EXTRACTION OF SQUALENE FROM PALM OIL MESOCARP USING SUPERCRITICAL CARBON DIOXIDE

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EXTRACTION OF SQUALENE FROM PALM OIL MESOCARP USING SUPERCRITICAL CARBON DIOXIDE

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A dissertation submitted in the fulfillment of the
requirements for the award of the degree of
Master of Engineering (Chemical)

Faculty of Chemical Engineering
Universiti Teknologi Malaysia

JULY 2014

This dissertation is especially dedicated to my loved father, mother and elder sister, Chen Yu De, Shi Qin Fen and Chen Yan for their encouragement, continuous love as well as spiritual and material support. Thank you.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my supervisor and my coordinator associate professor Dr. Mohd Azizi Bin Che Yunus and Dr. Umi Aisah Bt Asli, for their encouragements and invaluable guidance. Without their patience and continued support, this dissertation would not be completed and presented here. Meanwhile, i also would like to thank the laboratory technicians, Mr. Mohd Latfi Bin Che Haron, whose assistance for this research. Certainly, I cannot forget the altruistic help from my senior sister, Lee Nian Yian. In the end, i would like to be grateful for the members of my family, who are my father, Chen Yu De, mother, Shi Qin Fen and my elder sister, Chen Yan. Without their patience, understanding as well as spiritual and material support, i cannot obtain the opportunity to continue my further study in UTM and the completion of this dissertation would not be possible presented.

ABSTRACT

An innovative technique of Supercritical Fluid Extraction (SFE) process was adopted for the extraction of squalene from palm oil mesocarp. SFE is the process of separating the extractant from components using supercritical fluids (SCF) with CO_2 as the extracting solvent. However, the use of SFE for industrial application is still unpopular and the references of SFE are serious shortage in present. In this study, there was a comparison experiment between SC-CO_2 extraction and Soxhlet extraction in order to investigate the better method to extract squalene. Meanwhile, for SC-CO_2 extraction, the task was investigated the influence of independent conditions of pressure, temperature and CO_2 flow rate on the extraction of squalene and these independent variables were selected as follows: temperature (45-75 ℃), pressure (16-30 MPa) and CO_2 flow rate (2-5 ml/min). Afterwards, the chemical compositions of palm oil were analyzed by gas chromatography-mass spectrometry (GC-MS). Furthermore, the conditions of SC-CO_2 extraction for squalene were optimized by response surface methodology (RSM) following Box-Behnken Design (BBD) and the result was analyzed by Design Expert software. In the end, the optimum conditions obtained from the investigation were pressure (16 MPa), temperature (45.01 ℃) and CO_2 flow rate (2 ml/min) and the yield of squalene was 0.506%. Besides, after compared the yield of squalene, the method of SC-CO_2 extraction was better than the conventional Soxhlet extraction.

ABSTRAK

Teknik inovasi Pengekstrakan Bendalir Lampau Tinggi (PBLG) telah digunakan untuk menyari squalena daripada sabut minyak sawit. PBLG adalah proses memisahkan bahan larut dari komponen menggunakan bendalir genting lampau dengan karbon dioksida sebagai pelarut. Walau bagaimanapun, penggunaan PBLG di industri masih tidak popular dan kekurangan rujukan PBLG pada masa kini. Dalam kajian ini, perbandingan antara pengekstrakan PBLG dan pengekstrakan Soxhlet akan dibandingkan untuk mencari kaedah terbaik di dalam menghasilkan squalena. Sementara itu, bagi pengekstrakan PBLG pembolehubah pengaruh tekanan, suhu dan kadar aliran karbon dioksida pada pengekstrakan squalene telah dikaji, dan nilai yang dipilih adalah seperti berikut: suhu (45-75 ℃), tekanan (16-30 MPa) dan kadar aliran karbon dioksida (2-5 ml / min). Selepas itu, komposisi kimia minyak sawit telah dianalisis dengan menggunakan gas kromatografi-spektrometri jisim (GK-SJ). Di samping itu, keadaan pengekstrakan SC-CO_2 untuk squalena telah dioptimumkan dengan kaedah Gerak Balas Permukaan (GBP) melalui rekabentuk Box-Behnken (RBB) dan hasilnya dianalisis dengan perisian *Design Expert*. Akhirnya, keadaan optimum diperolehi daripada pengajian ini adalah tekanan (16 MPa), suhu (45.01 ℃) dan kadar aliran karbon dioksida (2 ml/min) dan hasil squalene adalah 0.506%. Selain itu, merujuk kepada hasil squalena, kaedah pengekstrakan PBLG adalah lebih baik daripada pengekstrakan Soxhlet.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
BBD	-	Box Behnken Design
CH_3OH	-	Methanol
$CHCl_3$	-	Chloroform
C_3H_8O	-	Isopropanol
CO_2	-	Carbon Dioxide
CPO	-	Crude Palm Oil
DNA	-	Deoxyribonucleic Acid
ECD	-	Electrical Conductivity Detector
FCA	-	Food Control Act
FPD	-	Fission-Product Detection
FT-MS	-	Fourier Transform Mass Spectrometer
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-mass Spectrometry
GLC	-	Gas-Liquid Chromatography
GSL	-	Gas-Solid Chromatography
H_2O	-	Water
ICP-MS	-	Inductively Coupled Plasma Mass Spectrometer
LC-MS	-	Liquid Chromatography-Mass Spectrometry
MAE	-	Microwave Assisted Extraction
MALDI-TOFMS	-	Matrix Assisted Laser Desorption Time of Flight Mass Spectrometer
MS	-	Mass Spectrometry

NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate
NPD	-	Nitrogen Phosphorous Detector
Pc	-	Critical Pressure
PKO	-	Palm Kernel Oil
PMO	-	Palm Mesocarp Oil
P-T	-	Pressure-Temperature
RPM	-	Rotations per minute
RPO	-	Refined Palm Oil
RSM	-	Response Surface Methodology
SC-CO_2	-	Supercritical Carbon Dioxide
SCF	-	Supercritical Fluid
SFE	-	Supercritical Fluid Extraction
SI-MS	-	Secondary Ion Mass Spectrometer
SSDF-MS	-	Spark Source Double Focusing Mass Spectrometer
Tc	-	Critical Temperature
UE	-	Ultra-sonication Extraction

LIST OF SYMBOLS

℃	-	Celsius Degree
hr	-	Hour
g	-	Gram
%	-	Percentage
µm	-	Micrometer
µl	-	Microliter
ml	-	Milliliter
mm	-	Millimeter
min	-	Minute
ml/min	-	Milliliter per minute
m_1	-	Mass of dry empty vessel
m_2	-	Mass of the vessel, including the product residue
E	-	Mass of sample
F	-	Percentage of palm oil
MPa	-	Mega Pascal
X_1	-	Pressure
X_2	-	Temperature
X_3	-	CO_2 flow rate
Y_i	-	Percentage of compound i
A_i	-	Area corresponding to compound i
$\sum A_T$	-	Sum of the areas

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CHAPTER 1

INTRODUCTION

Malaysia, located in the Southeast Asia, is a country that leads the production of palm oil around the world and it has about 1.9 million hectares of plantations. The main function of oil palm trees is obtaining the product of the palm oil, which is made of palm fruits. Meanwhile, there are two oil-containing parts, which are palm mesocarp and palm kernel in the bunch of palm fruit as showed in Figure 1.1. Then, the process flows diagram of palm oil mesocarp is shown in Figure 1.2. However, the majority of palm oil is obtained from palm mesocarp, because the production of palm mesocarp is quit more than the yield of palm kernel, which is one of the reason that the price of palm kernel oil (PKO) much higher than the palm mesocarp oil (PMO).

The extraction of natural products from solid matters with supercritical fluids (SCF), mainly using CO_2 as the solvent, has been investigated as an alternative to conventional processes that use liquid organic solvents (Luiz, 1997). Because of the properties of steady, non-toxic, non-hazardous, nonpolluting and non-flammable for the extraction of natural products, carbon dioxide (CO_2) has been the prevalent supercritical fluid in the extraction industries. In addition, it is quite important that the price of CO_2 is cheaper than other solvents in the industries and it can be recycled many times. Taking into account the requirements of economy and environment, it is

(a)

(b)

Figure 1.1: (a) fruits of oil palm (b) palm mesocarp and palm kernel

desirable to explore alternative supercritical fluid extraction (SFE) solvents that enable operation at less intense conditions (Catchpole and Proells, 2001; Wood and Cooper, 2003), accordingly, allowing designers to exploit the typical SFE benefits at more reasonable costs (Perrut, 2000). Because of these characteristics and properties, the technology of SC-CO_2 extraction has become a prevalent technique for the recovery of pharmaceutical products and food additives from natural plants in the extraction industries.

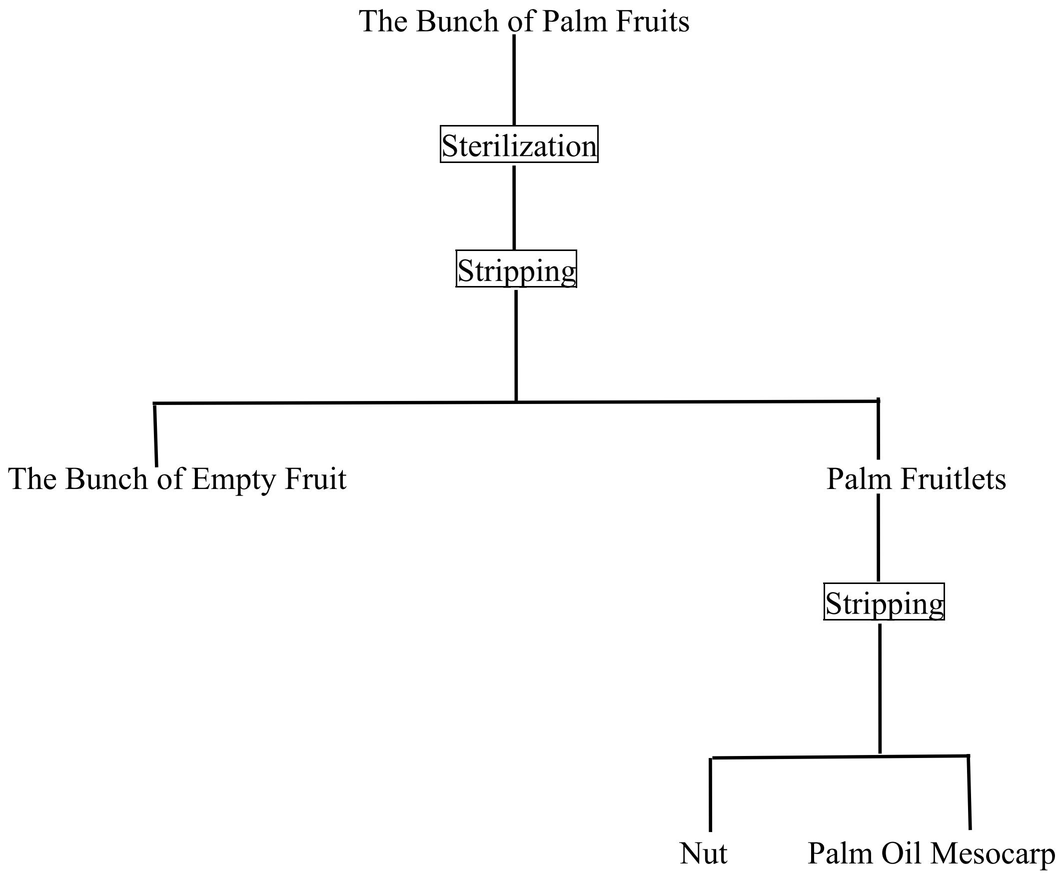

Figure 1.2: The process flow diagram of palm oil mesocarp

On the other hand, palm mesocarp is contained with the compound of squalene, which is a very important component in the palm oil and has substantial beneficial effects on the health of human, such as anti-cancer, anti-oxidation and reducing the serum cholesterol levels.

Therefore, in this study, extraction of palm oil from palm mesocarp using supercritical technology has been considered an advanced method to obtain squalene. In addition, although the technology of Soxhlet extraction is a conventional method, it has good extraction efficiency and the methodology of Soxhlet extraction only needs a little specialized training to have a possibility to obtain high sample yield. Therefore, currently, the method of Soxhlet extraction is still widely applied to many extraction fields. On the other hand, SC-CO_2 extraction as an emerging method, has been initially shown its predominant advantages and widely applied to many extraction fields. However, the production of products cannot meet the market requirement completely; therefore, the technology of SC-CO_2 extraction has broad prospects for development in the future.

1.1 Background of the Problem

As we all known that palm oil mesocarp contains affluent natural compounds, such as carotene, squalene, vitamin E and so on. These components have many unique and significant pharmacological effects, for example, β-carotene contains antioxidant activity and inhibiting growth of colon cancer cells (Di Mascio et al., 1991). β-carotene is an antioxidant with detoxification, which plays a significant role in many areas, such as antioxidant, anti-cancer, prevention of cardiovascular disease and cataracts; squalene has substantial beneficial effects on the health of human, such as anti-cancer, anti-oxidation, improving people's immune system response and reducing the serum cholesterol levels; vitamin E (e.g., tocotrienols) as antioxidant, anti-cancer and having hypercholesterolemic effects (Pearce et al., 1992; Salonen et al., 1985; Tomeo et al., 1995). Currently, majority of research reports of squalene are primary focused on the physiological activities and clinical applications. However, for the other fields, especially in the technology research of extraction and separation are quite rare and uncommon, which are the main reasons that scientists are interested in these research topics. In Malaysia, nevertheless, the use of SFE for industrial application is still unpopular even though several local researchers have studied the feasibility of supercritical fluid extraction especially on the palm oil industries (Azizi et al., 2007). Therefore, it has become quite imperative for the applications of new technologies and new methods of extracting squalene.

1.2 Problem Statement

In this study, the main purpose is to extract the maximal yield of squalene from palm oil mesocarp using SC-CO_2 extraction technology. On the issues of problem statements, we should focus on several points. The first one is that carbon dioxide is a good solvent to extract the required non-polar compounds from mixture materials. In another word, there is more challenge to extract the other polar compounds. Secondly, palm oil is solid status at the room temperature. It is very easily to block the tube of equipment of SFE, which will obstruct the process of extraction successfully. Thirdly, another one is that there are rare articles or journals for the materials of palm oil mesocarp to extract the squalene. Therefore, it is challenge to confirm the specific parameters for the experiments of extraction of squalene from palm oil mesocarp.

1.3 Research Objectives

The aims of this study are:

1) To study the influence of pressure, temperature and CO_2 flow rate on the oil yield and squalene compound

2) To determine the optimum conditions of SC-CO_2 and their interaction on the best oil yield and squalene compound

1.4 Scopes of Research

The extraction of palm oil yield and squalene in 60 minutes period from palm oil mesocarp using SC-CO_2. Therefore, in order to accomplish the purpose of the project, the scopes of the work should be as follows:

1) Sample preparation for extraction process. For example, particle size and make it slice formed.

2) Investigation of the effects on the independent conditions for the extraction of palm oil yield in 60 minutes period such as temperature, pressure and CO_2 flow rate and these independent variables were selected as follows: temperature (45-75 ℃), pressure (16-30 MPa) and CO_2 flow rate (2-5 ml/min).

3) Experimental work to analyse and determine the chemical compositions of palm oil and the percentage of squalene yield by the method of gas chromatography-mass spectrometry (GC-MS).

4) Experimental work to analyse the optimum conditions of SC-CO_2 extraction with response surface methodology (RSM) following the Box-Behnken Design (BBD) for the extraction of squalene yield and the data was analyzed by Design Expert software.

5) Comparison of the percentage of squalene yield between the conventional method of Soxhlet extraction and SC-CO_2 extraction according to the data obtained from experiments.

1.5 Significant of Study

In recent years, SFE technology gradually became a widespread separation technique in the extraction industries. It is increasingly favored by people because of its unique physical and chemical properties and high efficiency of extraction. Especially, the characteristics of extraction using supercritical CO_2 with non-toxic, inexpensive, harmless, no residue, nonpolluting, non-flammable and the extraction condition of low extraction temperature, it is more suitable for the separation and extraction in the food and medical industries. Therefore, in this study, the main contribution is that it has filled the gaps of extraction of squalene from palm oil mesocarp using SC-CO_2 and determined the optimum conditions for the technology of SFE to extract squalene from palm oil mesocarp. Furthermore, modern high-tech separation technology of SFE used in the extraction of squalene from palm oil mesocarp has provided a theoretical basis to explore further unknown fields.

1.6 Outline of Thesis

In this study, the thesis can be divided into five chapters. Chapter 1 is introduction, which main describes the topic, research objectives and the scopes of research. Then, chapter 2 is literature review, which used to provide the fundamental knowledge to support the research. Chapter 3 is methodology, which is the most significant part because the conditions of SC-CO_2 extraction for squalene were optimized by response surface methodology (RSM) following Box-Behnken Design (BBD) in this chapter. According to the data of chapter3, the result was analyzed by Design Expert software in chapter 4. In the end, chapter 5 is used to make a conclusion, which is that determination of the optimum conditions for SC-CO_2 extraction and the better method between soxhlet extraction and SC-CO_2 extraction.

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