UNIVERSITI PUTRA MALAYSIA

DRYING AND SOLID-LIQUID EXTRACTION OF HYDROXYCHAVICOL AND EUGENOL FROM BETEL LEAVES (*PIPER BETLE* L.)

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DRYING AND SOLID-LIQUID EXTRACTION OF HYDROXYCHAVICOL AND EUGENOL FROM BETEL LEAVES (*PIPER BETLE* L.)

By

PIN KAR YONG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Doctor of Philosophy

JUNE 2009

This thesis is specially dedicated to
my parents
and
my late aunty who passed away on 9th May 2008

Abstract of thesis presented to the Senate of Universiti of Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

DRYING AND SOLID-LIQUID EXTRACTION OF HYDROXYCHAVICOL AND EUGENOL FROM BETEL LEAVES (*PIPER BETLE* L.)

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JUNE 2009

Chairman: Associate Professor Luqman Chuah Abdullah, PhD

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Betel (*Piper betle* L.) is one of the invaluable medicinal plants originated from Malaysia. Its leaves have been used traditionally for various medication purposes. Scientific research on the leaf of this plant reveals that it possesses many beneficial bioactivities and its extract from betel leaves has a great potential to be used in developing commercial products. However, there is a lack of research on the processing aspects to produce its bioactive extract.

This research studied three key processes including drying, solid-liquid extraction, and freeze drying which are involved in processing of bioactive extract from betel leaves. Different experiments were designed and carried out to look into the effects of various operating parameters on the qualitative and quantitative aspects of betel leaves extract. Hydroxychavicol (HC) and eugenol (EU) were selected as the quality indicators of the product because these two compounds were reported to play an important role in the bioactivities of betel leaves including antioxidant, anti-inflammatory, and anticarcinogenic and antibacterial.

The effect of drying temperature on the quality of betel leaves and drying kinetics were studied in order to determine the optimum drying temperature. Changes in the concentration of HC and EU reveal that the optimum temperature for drying of betel leaves was 70^{o}C because degradation of HC and EU was observed above this temperature. Logarithmic model was found to be the most suitable model among the selected thin layer models in predicting the process.

Water was the most suitable solvent for extracting betel leaves compared to ethanol, ethyl acetate, and hexane. This was because it gave highest yield and the extract from water indicated high antioxidant and anti-inflammatory activities in which the activities were related to HC and EU. The optimum extraction temperature was determined as 60^{o}C to avoid degradation of EU. The ratio of water to solid of 30:1 (ml:g) was found to be optimum based on analysis of Response Surface Methodology (RSM). Extraction kinetics of betel leaves reveals that the optimum extraction time is one hour. A new model named equilibrium driven solid-liquid extraction (EDSLE) model was developed and successfully applied in describing the process.

The study of freeze drying process of betel leaves extract was conducted in two sections namely freezing and drying. The freezing kinetics data shows that the freezing point of betel leaves extract with 20%SC was about -4^{o}C. Prediction of freezing kinetics and freezing time was carried out successfully with numerical model. The results of drying kinetics of betel leaves extract show that the increase of drying temperature increased the drying rate. Midilli *et la*. model was found to be the most effective one among the selected models for modeling of the process.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doctor of Philosophy

PENGERINGAN DAN PENGEKSTRAKAN PEPEJAL-CECAIR BAGI *HYDROXYCHAVICOL* DAN *EUGENOL* DARIPADA DAUN SIRIH (*PIPER BETLE* L.)

Oleh

PIN KAR YONG

JUN 2009

Pengerusi: Profesor Madya Luqman Chuah Abdullah, PhD

Fakulti: Kejuruteraan

Sirih (*Piper betle* L.) merupakan salah satu tumbuhan ubatan bernilai yang berasal dari Malaysia. Daun sirih telah digunakan secara tradisional untuk pelbagai tujuan perubatan. Penyelidikan ke atas daun pokok ini menunjukkan ianya mempunyai banyak bioaktiviti yang bermanfaat dan ekstrak daripada daun sirih mempunyai potensi yang tinggi untuk digunakan dalam pembangunan produk. Tetapi, penyelidikan dalam aspek pemprosesan untuk menghasilkan ekstrak yang bioaktif ini adalah tidak mencukupi.

Penyelidikan ini merangkumi tiga proses utama termasuklah pengeringan, pengekstrakan pepejal-cecair, dan pengeringan beku yang terlibat dalam pemprosesan bioaktif ekstrak daripada daun sirih. Eksperimen berbeza telah direka dan dijalankan untuk mengkaji kesan pelbagai parameter terhadap aspek kualiti dan kuantiti bagi ekstrak daun sirih. *Hydroxychavicol* (HC) and *eugenol* (EU) dipilih sebagai petunjuk kualiti bagi produk kerana kedua-dua sebatian ini dilaporkan memainkan peranaan yang penting dalam bioaktiviti bagi daun sirih termasuklah anti-oksidan, anti-radang, anti-kanser dan anti-bakteria.

Kesan suhu pengeringan terhadap kualiti daun sirih dan kinetik pengeringan dikaji untuk menentukan suhu pengeringan yang optima. Perubahan kepekatan HC dan EU menunjukkan suhu pengeringan optimum adalah 70^{o}C kerana degradasi HC dan EU diperhatikan apabila melebihi suhu ini. Model *Logarithmic* adalah model yang paling sesuai antara model-model yang digunakan untuk peramalan proses itu.

Air merupakan pelarut yang paling sesuai untuk mengekstrak daun sirih berbanding dengan etanol, etil acetate, dan hexana. Ini kerana ia memberikan hasil yang paling tinggi dan ekstrak daripada air menunjukkan aktiviti anti-oksidan dan anti-radang yang tinggi dimana aktiviti itu berkaitan dengan HC dan EU. Suhu pengekstrakan yang optimum ditentukan sebagai 60^{o}C untuk mengelakkan degradasi EU. Nisbah air kepada pepejal yang bernilai 30: 1 (ml:g) didapati optimum berdasarkan analisa *Response Surface Methodology* (RSM). Kajian kinetik pengekstrakan daun sirih menunjukkan tempoh pengekstrakan yang optimum adalah satu jam. Satu model baru dinamakan model *equilibrium driven solid-liquid extraction* (EDSLE) telah dibangunkan dan digunakan dengan berjaya untuk menerangkan proses itu.

Pengkajian pengeringan beku dijalankan dalam dua bahagian termasuklah pembekuan dan pengeringan. Kinetik pembekuan menunjukkan takat beku bagi ekstrak daun sirih dengan kandungan pepejal 20% adalah -4^{o}C. Peramalan bagi kinetik pembekuan dan masa pembekuan dilakukan dengan berjaya dengan model *numerical*. Keputusan kinetik pengeringan menunjukkan penambahan suhu pengeringan dapat meningkatkan kadar pengeringan. Model *Midilli et al*. adalah model yang paling berkesan antara model-model yang digunakan untuk premodelan proses itu.

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Last but not least, I express my deepest gratitude to my parents and brother for their love, encouragement and support. Special thanks to my beloved girlfriend, Miss Teoh Hoe Peng for standing by me through thin and thick.

I certify that an Examination Committee has met on 11th June 2009 to conduct the final examination of Pin Kar Yong on his thesis entitled "Drying and Solid-liquid Extraction of Hydroxychavicol and Eugenol from Betel Leaves (*Piper betle* L.)" in accordance with Universities and Universities Colleges Act 1971and the Constitution of Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement of the degree of Doctor of Philosophy. The members of the Supervisor Committee were as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

PIN KAR YONG

Date: 5 August 2009

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATONS

σ	Coefficient in $D(C_s)$ relation
ρ	Density of the extract (kgm^{-3})
ω	Ice fraction (kg ice/ kg water)
ε	Internal porosity (m^3m^{-3})
α	Shape factor
σ	Specific surface area of the solid (m^2kg^{-1})
ϕ	Water content (kg water/ kg material)
ρ_d	Density of dry solid (kgm^{-3})
ρ_f	Density of frozen product (kgm^{-3})
ρ_i	Density of ice (kgm^{-3})
λ_n	Eigenvalues
ρ_p	Density of the solids (kgm^{-3})
Δt	Time step (s)
ρ_w	Density of water (kgm^{-3})
Δx	Grid size (m)
a	Thickness of slab (m)
A	Total surface area of the solids (m^2)
A'	Fitting parameter
b_o	Regression coefficient of intercept term
b_1	Linear regression coefficient
b_2	Linear regression coefficient
b_{11}	Squared regression coefficient
b_{22}	Squared regression coefficient

b_{12}	Interaction regression coefficient.
B'	Fitting parameter
Bi	Biot number
C'	Fitting parameter
C_e	Equilibrium solute concentration in liquid phase (gcm^{-3})
CH_3CN	Acetonitrile
C_L	Solute concentration in liquid phase (gcm^{-3})
$C_{L,exp}$	Solute concentration in liquid phase from experiment result (gcm^{-3})
$C_{L,pre}$	Predicted Solute concentration in liquid phase (gcm^{-3})
C_p	Specific heat capacity of the extract ($Jkg^{-1o}C^{-1}$)
$C_{p,fr}$	Specific heat capacity of frozen extract ($Jkg^{-1o}C^{-1}$)
$C_{p,un}$	Specific heat capacity of unfrozen extract ($Jkg^{-1o}C^{-1}$)
C_S	Solute concentration in solid phase (gcm^{-3})
d	Thickness of product (m)
D	Intraparticles diffusion coefficient (m^2s^{-1})
DDSLE	Diffusion driven solid-liquid extraction
D_e	Effective diffusivity coefficient (m^2s^{-1})
D_o	Preexponential factor of Arrhenius equation (m^2s^{-1})
E_0	Parameter of the Elovich equation
E_1	Parameter of the Elovich equation
E_a	Energy of activation ($Jmol^{-1}$)
EDSLE	Equilibrium driven solid-liquid extraction
EU	Eugenol
FDM	Finite difference method
FEM	Finite element method

FRIM	Forest Research Institute Malaysia
h	Heat transfer coefficient ($Wm^{-2}K^{-1}$)
H_3PO_4	Orthophosphoric Acid
HC	Hydroxychavicol
HPLC	High Performance Liquid Chromatography
i,j	Coordinate in the grid system
K	Mass transfer coefficient (cms^{-1})
k	Thermal conductivity of the extract ($Wm^{-1o}C^{-1}$)
K'	Second-order extraction rate constant
K_1	Peleg rate constant
K_2	Peleg capacity constant
k_d	Thermal conductivity of dry solid ($Wm^{-1o}C^{-1}$)
k_f	Thermal conductivity of frozen product ($Wm^{-1o}C^{-1}$)
k_{fr}	Thermal conductivity of frozen extract ($Wm^{-1o}C^{-1}$)
k_i	Thermal conductivity of ice ($Wm^{-1o}C^{-1}$)
k_{un}	Thermal conductivity of unfrozen extract ($Wm^{-1o}C^{-1}$)
k_w	Thermal conductivity of water ($Wm^{-1o}C^{-1}$)
L	Latent heat of freezing (kJ/kg)
M	Weight of total solid particles (g)
M_{db}	Moisture content (% dry basis)
M_e	Equilibrium moisture content (% wet basis)
M_o	Initial moisture content (% wet basis)
M_p	Weight of the solid particle (g)
MR	Dimensionless moisture ratio
MR_{exp}	Dimensionless moisture ratio from experiment result

MR_{pre}	Predicted dimensionless moisture ratio
M_{wb}	Moisture content (%wet basis)
P'	Plank's model constant
PAF	Platelet activating factor
Pk	Plank number
r	Distance form center axis (m)
R	Universal gas constant
R'	Plank's model constant
R^2	Correlation coefficient
RMSE	Root mean square error
RO	Reverse osmosis
R_p	Radius of solid particle (m)
R_{ss}	Ratio of solvent to solid (mlg^{-1})
S	Weight of solid (kg)
SC	Solid content (%)
SLE	Solid – liquid extraction
Ste	Stefan number
T	Temperature (K)
t	time (s)
T_f	Freezing temperature (K)
T_{a1}	Temperature of drying chamber (K)
T_{a2}	Temperature of thermal shelf (K)
T_{cr}	Freezing point (K)
T_{exp}	Temperature from experiment result (K)
T_{fin}	Final product temperature (K)

T_{fm}	Mean freezing temperature (K)
T_{in}	Initial product temperature (K)
TPA	Tetradecanoylphorbol acetate
T_{pre}	Predicted temperature (K)
V	Volume of product (cm^3)
V_e	Volume of aqueous extract used for freeze-drying (cm^3)
V_L	Volume of solvent (cm^3)
V_p	Volume of the solid (cm^3)
V_t	Volume of aqueous extract obtained after filtration (cm^3)
W	Weight of moisture within product (kg)
W_d	Weight of dried extract (g)
W_s	Weight of solid (g)
x	Distance form center axis (m)
X	Coded independent variable
x'	Independent variable
Y	Response of RSM

CHAPTER 1

INTRODUCTION

1.1 Herbal Industry in Malaysia

The development of the herbal industry in Malaysia is gaining its momentum due to its increasing economic demands. The current value of the local herbal market is RM 5 billion with an annual growth of 15 to 20 percent (Bernama, 2008). It is expected that the local market will achieve a staggering RM 8 billion by 2010. The global market is currently estimated to be US$ 200 billion with a growth rate between 10 and 20 percent per year and expected to hit US $5 trillion (RM 19 trillion) by 2050 (Bernama, 2007). This trend is attributed to the hiking awareness on the safety and environmental aspects of consumable products. Consumers are becoming more interested in the products from natural sources which are believed to cause lesser side effects.

Most of herbal products are produced by adding a certain amount of bioactive extract from medicinal plants. Bioactive extract is referred as extract that possesses therapeutic activities (WHO, 2000). There is a wide range of naturally-occurred phytochemicals found in bioactive extracts. These phytochemicals provide balanced and synergic effects which contribute to the beneficial properties of the extracts. This is the crucial part that is lacking in the single-compound synthetic drugs.

Nowadays, bioactive extracts are used in different types of products including herbal medicines, nutritional supplement, functional food and beverage, cosmetic products, health care products and so on. These products offer a more affordable alternative to modern pharmaceuticals. The number of herbal manufacturers in Malaysia is growing like mushroom after the rain due to the encouraging demand. There are many successful herbal product companies such as HPA Industries Sdn. Bhd., Felda Herbal Corporation, Sendayu Tinggi Corporation (M) Sdn. Bhd., Power Root (M) Sdn. Bhd., Nova Laboratories Sdn. Bhd. and so on. The Malaysian herbal manufacturers have commercialized several well-known herbal products formulated from various medicinal plants such as *Tongkat Ali* (*Eurycoma, lonngifolia*), *Kacip Fatimah* (*Labisia pumila*) and *Misai Kucing* (*Othorsiphon stiminues*) to meet the local demands. Table 1.1 listed some of the popular medicinal plants and their herbal products that are available in the local market. These products are widely accepted by local consumers.

Table 1.1. Popular medicinal plants in Malaysia

Local Name	Scientific Name	Product
Tongkat ali	*Eurycoma longifolia*	• Energy drinks • Health supplement
Pegaga	*Centella asiatica*	• Health supplement • Coffee
Kacip fatimah	*Labicia pumila*	• Herbal Tea • Coffee
Dukung anak	*Andrographis paniculata*	• Health supplement
Misai kucing	*Orthosiphon aristatus*	• Herbal Tea
Asam keping	*Garcinia atroviridis*	• Weight maintenance supplement • Herbal Tea
Mas cotek	*Ficus deltoidea*	• Herbal Tea

*Information above is gathered from Herbal Technology, FRIM and Chemical Engineering Pilot Plant, UTM.

It is reported that there are about 1200 species of medicinal plants found in our forest (Soepadma, 1992). There is a great possibility to develop and produce more beneficial herbal extracts that could be formulated into commercial products. This would ensure that we are able to share the economic benefits from the rapidly growing local as well as foreign market. Before that, we need to choose the right plant that has great potential and optimize the production process of its bioactive extract.

1.2 Herbal Processing

Herbal processing is part of a larger industry incorporating nutraceuticals, functional foods, nutritional supplements and herbal medicines (Ramlan *et al*., 2005). In Malaysia, most of the herbal researches mainly focus on the chemistry and biological activities of the medicinal plants while the study on herbal processing from the engineering aspects is relatively limited. In fact, the burgeoning knowledge of herbal processing is the important piece of the puzzle before the large scale production could be carried out. There are two institutions participate actively in research of herbal processing namely Chemical Engineering Pilot Plant (CEPP) of University Technology Malaysia (UTM) and Herbal Technology Center (HTC) of Forest Research Institute Malaysia (FRIM).

The processes involved in producing bioactive extract are shown in Figure 1.1. The key processes are drying, solid-liquid extraction and drying of extract (Ramlan *et al*., 2000). Drying is known as dehydration process which involves removal of moisture. Most of the drying techniques apply heat on the raw materials which might cause certain level of degradation or loss of quality. It is the phytochemicals that contribute to the beneficial bioactivity of medicinal plants. Thus, the major consideration in herbs drying is to shorten the drying time while preserve the active phytochemicals.