



**UNIVERSITI PUTRA MALAYSIA**

**SUPERCRITICAL FLUID EXTRACTION OF MAJOR BIOACTIVE  
FLAVONOIDS FROM SPEARMINT (*Mentha spicata* L.) LEAVES**

**MANDANA BIMAKR**

**FK 2009 59**



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FLAVONOIDS FROM SPEARMINT (*Mentha spicata L.*) LEAVES**

**BY**

**MANDANA BIMAKR**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the requirement for the Master of science**

**February 2009**



**ESPECIALLY DEDICATED TO MY BELOVED FAMILY**



Abstract of thesis presented to University Putra Malaysia in fulfilment of the requirement for the degree of Master

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**February 2009**

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Supercritical fluid extraction (SFE) is an attractive alternative technique to conventional liquid extraction due to its several distinct properties. This novel interesting extraction method which was developed in 1960 is an energy efficient, economically viable and environmentally friendly process. This study evaluated the effect of supercritical carbon dioxide extraction on the extraction yields and major bioactive flavonoid compounds from the herbal matrices. Two basic extraction methods were investigated: conventional soxhlet extraction (CSE) and supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction. High-Performance Liquid Chromatography (HPLC) was used to identify and quantify bioactive flavonoid compounds of produced extracts. Results obtained from the two extraction methods were compared for a higher extraction yield and concentration of flavonoid compounds. In the study of supercritical carbon dioxide extraction and conventional soxhlet extraction spearmint (*Mentha spicata L.*) leaves were selected. For optimizing of SC-CO<sub>2</sub>



extraction process three most important variables including temperature, pressure and extraction dynamic time have been studied. The full factorial in complete randomize design (CRD) based on three levels and three factors was employed to obtain the optimum condition for SFE. Based on the simultaneous optimization of crude extract yield and concentration of flavonoid compounds the optimum condition was found at temperature of 60 °C, pressure of 200 bar and extraction dynamic time of 60 min. In conventional soxhlet extraction study, different solvents were used to evaluate the effect of different applied solvents on the extraction yield and major bioactive flavonoid compounds. Ethanol: water (70:30) was found as a preferable solvent among the other applied solvents due to its higher extraction yield, flavonoid compounds concentration and lower toxicity effects. Compared with supercritical carbon dioxide extraction the higher concentration of bioactive flavonoid compounds was obtained and extraction time was reduced by applying SC-CO<sub>2</sub> extraction. However, the higher crude extract yields were obtained by using conventional soxhlet extraction. The influence of co-solvent (modifier) on the extraction yield and extracted flavonoid compounds from spearmint (*Mentha spicata* L.) leaves was also studied. In this study ethanol acted as co-solvent to improve the efficiency of polar compounds (flavonoids) extraction. Co-solvent flow rate of 6 g/min was found as a preferable modifier flow rate to obtain higher extraction yield and bioactive flavonoid compounds concentration.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master

**EKSTRAKSI SUPERKRITIKAL CECAIR BIOAKTIF FLAVONOID  
MAJOR DARI DAUN PUDINA (*Mentha spicata L.*)**

Oleh

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Ekstraksi Cecair Superkritikal merupakan sebuah teknik alternatif yang menarik berbanding teknik konvensional ekstraksi cecair kerana beberapa kelebihan. Kaedah pengekstrakan yang baru ini telah diperkenalkan pada tahun 1960 dan ia merupakan sebuah teknik yang dapat menjimatkan tenaga, mengoptimumkan keuntungan dan mesra alam. Kajian ini menilai kesan ekstraksi Superkritikal Karbon Dioksida terhadap hasil ekstraksi dan kompaun utama flavanoid daripada tumbuhan herba. Dua kaedah ekstraksi iaitu kaedah konvensional ekstraksi Soxhlet (CSE) dan Ekstraksi Superkritikal Karbon Dioksida (SC-CO<sub>2</sub>) digunakan untuk mengekstrak daun pudina (*Mentha spicata L.*). Kromatografi Cecair Berprestasi Tinggi (HPLC) digunakan untuk mengenalpasti komponen flavanoid bioaktif dari hasil ekstraksi. Tiga parameter yang memainkan peranan penting dalam SC-CO<sub>2</sub> iaitu suhu, tekanan dan masa dinamik ekstraksi telah dikaji. Kaedah faktorial penuh dalam kaedah perawakan lengkap berdasarkan tiga peringkat dan tiga faktor telah dilakukan untuk mendapatkan nilai yang optimum untuk SFE. Berdasarkan pengoptimuman serentak

terhadap hasil ekstraksi dan kepekatan komponen flavanoid, keadaan optimum dapat dicapai pada suhu 60 °C, tekanan 200bar dan masa dinamik ekstraksi 60 menit. Dalam kaedah ekstraksi konvensional, kesan jenis pelarut yang digunakan telah diselidik. Pelarut yang terdiri dari etanol dan air (70:30) merupakan pelarut yang terbaik berdasarkan hasil ekstraksi dan kepekatan komponen flavanoid yang lebih tinggi, disamping kesan toksik yang rendah. Teknik SC-CO<sub>2</sub> menghasilkan kepekatan komponen flavanoid yang lebih tinggi dan masa ekstraksi yang lebih rendah berbanding teknik konvensional. Walau bagaimanapun, kaedah konvensional menghasilkan hasil ekstraksi yang lebih tinggi. Penggunaan pelarut bersama dalam kaedah konvensional turut meningkatkan kepekatan komponen flavanoid yang diekstrak.

## AKNOWLEDGEMENTS

I pray to Almighty ALLAH Subhanahu wa Ta'ala who give me the thoughts, the will, and guided me to complete this work. I pray that ALLAH will bless this work and make it useful for mankind, and that He will forgive us.

My sincere and deepest gratitude to Professor Dr Russly Abdul Rahman, the chairman of my supervisory committee for his guidance, encouragement, patience and continuous follow up during the course of this study. My appreciation and gratitude is also extended to members of my supervisory committee, Dr. Farah Saleena Bt. Taip and Dr. Ling Tau Chuan for their advice, punctuate comments and support.

My gratitude is also due to all the staff of the Department of Food Technology, and Faculty of Food Science and Technology, UPM for their cooperation. My special appreciation is extended to my friend Mrs. Liza Md Salleh for her kind help and friendly attitude. I would like to acknowledge the financial support received from the RMC, the Universiti Putra Malaysia for this project.

I would also like to give my thanks to my husband, Ali Ganjloo who brightens my life with his support, encouragement, sacrifice and patience.

Finally, I must express my deepest gratitude to my parents continuously encouraged me and presented me the most beautiful World.







This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master. The members of Supervisory 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.

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Mandana Bimakr

Date: 27 April 2009

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BHA	Butylated- Hydroxyl-Anisole
BHT	Butylated-Hydroxy-Toluene
CO <sub>2</sub>	Carbon dioxide
cm	Centimeter
CRD	Complete Randomize Design
EtOH	Ethanol
g	Gram
h	Hour
GC-MS	Gas Chromatograph Mass Spectrometry
HCL	Hydrochloride acid
HPLC	High Performance Liquid Chromatography
kg	Kilogram
kHz	KiloHertz
LSD	Least Significant Difference
M	Molar
MeOH	Methanol
min	Minute
mg	Milligram
ml	Milliliter
mm	Millimeter
nm	Nanometer
°C	Degree centigrade

R <sup>2</sup> s	Coefficient of Determination Second
SE	Soxhlet Extraction
SFE	Supercritical Fluid Extraction
SC-CO <sub>2</sub>	Supercritical Carbon dioxide
TBHQ	Tertiary-Butyl Hydro-Quinone
TFA	Tri-Flouro-Acetic acid
μm	Micrometer
μl	Microliter
UV	Ultra Violet



## CHAPTER I

### GENERAL INTRODUCTION

Undesirable changes in food quality due to oxidation reactions can be prevented by applying antioxidant compounds in to its formulation. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) should be replaced by natural compounds due to their possible toxicity (Namiki, 1990; Pokorny, 1991). By considering adverse effects of synthetic antioxidant on human health, alternative natural and safe sources of food antioxidant should be identified (Wanasundara and Shahidi, 1998; Goli et al., 2005). Plant extracts due to possess similar or even higher antioxidant activity can be natural alternatives to synthetic antioxidants, so they are strongly of interest in the food industry (Le Floch et al., 1998).

Polyphenols such as flavonoid compounds are one of the most used groups of biological systems and have been extensively used for decades as food additives due to their well-known abilities to scavenge free radicals (i.e., antioxidant power). Flavonoids, abundant in fruits, vegetables, teas, medicinal plants, are a kind of highly effective antioxidant and less toxic than synthetic antioxidants, such as BHA, BHT (Liu and Zhu, 2007). Cardiovascular disease, cancer, inflammatory disorders, neurological degeneration can be protected by consuming these bioactive compounds in diary diets. Flavonoids are categorized as flavonol (such as kaempferol), flavanol (such as catechin and (-)- epicatechin), flavonone (such as naringenin), flavones (such as apigenin and rutin), anthocyanidin and isoflavone.



Bioactive flavonoid compounds due to their complicated chemical structure have not been studied completely (Wach et al., 2007). Therefore, in this study bioactive flavonoid compounds was selected as target compounds.

It was demonstrated that *Labiatae* family herbs such as thymus, rosemary, sage and cloves are source of antioxidants (Nguyen. 1991; Yopez et al., 2002). For example, some antiradical activity in aqueous and methanolic extracts of oregano leaves were studied by Cervato et al. (2000), Bendini et al. (2002) reported that ethanolic extracts under selected conditions showed antioxidant activity (Cervato et al., 2000; Bendini et al., 2002 ). It has been reported that antioxidant compounds can be extracted by traditional extraction methods like steam distillation and solid–liquid extraction with the use of different solvents such as methanol, ethanol and acetone (Diaz-Maroto et al., 2002).

The concentration of active compounds in herbal plants usually is low, so a wide variety of research has been done to develop more effective and selective extraction methods such as supercritical fluid extraction (SFE) to extract these compounds from the herbal matrices (Lang and Wai, 2001).

Recently, a great deal of study has been done to use supercritical fluid extraction (SFE) with carbon dioxide (CO<sub>2</sub>) as a solvent for extraction of natural compounds from different raw materials. The supercritical fluid extraction region of a pure compound is defined as the region where the temperature and pressure are higher than its critical values. The special note of this process for selective extraction of soluble compounds from a raw material is usage of gases above their critical points

(Ibanez et al., 1999; Baysal, 2000; Diaz-Maroto, 2002; Cavero, 2006). During past decades, one of the most important application areas for SFE was extraction of active natural products from herbal, or more generally, from plant materials. Nowadays, SFE is as an acceptable alternative extraction technique to solid-liquid extraction methods (McHugh, 1994; Luque de Castro, 1994; Lang, 2001). The technique is less energy intensive than distillation and liquid extractions and is particularly suitable for thermo-sensitive materials which make it attractive for the extraction of natural products (Hills et al., 1991). On the other hand, supercritical fluid extraction has some advantages over liquid-phase extractions including: lower viscosity and variable density of the supercritical fluid (SF), faster mass transfer, higher efficiency and shorter extraction time (Hills et al., 1991). Therefore, in the present study, supercritical CO<sub>2</sub> extraction was investigated to separated thermo-sensitive bioactive flavonoid compounds from herb matrices.

Among different solvents tested for SFE, carbon dioxide (CO<sub>2</sub>) is an ideal solvent for the extraction of natural products because it is non-toxic, non-explosive, readily available and easy to remove from extracted products. Worthy of note, SFE using carbon dioxide has the ability to ensure minimal changes of the active ingredients and the curative properties can be preserved (Cavero et al., 2006). CO<sub>2</sub> is a non-polar solvent, so extraction of polar compounds such as flavonoids is difficult with super critical carbon dioxide. Fortunately, this problem can be easily solved adding small amounts of organic modifiers (Cavero et al., 2006). Mint, in most Indian language country known as *pudina*, belongs to the genus *Mentha* in the family *Labiatae* (*Lamiaceae*). A numerous studies have shown that the herbs of this family have antioxidant properties (Coelho et al., 2003). To the best of our

knowledge, there is not any report about the supercritical fluids extraction of Malaysian *Mentha spicata* L. flavonoid compounds; therefore it is interesting to study the SFE of spearmint leaves flavonoids.

The objectives of this study were as follows:

- 1) To investigate the effect of operating parameters such as pressure, temperature and dynamic extraction time on yield and flavonoid compounds of the obtained extract from spearmint (*Mentha spicata* L.) leaves using supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) extraction and optimize the effect of these three parameters on yield and flavonoid compounds.
- 2) To compare between conventional soxhlet extraction (CSE) and SC-CO<sub>2</sub> extraction of bioactive flavonoid compounds from spearmint leaves (*Mentha spicata* L.).
- 3) To investigate the effect of co-solvent flow rate on yield and flavonoid compounds of the obtained extract using SC-CO<sub>2</sub> extraction from spearmint (*Mentha spicata* L.) leaves.

## CHAPTER II

### 2. LITERATURE REVIEW

#### 2.1 Introduction

Quality decrease and deterioration of large amounts of fat containing products can be caused by oxidative transformation of lipids during storage (Grigonis et al., 2005). Aldehydes and ketones are two main compounds that leading oxidation of lipids in foods and resulting degradation in food quality. Kumpulainen and Salonen (1996) mentioned that oxidized lipids are strongly associated with health disorders such as mutagenesis, aging and atherosclerosis. Oxidation of lipids in food products can be prevented by keeping away from oxygen, stored at low temperatures to retard oxidation reactions or add antioxidants (Kumpulainen and Salonen, 1996). In brief, antioxidants could be defined as any substrates that in the presence of them even at low concentration, the oxidative transformation of oxidizable substrates has strongly delayed or prevented. In the presence of antioxidants the formation of new free radical species is inhibited, existing free radicals are converted into less harmful molecules (Kanner and Jeffe, 1991).

#### 2.2 *Lamiaceae (Labiatae) family*

*Lamiaceae (Labiatae)* family which is consisting of about 25-30 species have shown strong antioxidant properties due to being a rich source of polyphenolic compounds. Aromatic herbs members of the family *Lamiaceae*, such as basil, rosemary,



marjoram, oregano, peppermint, spearmint, sage, lavender and thyme are cultivated as industrial crops in several countries. Members of the genus *Mentha*, which belongs to the family *Lamiaceae* (*Labiatae*), are characterized by their volatile oils. Their volatile oils used in the pharmaceutical, cosmetic, food, confectionery and liquor industries (Ali et al., 2002; Sweetie et al., 2007).

### **2.2.1 Spearmint**

Spearmint belongs to the genus *Mentha* in the family *Labiatae* (*Lamiaceae*) (Wang et al., 2004). It is usually known as ‘*Pudina*’ in most Indian language countries. A number of studies have found that herbs of the *Lamiaceae* family have been indicated as a potential source of natural antioxidants (Pizzale et al., 2002; Koleva et al., 2003). Most studies on antioxidant compounds in the *Lamiaceae* family have been focused on phenolic diterpenes, flavonoids and phenolic acids (Yanishilva and Marinova, 2001). Rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and lavender (*Lavendula angustifolia* Mill.), are native to the Mediterranean region and cultivated worldwide, balm (*Melissa officinalis* L.), and spearmint (*Mentha spicata* L.) are common plants in Britain and other European countries (Wang et al., 2004).

*Lamiacea* family herbs have been used in folk remedies for exhaustion, weakness, depression, memory enhancement, circulation improvement and strengthening fragile blood vessels. In numerous studies, several researchers pointed out that these plants are source of compounds possessing high antioxidant, anti-inflammatory, antiallergy and antidepressant activity. It was demonstrated that their content of