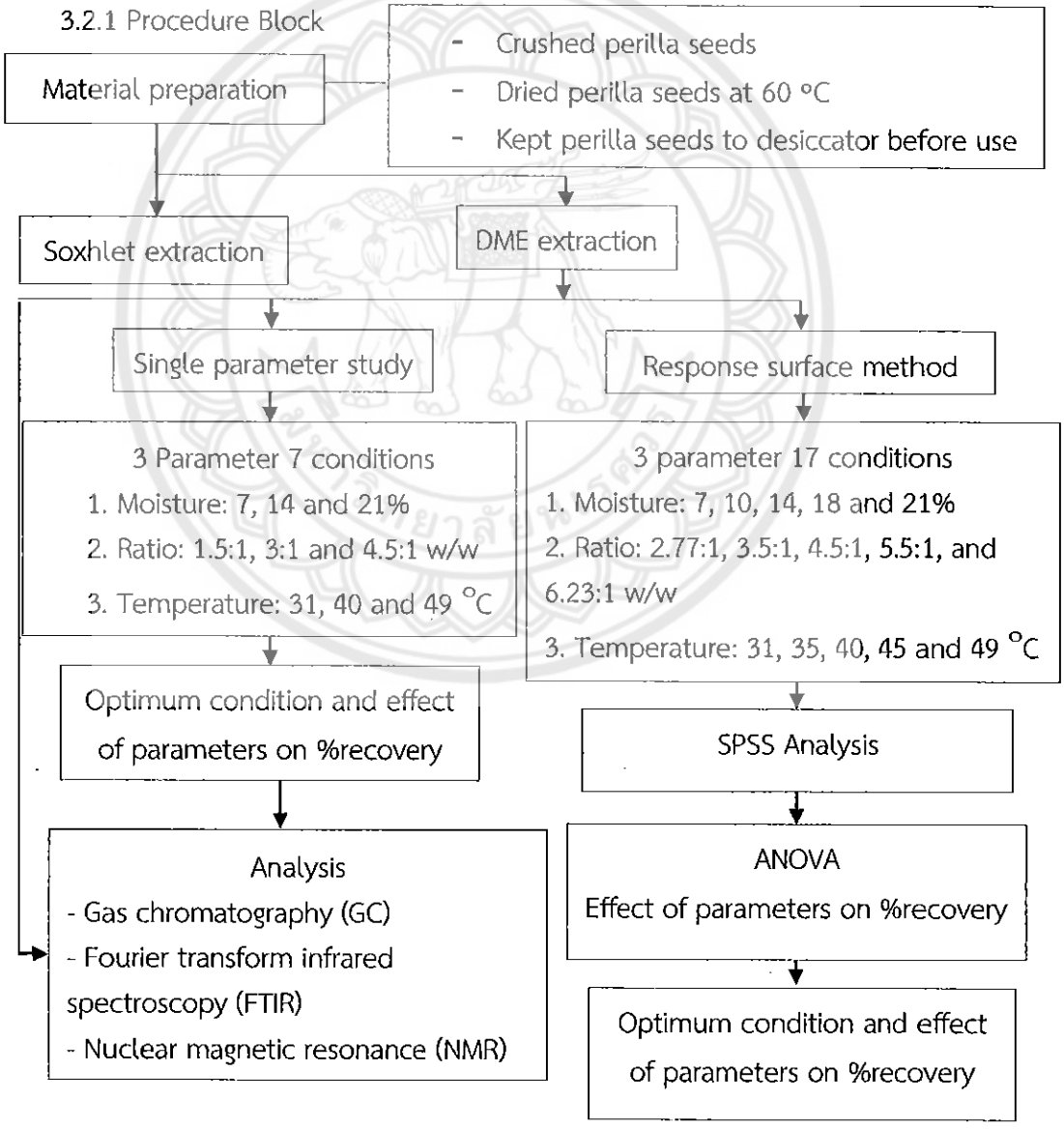

Figure 3.1 Procedure block from this work

3.2.2 Sample preparation

Perilla seed was briefly crushed and dried at 60 °C until weight constant. After that, kept in desiccator before use. Dried perilla seed was determined the moisture content according to ASTM D4442-07. The minimum moisture content obtained from the drying at 60 °C was use as the based moisture content data for filling higher moisture content in our experiments. The moisture content was calculated by the equation 3.1.

$$\text{Moisture content \%} = \left(\frac{\text{volume of water}}{\text{dry weight of seed}}\right) \times 100 \qquad (3.1)$$

3.2.3 DME Extraction

First, perilla seeds were weighed to approximately 10 grams and then mixed with water at desired moisture content. Then loaded into thimble (cellulose 30x100 mm) and loaded into an extractor (volume 100 ml stainless steel) along with 8mm diameter magnetic bar. Liquefied DME was filled into the extractor at required solvent to sample ratio. The temperature of the system was controlled at desired temperature by heating jacket connecting with control box. The extractor was the placed onto a stirrer and fixed agitation at 500 rpm for 30 minutes. The schematic of extractor was shown in the Figure 3.2. After extraction, the ball valve was turned to open then DME and oil were transferred to the 100 ml beaker through stainless steel filter (5 μm pore diameter) which can separate the solid residues from the extracts. The obtained solution was heated by hot plate stirrer at 100 °C and 500 rpm to remove the moisture. The obtained oil was analyzed by weighting and composition of extracted oil was analyzed by GC, NMR and FT-IR.

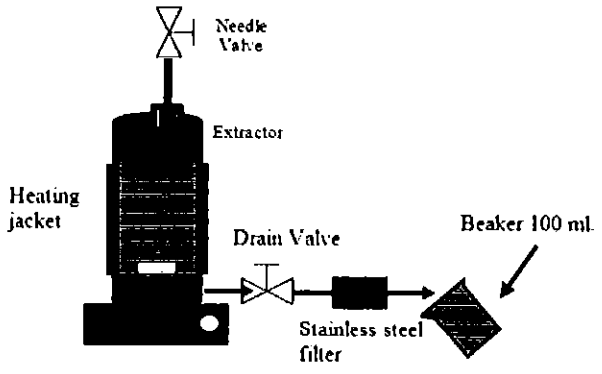

Figure 3.2 Schematic diagram of DME Extractor

3.2.3.1 Single parameter study

Single parameter was applied to determine the optimal condition of DME extraction experiment by study one by one parameter: extraction temperature, solvent to sample ratio and moisture content in perilla seed. Methods of extraction were described in section 3.2.3 and single parameter conditions were shown in the table 3.1.

Table 3.1 Condition of single parameter study

Run	Moisture content (%)	Solvent to sample ratio (w/w)	Temperature (°C)
1	7	3:1	40
2	14	3:1	40
3	21	3:1	40
4	Best from previous	1.5:1	40
5	Best from previous	4.5:1	40
6	Best from previous	Best from previous	31
7	Best from previous	Best from previous	49

3.2.3.2 Response surface study

Optimization of conditions for extraction of total perilla oil from perilla with DME solvent systems was carried out using a spherical central composite design (Spherical CCD). Three independent variables were chosen: extraction temperature (X_1), solvent to sample ratio (X_2) and moisture content (X_3). The levels of factor were designed by $\alpha = \sqrt{k}$, where k is the number of variables (k = 3).This design puts all the factorial and axial design points on the surface of a sphere of radius $\sqrt{k}$. The low, middle and high levels of each variable were designated −1.73, 0 and +1.73, respectively. The corresponding actual values for each variable in table 3.3 were calculated from equation in table 3.2 and using the spherical CCD for design amount of experimental including the factors and their levels are shown in table 3.4. The analysis of the central composite experimental design was carried out using SPSS. Methods of extraction is show above and response surface use conditions follow in the table 3.5.

Table 3.2 calculation of actual variables

-1.73	-1	0	1	1.73
$X_1 - (\Delta Y_1 \times 1.73)$	$X_1 - \Delta Y_1$	X1	$X_1 + \Delta Y_1$	$X_1 + (\Delta Y_1 \times 1.73)$
$X_2 - (\Delta Y_2 \times 1.73)$	$X_2 - \Delta Y_2$	X2	$X_2 + \Delta Y_2$	$X_2 + (\Delta Y_2 \times 1.73)$
$X_3 - (\Delta Y_3 \times 1.73)$	$X_3 - \Delta Y_3$	X3	$X_3 + \Delta Y_3$	$X_3 + (\Delta Y_3 \times 1.73)$

X = chosen value (base on experiment from single parameter study), ΔY = interval

Table 3.3 Levels of actual and coded variables

Actual/coded variables	X_i				
	-1.73	-1	0	1	1.73
Moisture content (%)	7	10	14	18	21
Solvent to sample ratio (w/w)	2.77	3.5	4.5	5.5	6.23
Temperature (°C)	31	35	40	45	49

Table 3.4 Spherical CCD

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	-1.73	0	0
10	1.73	0	0
11	0	-1.73	0
12	0	1.73	0
13	0	0	-1.73
14	0	0	1.73
15	0	0	0
16	0	0	0
17	0	0	0

Table 3.5 Conditions of response surface

Run	Moisture content (%)	Solvent to sample ratio (w/w)	Temperature (°C)
1	10	3.5:1	35
2	18	3.5:1	35
3	10	5.5:1	35
4	18	5.5:1	35
5	10	3.5:1	45
6	18	3.5:1	45
7	10	5.5:1	45
8	18	5.5:1	45
9	7	4.5:1	40
10	21	4.5:1	40
11	14	2.77:1	40
12	14	6.23:1	40
13	14	4.5:1	31
14	14	4.5:1	49
15	14	4.5:1	40
16	14	4.5:1	40
17	14	4.5:1	40

3.2.4 Soxhlet extraction

Perilla seeds were weighed to approximately 10 grams and extracted with 200 ml suitable solvent by soxhlet apparatus for 15 hours. The total oil content determined by this method was found to be 4.98 grams and used as a basis to calculate %recovery of perilla seed oil extracted by DME extraction as shown in equation 3.2.

$$\%\text{recovery} = \frac{\text{oil content extracted by DME}}{\text{oil content extracted by soxhlet}} \times 100 \qquad (3.2)$$

3.3 Characterization of perilla seed oil

Extracted perilla seed oil without adding any moisture content and remove moisture by heating was analyzed composition of fatty acid and functional group by the following analysis methods.

3.3.1 Gas chromatography (GC)

Gas chromatography analysis of the fatty acid dimethyl ether composition was analyzed using an Agilent 6890N series Gas Chromatography equipped with a flame ionization detector (FID) on a split injector. A fused silica capillary column (DB-225, 30 mm - 0.32 mm i.d., J&W Scientific, USA) was used with the injector and detector temperature maintained at 230 and 250 °C, respectively. The oven temperature was programmed at 160 °C for 2 minutes and then increased to 230°C with 4 °C/min. The carrier gas used was nitrogen at a flow rate of 1.5 mL/min.

3.3.2 Nuclear magnetic resonance spectroscopy (NMR)

Perilla seed oil preparation for NMR recording, used dropper to suck the perilla seed oil into the tube NMR about 0.5-0.6 ml and used another dropper to suck the solvent (chloroform-d, $CDCl_3$) into the tube NMR until the height more than 4 cm then close the cap. The measurement is an atom hydrogen (Proton, 1H) and the NMR spectrum was analyzed in rang of frequency -1 ppm to 11 ppm.

3.3.3 Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of the oil, kernel and cake were obtained using FT-IR instrument (Shimandzu:IR Affinity) using KBr as background for functional group identification. To obtain the IR spectra of perilla oil sample (0.2 - 0.4 mg), KBr is used in pellet form and for solid sample (moisture free) analyzed KBr was mixed in powder form with the sample (100:1 ratio) to get homogenous mixture. Pure KBr (pellet/powder) and then KBr with sample (liquid/solid) placed in sample holder and the infrared solution was analyzed in the range of wave number 400-4000 cm^{-1}. FT-IR spectrums of the samples were used.

CHAPTER IV

RESULTS AND DISCUSSIONS

The effects of extraction parameter including moisture content, solvent to sample ratio (w/w) and temperature on %recovery of perilla seed oil are reported in single parameter study. The response surface study for optimization of perilla seed oil using DME extraction is reported. The perilla seed oil extracted by DME extraction is then characterized by GC, NMR and FT-IR.

4.1 Single parameter study

In table 4.1 the results of single parameter study were obtained amount of oil (g) and used for calculated of %recovery.

Table 4.1 %recovery of perilla seed oil in single parameter study

Run	Moisture content (%)	Solvent to sample ratio (w/w)	Temperature (°C)	Oil (g)	%recovery
1	7	3:1	40	2.11	42.37
2	14	3:1	40	2.72	54.62
3	21	3:1	40	2.66	53.41
4	14	3:1	40	2.72	54.62
5	14	4.5:1	40	3.53	70.88
6	14	5.5:1	40	3.92	78.71
7	14	6.23:1	40	3.82	76.71
8	14	5.5:1	31	2.68	53.82
9	14	5.5:1	40	3.92	78.71
10	14	5.5:1	49	3.43	68.88

4.1.1 Effect of moisture content

The effect of moisture content on %recovery was studied at solvent to sample ratio 3:1 (w/w) and 40 °C. The results show in Figure4.1 revealed that %recovery increased up to maximum 54.62 with 14% of moisture content. At moisture content more than 14%, %recovery was slightly decreased. The increase in the amount

of water might firstly help DME to diffuse into the matrices of perilla seed but too large amount of water might increase the overall solvent polarity caused the lower oil yield since oil is the non-polar compound [18].

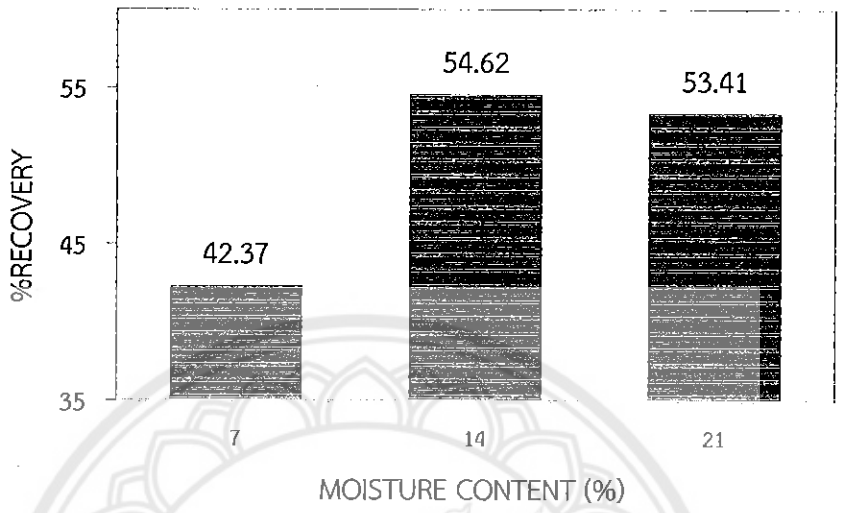

Figure 4.1 Effect of moisture content on %recovery

4.1.2 Effect of solvent to sample ratio

The effect of solvent to sample ratio on %recovery was studied at 14% moisture content and 40 °C. The results show in Figure 4.2 it was noticed that higher solvent to sample ratio, the greater the %recovery of perilla seed oil. When the volume of the extraction solvent increased compared to the amount of sample, the diffusion became more efficient. The increase in the solvent to sample ratio seems to have more effect than that of the pretreatment energy input [19]. The results might be explained by the fact that increase in amount of solvent help approach the equilibrium of oil in the solvent.

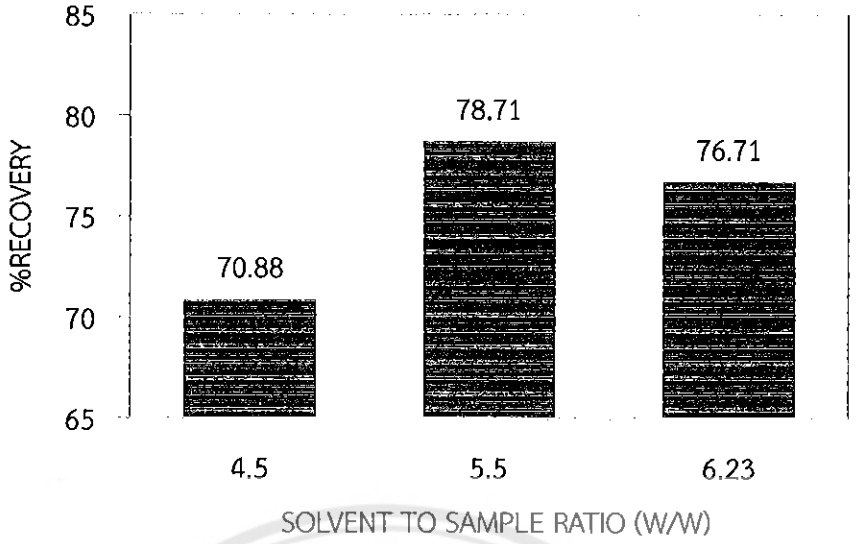

Figure 4.2 Effect of solvent to sample ratio on %recovery

4.1.3 Effect of temperature

The effect of temperature on %recovery was studied at 14% moisture content and 5.5:1 solvent to sample ratio. The results show in Figure 4.3 revealed that %recovery increased up to maximum 78.71 with temperature at 40°C but slightly decreased at higher temperature. The increase in temperature affects the higher solubility of oil in DME. However, density of solvent was decreased with increase in temperature and caused lowering solvent power of DME. At too high temperature (50°C), decrease in density of solvent might overwhelm the effect of solubility resulting in the observed lower yield [20-21].

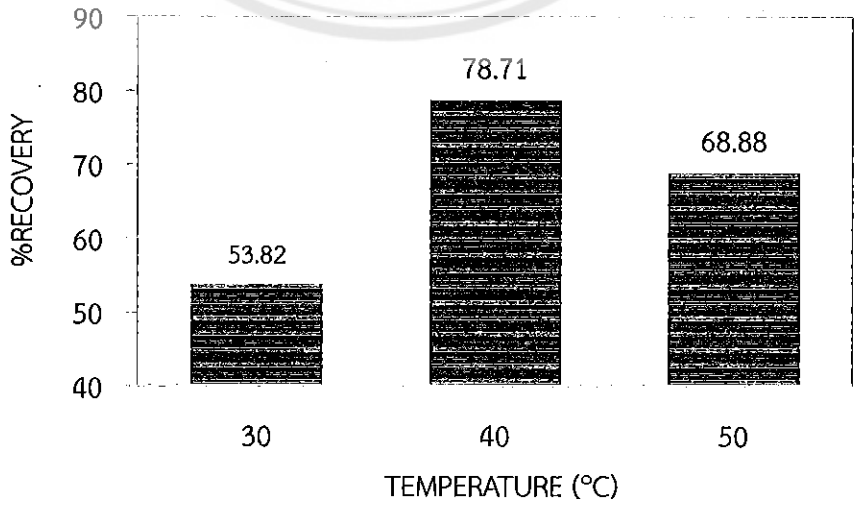

Figure 4.3 Effect of temperature on %recovery

The single parameter study was employed for studying the effect of extraction parameter. The suitable conditions obtained by this method are not the optimum condition for DME extraction since experiment should be done 27 runs (3^3). The RSM is used to optimize extraction condition in the next section. For RSM study, the experiment can be reduced from 27 to 17 runs then statistical analysis by ANOVA was used to predict the optimum condition for this work.

4.2 Response surface methodology

The %recovery of perilla seed oil determined by dimethyl DME extraction along with predicted yields by analysis of variance (ANOVA) are shown in table 4.2 and compared %recovery from experimental value with predicted value in term of residue. The results of statistical analysis using SPSS 23.0 program of the extraction parameter are shown in table 4.3 and the regression parameters are shown in table 4.4. From the results, the quadratic equation for prediction of %recovery can be obtained as equation 4.1. The predicted versus observed values of the total %recovery perilla seed for systems indicated good agreement between the regression model and the experimental data, with the R^2 of 0.863. In addition, statistical analysis revealed moisture content and solvent to sample ratio were significant parameters because their p-value were lower than 0.05 (0.024 and 0.043, respectively). However, the regression model substituted by real condition was generated and shown as equation 4.2 for the more convenience.

$$Yield = 67.315 + 8.329X_1 + 6.215X_2 + 0.773X_3 + 2.335X_1X_2 - 2.535X_1X_3 - 1.28X_2X_3 - 3.732X_1^2 - 2.123X_2^2 + 2.131X_3^2 \quad (4.1)$$

$$Yield = -29 + 11.06X_1 + 27.4X_2 - 3.73X_3 - 0.233X_1^2 - 2.12X_2^2 + 0.0852X_3^2 + 0.584X_1X_2 - 0.127X_1X_3 - 0.256X_2X_3 \quad (4.2)$$

Table 4.2 %Recovery from experimental and prediction of perilla seed oil in RSM.

Run	Moisture content (%)	Solvent to sample ratio (w/w)	Temperature (°C)	%recovery		Residue
				Experimental	Predicted	
1	10	3.5:1	35	52.01	46.68	5.33
2	18	3.5:1	35	59.24	63.76	-4.52
3	10	5.5:1	35	52.81	57.08	-4.27
4	18	5.5:1	35	79.92	83.50	-3.58
5	10	3.5:1	45	59.44	55.88	3.56
6	18	3.5:1	45	67.07	62.8	4.27
7	10	5.5:1	45	65.66	61.16	4.50
8	18	5.5:1	45	72.09	77.42	-5.33
9	7	4.5:1	40	36.55	41.25	-4.70
10	21	4.5:1	40	75.92	70.43	5.49
11	14	2.77:1	40	45.38	50.09	-4.71
12	14	6.23:1	40	76.71	71.73	4.98
13	14	4.5:1	31	76.51	72.75	3.76
14	14	4.5:1	49	71.04	75.56	-4.52
15	14	4.5:1	40	70.88	67.25	3.63
16	14	4.5:1	40	66.20	67.25	-1.05
17	14	4.5:1	40	64.86	67.25	-2.39

Table 4.3 Analysis of variance (ANOVA) for the quadratic polynomial mode.

Source	SS	df	MS	F	p-value
Model	1990.10	9	221.123	4.91	0.024
X_1	970.71	1	970.708	21.55	0.002 *
X_2	540.24	1	540.24	12.00	0.001 *
X_3	8.37	1	8.373	0.19	0.679
X_1X_2	43.59	1	43.593	0.97	0.358
X_1X_3	51.42	1	51.416	1.14	0.321
X_2X_3	13.11	1	13.110	0.29	0.606
X_1X_1	169.14	1	169.136	3.76	0.094
X_2X_2	54.81	1	54.808	1.22	0.306
X_3X_3	55.10	1	55.098	1.22	0.305
Error	315.27	7	45.038		
Total	2305.37	16			
R^2	0.863				

X_1 = Moisture, X_2 = Ratio, X_3 = Temperature, SS = Sum of Squares, MS = Mean Square, "* is significant"

Table 4.4 Regression

	ANOVA				
Model	SS	df	MS	F	p-value
Regression	1990.125	9	221.125	4.912	0.024
Residual	315.107	7	45.015		
Total	2305.233	16			

Response surface methodology was used to determine the optimal conditions for DME extraction. The three-dimensional plots of predicted response base on the model in equation 4.2 as a function of the combination of two test variables with the other maintained at its respective zero level are show in figure 4.4-4.6. The optimal conditions giving the maximum response for %yield calculate from equation 4.2 were moisture content of 20.92%, solvent to sample ratio of 6.23 and temperature of 31.35 °C resulting in %recovery of 98.4, for extraction time of 30 minute and agitation rate 500 rpm.

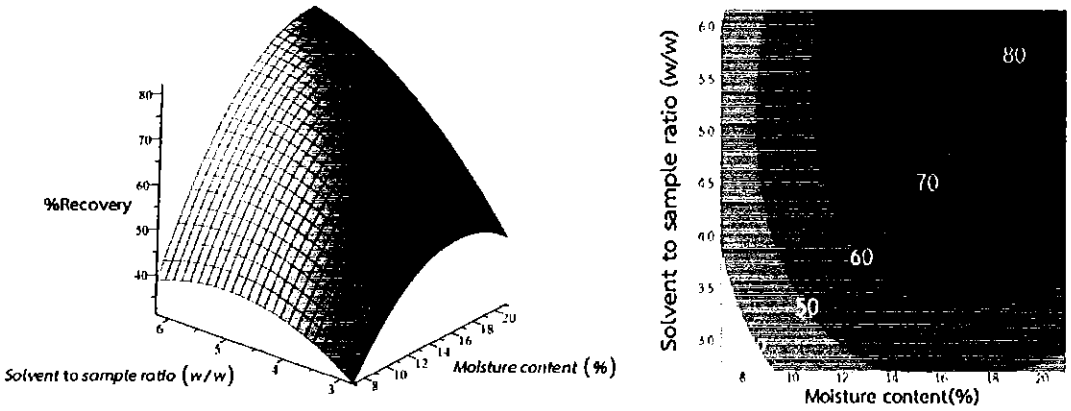

Figure 4.4 Response surface and contour plot for the %recovery as function of solvent to sample ratio and moisture content at a fixed temperature of 40 °C

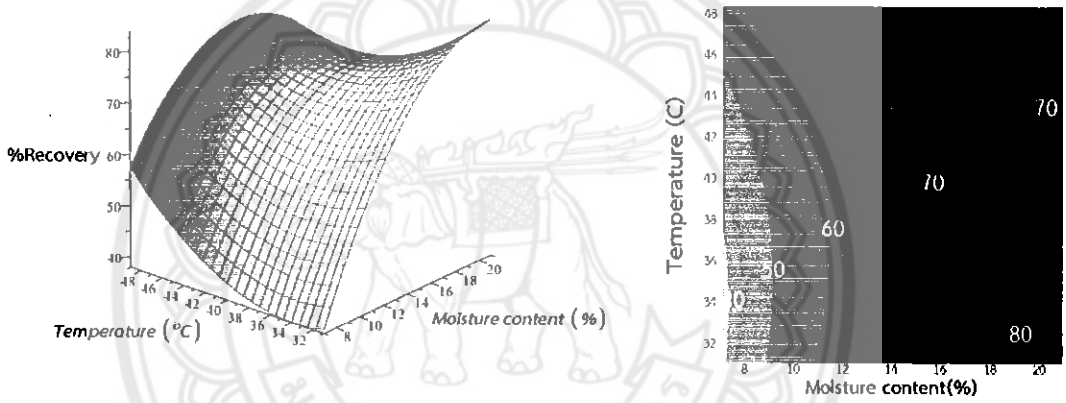

Figure 4.5 Response surface and contour plot for the %recovery as function of moisture content and temperture at a fixed solvent to sample ratio of 4.5:1

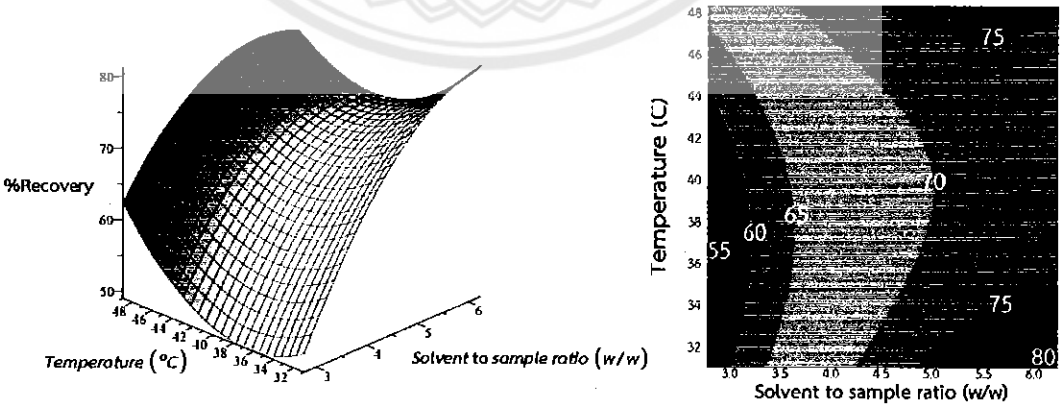

Figure 4.6 Response surface and contour plot for the %recovery as function of solvent to sample ratio and temperture at a fixed moisture content of 14%

4.3 Characterization of perilla seed oil

4.3.1 Composition analysis of perilla seed oil

Fatty acid composition of perilla seed oil were analyzed by ^{1}H NMR spectroscopy. Identified peaks in NMR spectra (Figure 4.7) were used to determine linolenic (Ln), linoleic (L), oleic (O) and saturated (S) fatty acid present in the perilla seed oil. The percentage of fatty acids were obtained by equations 4.3-4.6

$$Ln\ (\%) = 100\left(\frac{B}{A+B}\right) \tag{4.3}$$

$$L\ (\%) = 100\left[\frac{E}{D} + 2\left(\frac{B}{A+B}\right)\right] \tag{4.4}$$

$$O\ (\%) = 100\left[\frac{C}{2D} - \frac{E}{D} + \left(\frac{B}{A+B}\right)\right] \tag{4.5}$$

$$S\ (\%) = 100\left(1 - \frac{C}{2D}\right) \tag{4.6}$$

Where : A : 1, B : 2, C : 5, D : 6 and E : 7 are area of the peaks with figure 4.7.

^{1}H NMR analysis showed that perilla seed oil contains saturated, monounsaturated and polyunsaturated fatty acid (Table 4.5). The unsaturated fatty acids such as linolenic (polyunsaturated), linoleic (polyunsaturated) and oleic (monounsaturated) acid were found by GC to be 51%, 19.24% and 13.54% respectively, with 11.57% as saturated acid. And fatty acids such as linolenic, linoleic and oleic acid were found by equation to be 49.62%, 21.37% and 11.33% respectively, with 17.68% as saturated acid. The fatty acids analysis by GC is similar to the calculation of the equation derived from the analysis by ^{1}H NMR. There are significant differences in fatty acid compositions of perilla seed oil compared to the reported value (Table 4.5). The data show that linolenic acid is the prominent fatty acid in perilla seed oil.

Table 4.4 shows that fatty acid compositions of perilla seed oil from DME extraction compare with hexane extraction were analyzed by gas chromatography. The results showed that the fatty acid content obtained from both extraction methods were similar. Therefore, DME can using as solvent for extraction of perilla seed oil and obtained amount of fatty acid composition nearby of hexane extraction. DME is also not toxic to the body as compared to hexane.

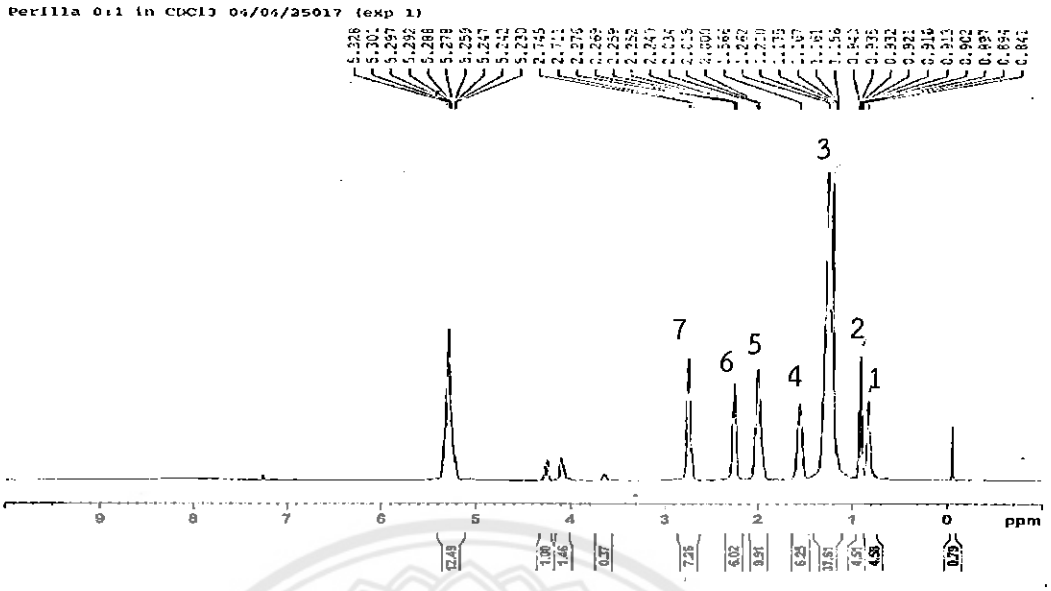

Figure 4.7 ^{1}H NMR of perilla seed oil.

Table 4.5 Fatty acid composition of perilla seed oil analysis by GC and equation

Fatty acid	Percentage		
	GC analysis (This work)	NMR analysis (This work)	GC analysis [17]
Unsaturated fatty acid	**84.02**	**82.32**	**93.01**
Linolenic acid	51.00	49.62	61.93
Linoleic acid	19.24	21.37	14.32
Cis-9-Oleic acid	13.54	11.33	16.65
Palmitoleic acid	0.21	-	0.11
Saturated fatty acid	**11.57**	**17.68**	**6.16**
Stearic acid	3.85	-	1.87
Palmitic acid	7.47	-	4.11
Arachidic acid	0.25	-	0.18

Table 4.6 Assignment of signals in the ^{1}H NMR spectra of perilla seed oil [22]

δ (ppm)	Assignment
0.841-0.94	$-CH_3$ (terminal methyl)
1.156-1.262	$-CH_2$ (saturated aliphatic chain)
1.56	$-CH_2-$ (acyl C-3, saturated chains)
2.000-2.034	$-C=C-CH_2-$ (allylic methylene)
2.240-2.276	$-CH_2-CO-$ (α-metbylene in ketone)
2.711-2.745	-C-C-H (terminal acetylene, nonconjugated)
5.230-5.328	H-C=C-H (Olefinic or cyclic; nonconjugated)

4.3.2 Fourier transform infrared spectroscopy (FT-IR) analysis of perilla seed oil.

Figure 4.8 shows the FT-IR spectra of perilla seed oil. The main peaks and their assignment to functional groups are given in table 4.7 and show the functional groups present in perilla seed oil are similar to in other vegetable oils [23-24].

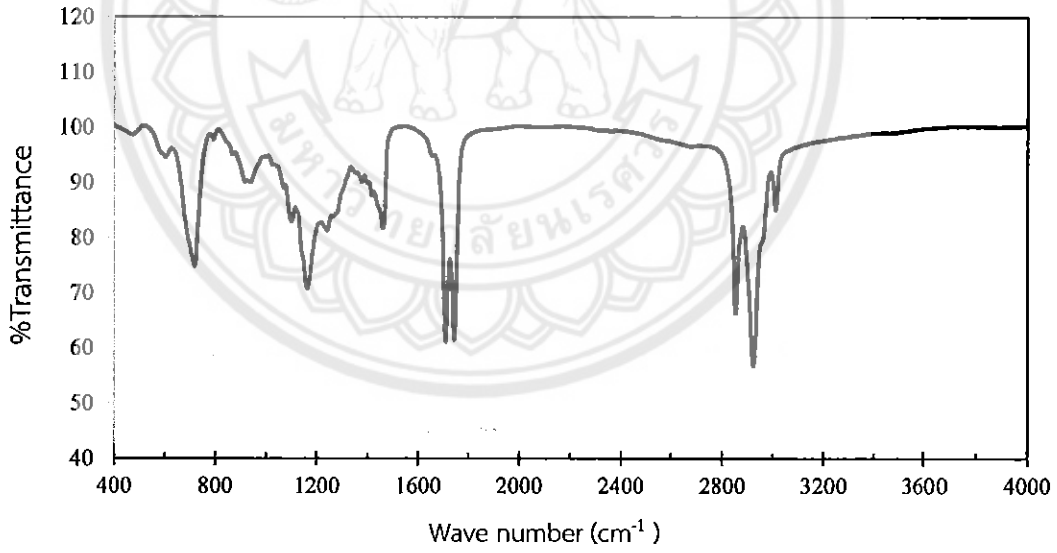

Figure 4.8 FT-IR spectra of perilla seed oil

Table 4.7 The main peak in the FT-IR spectra of perilla seed oil

Peak (cm^{-1})	Functional group
3011	=C-H stretching vibration (Alkanes)
2923, 2853	C-H stretching vibration (Alkanes)
1744,1709	C=O stretching vibration (Ester)
1167	C-O stretching vibration (Ester)
721	C-H group vibration (aliphatic)

CHAPTER V

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

5.1.1 Liquefied dimethyl ether shows the possibility to use as solvent for extraction of oil from perilla seed.

5.1.2 The optimum condition of perilla seed oil extraction from single parameter study included a moisture content of 14%, solvent to sample ratio of 5.5:1 and an temperature of 40 °C. Under these conditions, the maximum %recovery was 78.71%.

5.1.3 The optimum conditions of perilla seed oil from response surface methodology included a moisture content of 20.92%, solvent to sample ratio of 6.23:1 and an extraction temperature of 31.35 °C. Under these conditions, the maximum %recovery was 98.4%

5.1.4 The main fatty acid were linolenic (51%), linoleic (19.24%), and oleic (13.54%) acid. Due to perilla oil contained high levels of polyunsaturated fatty acids, perilla oil should have applications in the cosmetic, pharmaceutical, and food industries.

5.2 Recommendation

5.2.1 Vitamin E and antioxidant are important component in perilla seed oil. A studies on the properties of perilla seed oil in each experimental condition may have different levels of vitamin E and antioxidants. Therefore, extraction of vitamin E and antioxidant should be further studied.

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Appendix A

1. Calculate %recovery

Table 1A oil and %recovery of perilla seed oil in single parameter study

Run	Moisture content (%)	Solvent to sample ratio (w/w)	Temperature (°C)	Oil (g)	%recovery
1	7	3:1	40	2.11	42.37
2	14	3:1	40	2.72	54.62
3	21	3:1	40	2.66	53.41
4	14	3:3	40	2.72	54.62
5	14	4.5:1	40	3.53	70.88
6	14	5.5:1	40	3.92	78.71
7	14	6.23:1	40	3.82	76.71
8	14	5.5:1	31	2.68	53.82
9	14	5.5:1	40	3.92	78.71
10	14	5.5:1	49	3.43	68.88

From equation 3.2

$$\%\text{recovery} = \frac{\text{oil content extracted by DME}}{\text{oil content eatracted by soxhlet}} \times 100$$

$$\%\text{recovery} = \frac{2.11}{4.98} \times 100$$

$$= 42.37\ \%$$

2. Oil and %recovery of perilla seed oil in RSM

Table 2A Oil and %recovery of perilla seed oil in RSM

Run	Moisture content (%)	Solvent to sample ratio (w/w)	Temperature (°C)	Oil (g)	%recovery	
					Experimental data	Predicted data
1	10	3.5:1	35	2.59	52.01	46.68
2	18	3.5:1	35	2.95	59.24	63.76
3	10	5.5:1	35	2.63	52.81	57.08
4	18	5.5:1	35	3.98	79.92	83.50
5	10	3.5:1	45	2.96	59.44	55.88
6	18	3.5:1	45	3.34	67.07	62.8
7	10	5.5:1	45	3.27	65.66	61.16
8	18	5.5:1	45	3.59	72.09	77.42
9	7	4.5:1	40	1.82	36.55	41.25
10	21	4.5:1	40	3.781	75.92	70.43
11	14	2.77:1	40	2.26	45.38	50.09
12	14	6.23:1	40	3.82	76.71	71.73
13	14	4.5:1	31	3.81	76.51	72.75
14	14	4.5:1	49	3.5377	71.04	75.56
15	14	4.5:1	40	3.53	70.88	67.25
16	14	4.5:1	40	3.297	66.20	67.25
17	14	4.5:1	40	3.23	64.86	67.25

3. Calculate percentage of fatty acid

Where : A : 1, B : 2, C : 5, D : 6 and E : 7 are area of the peaks with figure 4.7.

Form equation 4.3-4.6

$$\text{Ln (\%)} = 100\left(\frac{B}{A+B}\right)$$

$$= 100\left(\frac{4.51}{4.58+4.51}\right)$$

$$= 49.62\%$$

$$L\ (\%) = 100\left[\frac{E}{D} + 2\left(\frac{B}{A+B}\right)\right]$$

$$= 100\left[\frac{7.26}{6.02} + 2\left(\frac{4.51}{4.58+4.51}\right)\right]$$

$$= 21.37\%$$

$$O\ (\%) = 100\left[\frac{C}{2D} - \frac{E}{D} + \left(\frac{B}{A+B}\right)\right]$$

$$= 100\left[\frac{9.91}{2(6.02)} - \frac{7.26}{6.02} + \left(\frac{4.51}{4.58+4.51}\right)\right]$$

$$= 11.33\%$$

$$S\ (\%) = 100\left(1 - \frac{C}{2D}\right)$$

$$= 100\left(1 - \frac{9.91}{2(6.02)}\right)$$

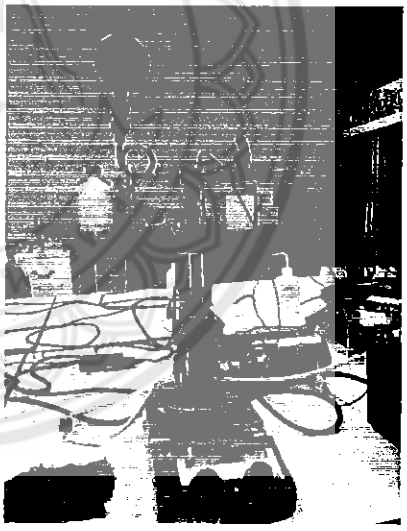
$$= 17.68\%$$

Appendix B

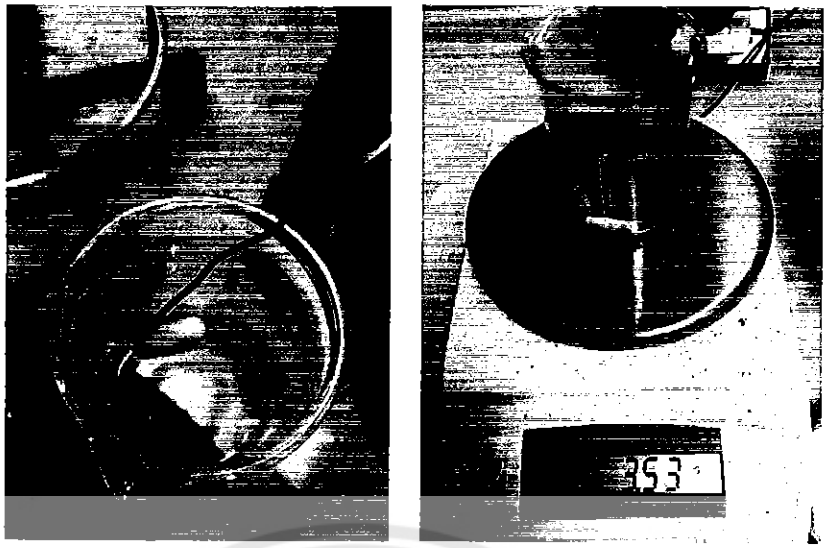
1. Extraction of perilla seed oil by DME

Dried perilla seed

Thimble (filter paper)



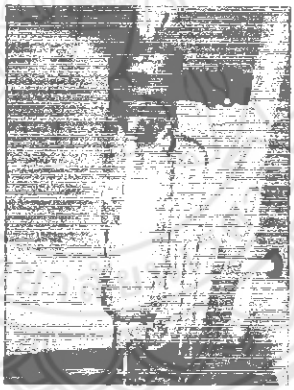
Reactor



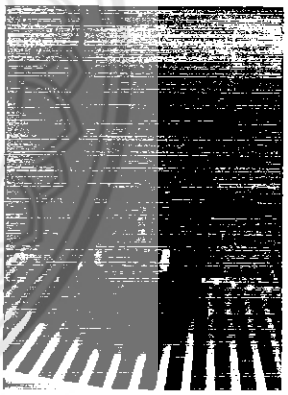
Perilla seed oil

2. Extraction of perilla seed oil by soxhlet

Soxhlet



condenser



perilla seed oil mixed with hexane

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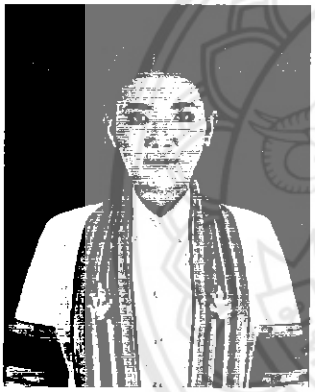

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