UNIVERSITI PUTRA MALAYSIA

EXTRACTION OF BIOACTIVE FLAVONOID COMPOUNDS FROM PECAH KACA (STROBILANTHES CRISPUS) USING SUPERCRITICAL CARBON DIOXIDE

LIZA BT MD SALLEH

EXTRACTION OF BIOACTIVE FLAVONOID COMPOUNDS FROM PECAH KACA (STROBILANTHES CRISPUS) USING SUPERCRITICAL CARBON DIOXIDE

By

LIZA BT MD SALLEH

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

February 2009

DEDICATION

To my dear husband, Izadin Tendot bin Saleh for his support and affectionate caring each moment in my life, especially throughout my study, specially dedicated to my kids Nurizzati Syaheerah, Muhammad Ezad Hakimi and Nur Batrisyia Maisarah, my late father Haji Md Salleh Bin Samad and my mother, Hajah Khadijah Bt Abd Manap for her spiritual support and doa, 'terimakasih mak', that I owe them each moment of my life.

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

EXTRACTION OF BIOACTIVE FLAVONOID COMPOUNDS FROM PECAH KACA (STROBILANTHES CRISPUS) USING SUPERCRITICAL CARBON DIOXIDE

By

LIZA BT MD SALLEH

February 2010

Chairman : Professor Russly Bin Abdul Rahman, PhD

Faculty : Engineering

Due to increasingly stringent environmental regulations, supercritical fluid extraction (SFE) has gained wide acceptance in recent years as an alternative to conventional solvent extraction for separation of organic compounds in many analytical and industrial processes. Consequently, supercritical fluid extraction (SFE) as an environmentally responsible and efficient extraction technique for solid materials was introduced and extensively studied for separation of active compounds from herbs and other plants. Supercritical carbon dioxide (SC-CO_2) is one of widely used SCF. This is because of its mild critical conditions,nontoxic, non explosive, readily available and easily removable from the products.

The extraction study of bioactive flavonoid using liquid organic solvents was carried out using the standard soxhlet method. From this study it found that ethanol was the best solvent for extraction of bioactive flavonoid compounds, in terms of high extract and component yield. Analysis of the bioactive flavonoid compounds

extractable from *S. crispus* was accomplished by High Performance Liquid Chromatography (HPLC).

The first part of this study investigated the effects of pressure, temperature and dynamic extraction time as dependent variables of the SC-CO_2 extraction of bioactive flavonoid compounds. A full factorial in complete randomized design (CRD) was applied to obtain the best extraction conditions and mean value (*k*) between levels was calculated for different extraction conditions. From the *k* value it can be concluded that pressure (with *k* value of 11.79) had a dominant effect on the extraction yield after which followed by temperature (with *k* value of 10.85) and dynamic time (with *k* value of 1.53).

Based on a previous study it was found that pressure and temperature were the most important conditions in the SC-CO_2 extraction, therefore for the optimization of the extraction conditions, pressure and temperature with additional co-solvent (ethanol) flow rate were selected as the extraction variables. The Box Behnken Design (BBD) based on three factors and three levels was employed to obtain the optimum condition for SC-CO_2. Result showed that the optimum conditions were pressure at 200 bar, temperature at 50°C and co-solvent flow rate of 5 g/min respectively. Statistically, the yield was significantly affected by pressure and co-solvent flow-rate ($p<0.05$), however no significant showing was found for temperature.

The mathematical model was developed in order to estimate the correlation between experimental data and theoretical prediction for different parameters of SC-CO_2 namely CO_2 flow rate, pressure and temperature. The best extraction was obtained at

CO_2 flow rate of 15 g/min, pressure of 200 bar and temperature of 50°C. Under this condition, 6.87% of initial extraction yield of flavonoid compounds obtained in the leaves was extracted. The correlation between external mass transfer coefficient and the physical properties of supercritical carbon dioxide-ethanol mixture and leaves particles in term of dimensionless number was $Sh = 2.368\ Re^{0.271}\ Sc^{0.33}$, which generates AARD of 2.67%. In general, the desorption model and Sovova model is better than adsorption equilibrium model for the estimation of extraction profile.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGEKSTRAKAN KOMPAUN BIOAKTIF FLAVONOID DARI PECAH KACA (STROBILANTHES CRISPUS) MENGGUNAKAN BENDALIR LAMPAU GENTING KARBON DIOKSIDA

Oleh

LIZA MD SALLEH

Februari 2010

Pengerusi : Professor Russly Abdul Rahman, PhD

Fakulti : Kejuruteraan

Memandangkan terdapat peningkatan undang-undang alam sekitar yang ketat terhadap kaedah pengekstrakan, maka pada masa ini kaedah pengekstrakan bendalir lampau genting (SFE) telah mendapat sambutan yang menggalakan. Ia merupakan kaedah pengekstrakan alternatif selain dari pengekstrakan pelarut bagi memisahkan kompoun-kompoun organic samaada untuk proses analisis atau industri.

Oleh yang demikian, kaedah pengekstarakan SFE yang mesra alam dengan kaedah pengekstrakan berkesan ini telah digunapakai secara meluasnya dalam kajian bagi memisahkan kompaun-kompaun aktif didalam bahan pejal seperti herba dan tumbuh-tumbuhan. Bendalir lampau genting-karbon dioksida telah digunakan secara meluas sebagai bendalir lampau genting. Ini aadalah kerana sifat pada titik gentingnya yang sempurna, tidak beracun, tidak mudah terbakar dan sangat ekonomi. Kajian pengekstrakan komponen bioaktif flavonoid menggunakan pelarut organic dilakukan dengan kaedah pengekstrakan soxhlet.Daripada kajian ini didapati

ethanol merupakan pelarut yang terbaik bagi pengekstrakan diukur dari segi hasil dan jumlah kompaun terekstrak. Bagi mengenalpasti kompoun-kompoun terekstrak, penganalisaan adalah menggunakan kaedah kromatografi cecair berprestasi tinggi (HPLC).

Bahagian pertama kajian ini adalah mengkaji kesan tekanan, suhu dan masa pengekstrakan sebagai parameter bebas didalam SC-CO_2. 'Full Factorial Design' dengan Serakan Lengkap Design (CRD) telah digunakan bagi mendapatkan kondisi pengekstrakan terbaik. Nilai purata (K) diantara aras telah dikira bagi setiap kondisi pengekstrakan. Daripada nilai K, boleh disimpulkan bahawa tekanan (nilai K adalah 11.79) memberikan kesan yang paling berpengaruh terhadap hasil pengekstrakan, dan diikuti oleh suhu (nilai K adalah 10.85) dan masa pengekstrakan (nilai K adalah 1.53).

Berdasarkan keputusan kajian bahagian pertama, didapati bahawa tekanan dan suhu merupakan factor penting di dalam pengekstrakan SC-CO_2. Oleh yang demikian bagi kajian kondisi proses pengoptimuman, kedua-dua faktor tekan dan suhu diambilkira dan dengan penambahan kadar alir co-solvent ethanol. Reka bentuk Box Behnken (BBD) berdasarkan 3 faktor dan 3 aras telah digunakan untuk mendapatkan kondisi optimum bagi SC-CO_2. Keputusan menunjukan keadaan pengekstrakan optimum dicapai pada tekanan 200 bar, suhu 50°C dan kadar alir co-solvent adalah 50 g/min. Secara statistik, didapati bahawa hasil ekstrak adalah sangat dipengaruhi oleh tekanan dan kadar alir co-solvent ($P< 0.05$).

Model matematik dibangunkan bagi melihat perkaitan diaantara data eksperimen dengan penilaian secara teori. Oleh yang demikian tiga kaedah permodelan yang digunakan iaitu adsorption, desorption dan Sovova. Pengekstrakan terbaik adalah pada kondisi kadar alir carbon dioxide 15 g/min, tekanan 200 bar dan suhu 50°C dengan hasil 6.87%. Perkaitan diantara pekali pindah jisim luaran dengan sifat fizikal camouran CO2-ethanol bagi daun tersebut dalam sebutan nombor sherwood adalah, $Sh = 2.368\ Re^{0.271}\ Sc^{0.33}$. Secara umumnya model desorption dan sovova adalah sesuai untuk mengaanggarkan profil pengekstrakan.

ACKNOWLEDGEMENTS

First of all I would like to express my utmost thanks and gratitude to Almighty Allah s.w.t, the Sustainer, the most Gracious and most Merciful; without Whom will no one can achieve anything. My salawat and salam is addressed to His righteous messenger, prophet Muhammad s.a.w.

I would like to express my deepest gratitude to my supervisor, Prof Dr. Russly Bin Abdul Rahman for the encouragement and endless guidance I have received from him, during completion of this dissertation. I would like also to express my sincere gratitude to my supervisory committee members, Prof Dr. Jinap Selamat and Prof Dr Asmah Rahmat for their thoughtful comments and support throughout this study. I am also grateful to Universiti Putra Malaysia for providing the necessary funding for this research under the Research University Grant Scheme (RUGS). Initiative two, accorded to my supervisor, Prof Russly Abd Rahman.

I would like also to thank my post graduate friends Sabri, Mandana, Asep, Ali, Shikin, Chong and others in the Department for their company over the last four years which, I valued so much. I am also very grateful to the helpful staffs of the Department of Process and Food Engineering Mr Mohd Noh, Tuan Hj Kamaruzaman, Mr Zahid and Puan Siti and to the staffs of the Faculty of Food Science and Technology, Mr Azman and Mr Halim for helping me throughout my study. Last but not least, I wish to express my gratitude to Dr Masturah Markom from Universiti Kebangsaan Malaysia and Puan Roslina Rosli from Universiti

Teknologi Mara for their kind assistant in the part of developing model for this study.

Liza Bt Md Salleh

December 2009

I certify that a Thesis Examination Committee has met on 22 February 2010 to conduct the final examination of **Liza Bt Md Salleh** on her Phd thesis entitled "**Extraction of Bioactive Flavonoid Compounds from *Pecah Kaca* (*Strobilanthes crispus*) using Supercritical Carbon Dioxide**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the candidate be awarded the Doctor of Philosophy.

Members of Examination Committee as follows:

Mohd Ali Hassan, PhD
Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Ling Tau Chuan, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Thomas Choong Shean Yaw, PhD
Associate Professor/Ir
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Rosalam b. Hj Sarbatly, PhD
Associate Professor
School of Engineering and Information Technology
Universiti Malaysia Sabah
(External Examiner)

BUJANG BIN KIM HUAT, PhD
Professor/Deputy Dean
School of graduate Studies
Universiti Putra Malaysia

Date: 24 June 2010

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for degree of Doctor of Philosophy. The members of Supervisory Committee were as follows:

Russly Abdul Rahman, PhD
Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Jinap Selamat, PhD
Professor
Faculty of Science and Food Technology
Universiti Putra Malaysia
(Member)

Asmah Rahmat, PhD
Professor
Faculty of Medical and Health Sciences
Universiti Putra Malaysia
(Member)

_____
HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 15 July 2010

DECLARATION

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

_____

LIZA BT MD SALLEH

Date:

TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iii
ABSTRAK		vii
ACKNOWLEDGEMENTS		ix
APPROVAL		x
DECLARATION		xi
LIST OF TABLES		xvii
LIST OF FIGURES		xix
LIST OF ABBREVIATIONS		xxi
CHAPTER		
1	**GENERAL INTRODUCTION**	
	1.1 General Introduction	1
	1.2 Supercritical Fluid Extraction (SFE) Technology	3
	1.3 Problem Background	5
	1.4 Hypothesis	7
	1.5 Research Objectives	7
	1.6 Scope of Work	8
	1.7 Organization of Thesis	8
2	**LITERATURE REVIEW**	
	2.1 Antioxidants	12
	2.1.1 Synthetic Antioxidant	13
	2.1.2 Natural Antioxidant	14
	2.1.3 Types of Antoxidants	15
	2.1.4 Free Radical	16
	2.1.5 Free Radicals Formation	17
	2.2 Flavonoids	18
	2.3 *Strobilanthes crispus* (Pecah Kaca)	24
	2.3.1The Uses of *Strobilanthes crispus*	26
	2.4 Application of Extraction Method	27
	2.4.1 Extraction Method	28
	2.5 Conventional Soxhlet Extraction	30
	2.5.1 Conventional Soxhlet Extraction Conditions	31
	2.5.2 Advantages and Disadvantages of Conventional Soxhlet Extraction	33
	2.5.3 Envisioned Uses of Soxhlet Extraction	34
	2.6 Supercritical Fluid Extraction	34
	2.6.1 Properties of Supercritical Fluid	36
	2.6.2 Principles and Mechanisms	42
	2.7 Supercritical Fluid Extraction Conditions	44
	2.7.1 Extraction time	45

2.7.2 Sample Particle Size 48
2.7.3 Co-solvent or Modifier 49
2.7.4 Solvent Flow Rate 52
2.7.5 Pressure 55
2.7.6 Temperature 56
2.7.7 Solubility of Solute 58
2.8 Advantages and Disadvantages of Supercritical Fluid Extraction 60
2.9 Response Surface Methodology (RSM) 62
2.10 Mass Transfer Mechanisms for Developed Model in Supercritical Fluid 64
2.11 Modeling on Supercritical Fluid Extraction 66
2.11.1 Empirical and Semi Empirical Model 67
2.11.2 Theoretical Model 69

3 MODELLING OF BIOACTIVE FLAVONOID COMPOUNDS FROM PECAH KACA (*S. crispus*) USING SUPERCRITICAL CARBON DIOXIDE (SC-CO_2)
3.1 Introduction 73
3.2 Modeling of Supercritical Fluid Extraction with Linear Adsorption Equilibrium 73
3.3 Modeling of Supercritical Fluid Extraction with Integral Desorption using Single Parameter 83
3.3.1 Effect of Temperature and Supercritical Fluid Density on Desorption Rate Constant 85
3.4 Sovova's Extended Lack's Model (SLM) 86
3.5 Error Analysis 90
3.6 Development of External Mass Transfer Coefficient Correlation 91

4 MATERIALS AND METHODS
4.1 Introduction 96
4.2 Materials and Reagents 96
4.3 Methods 97
4.3.1 Measurement of Real, Bulk Density and the Thickness of Leaves 97
4.3.2 Supercritical Fluid Extraction (SFE) 102
4.3.3 Classical Soxhlet Extraction (CSE) 102
4.4 Determination of Extraction Yield 102
4.5 Solubility Analysis 103
4.6 High Performance Liquid Chromatography (HPLC) Analysis 104
4.7 Experimental design by Response Surface Methodology (RSM) 105
4.8 Statistical Analysis 107

5 RESULTS AND DISCUSSION

5.1 The Physical Properties of *S. crispus* Leaves 108
5.2 Conventional Soxhlet Extraction Method on Separation of Bioactive Flavonoid Compounds 109
5.2.1 Introduction
5.2.2 Effect of Different Solvents Used on the Extraction Yield 110
5.2.3 Effect of Different Solvents Used on the Extracted Compounds 113
5.3 Preliminary Study of Supercritical Carbon Dioxide ($SC\text{-}CO_2$) Extraction Condition on Yield and Bioactive Flavonoids Compound from Pecah Kaca (*S. crispus*)
5.3.1 Introduction 115
5.3.2 Experimental conditions 116
5.3.3 Effect of Pressure on Extraction Yield 119
5.3.4 Effect of Temperature on Extraction Yield 121
5.3.5 Effect of Dynamic Extraction Time on the Extraction Yield 123
5.36 Identification and Quantification of the Extracted Compound 124
5.4 Optimization Using Response Surface Methodology
5.4.1 Model Fitting 127
5.4.2 Analysis of Response Surface 130
5.4.3 Optimization 137
5.4.4 Model Validation and Confirmation 139
5.4.5 Identification and Quantification of Extracted Compounds 140
5.5 Prediction of Mathematical Model of Bioactive Flavonoid Compounds From Pecah Kaca (*S. crispus*) Using Supercritical Carbon Dioxide ($SC\text{-}CO_2$)
5.5.1 Effect of Carbon Dioxide Flow Rate 143
5.5.2 Effect of Pressure 153
5.5.3 Effect of Temperature 159
5.5.4 Mass Transfer Correlation 168
5.6 Effect of Pressure and Temperature on the Solubility Performance 170
5.6.1 Solubility Behavior as a Function of Pressure and Pressure 171

6 CONCLUSION AND RECOMMENDATION

6.1 Conclusion 174
6.2 Recommendation 176

REFERENCES 179
APPENDICES 194
BIODATA OF STUDENT 221
AWARDS AND PUBLICATIONS 220