EXTRACTION AND CHARACTERIZATION OF OIL FROM *Moringa Oleifera* SEEDS USING SOXHLET EXTRACTION

by

KHADIJAH BINTI MD NOH

Thesis submitted in fulfilment of the requirements for the degree of Master of Science

April 2018

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and Most Merciful. My greatest thank to Allah the Almighty for the blessing and guidance, with His willing, I was able to complete my master thesis. All praise belongs to Him.

I would like to express my genuine gratitude to both my dedicated supervisors Professor Dr. Abdul Latif Ahmad and Dr. Derek Chan Juinn Chieh for their support and encouragement during the period of my studies. Your practical view and guidance on my research work was of the utmost important. Thank you very much for the unending help throughout the course of my research.

To my dearest husband, Mr Ihsan bin Ahmad Zubir, my beloved children, Hannan and Muhammad Bilal, thank you for your everlasting love, endless moral support, care, patient, prays and always being there for me when I need you most.

Special dedication to my beloved parents, Mr. Md Noh bin Ma'ruf (passed away on 4th of June 2016) and Mrs. Fatimah binti Emran, my parents in law, my brothers and sisters, thanks for your everlasting prays and support.

My best regards to Ministry of Higher Education for granting me the Mybrain (MyMaster) scholarship to assist financially for my study.

Last but not least, I would like to address my gratitude to all the staffs and assistant engineers of School of Chemical Engineering of USM, especially the staffs of (MTDC) lab, Unit Operational, Chemical and Analytical laboratory, for their cooperation and assistance. And also to all people that contributed their helps directly and directly, thank you so much and all your contribution will not be forgotten.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOCS	American Oil Chemist Society
AOAC	Association of Analytical Communities
CO_2	Carbon dioxide
FAME	Fatty acid methyl ester
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
N	Neutrality
Na_2SO_4	Sodium sulphate
SAP	Saponification value
SEM	Scanning Electron Microscopy
TAG	Triacyglycerol

LIST OF SYMBOLS

-	Single bond in organic compound
=	Double bond in organic compound
$\geq$	Greater than or equal to
$\leq$	Less than or equal to
%	Percentage
ρ	Density
μ	Viscosity
α	Alpha (value of 1 in Greek numerals)
γ	Gamma (value of 3 in Greek numerals)
®	Registered trademark symbol
wt.%	Weight percentage

PENGEKSTRAKAN DAN PENCIRIAN MINYAK DARIPADA BIJI *Moringa Oleifera* MENGGUNAKAN PENGEKSTRAKAN SOXHLET

ABSTRAK

Moringa oleifera diklasifikasikan sebagai salah satu tumbuhan sangat berharga kerana kebanyakan bahagian tumbuhan ini; akar, kulit, getah, daun, dan biji mempunyai kegunaan tersendiri. Minyak yang diekstrak daripada biji *M. oleifera* telah digunakan secara meluas dalam masakan, ubat-ubatan dan kosmetik. Kaedah pengekstrakan Soxhlet dengan menggunakan heksana sebagai pelarut pengekstrakan telah digunakan secara meluas untuk mengeluarkan minyak *M. oleifera* daripada bijinya. Walaubagaimanapun, kesan buruk penggunaan heksana terhadap alam sekitar dan kesihatan mungkin mengehadkan penggunaan heksana. Dalam kajian ini, heptana digunakan sebagai pelarut pengekstrakan kerana pelarut ini serupa dengan heksana tetapi ia tidak mempunyai masalah terhadap alam sekitar dan kesihatan yang berkaitan dengan heksana. Kesan suhu pengekstrakan, masa pengekstrakan, saiz zarah biji, dan nisbah pelarut kepada pepejal telah dikaji dalam penyelidikan ini. Hasil minyak bergantung kepada dengan perubahan pembolehubah yang dikaji. Suhu antara 85 ^{O}C hingga 105 ^{O}C, masa pengekstrakan antara 2 jam hingga 4.5 jam, nisbah pelarut kepada nisbah pepejal antara 7.5: 1 hingga 15: 1 dan saiz biji antara 0.5 mm hingga 2.0 mm telah digunakan semasa kajian. Keadaan optimum yang menghasilkan hasil minyak tertinggi adalah suhu pada 100 °C, masa pengekstrakan 4 jam, saiz zarah kurang daripada 0.50 mm dan nisbah pelarut kepada pepejal 15: 1. Hasil minyak maksimum ialah 40.10%. Biji *M. oleifera* dicirikan sebelum dan selepas proses pengekstrakan dengan menggunakan Pengimbasan Elektron Microskopik (SEM), *Fourier Transform Infrared* (FTIR) dan ujian penganalisis unsur. Pengelasan fizikal dan kimia minyak yang diekstrak telah dianalisa. Asid oleik, asid palmitik dan asid oksalat dikesan dengan asid oleik mendominasi kira-kira 65.90%. Minyak *M. oleifera* yang diekstrak berwarna kuning dan mempunyai ketumpatan 0.905 g/mL. Nilai indeks biasnya adalah 1.46705 dan 65cP kelikatan. Nilai pengekstrakan ditentukan ialah minyak 198.63 mg KOH/g. Daripada kajian kinetik, model pengekodan urutan kinetik kedua berpadanan dengan data eksperimen dengan pekali korelasi (R^2) hampir 1.00. Kewujudan fitokimia dalam minyak *M. oleifera* yang diekstrak telah diuji dan hasilnya menunjukkan bahawa flavonoid, saponin, fenol, tanin dan terpenoid wujud di dalam minyak. Daripada penyelidikan, didapati bahawa heptana adalah pelarut yang berkesan untuk proses pengekstrakan minyak *M. oleifera* menggunakan kaedah pengekstrakan Soxhlet.

EXTRACTION AND CHARACTERIZATION OF OIL FROM *Moringa Oleifera* SEEDS USING SOXHLET EXTRACTION

ABSTRACT

Moringa oleifera is classified as one of the most valuable plants as most parts of this plant; root, bark, gum, leaf, and seed have their special usages. The oil extracted from *M. oleifera* seeds has been widely used in culinary, medicine and cosmetics. Soxhlet extraction method by using hexane as the extraction solvent has been widely used to extract the *M. oleifera* oil from its seed. However, the environmental and health concerns about hexane may limit its usage. In this research, heptane is used as the extraction solvent as this solvent is similar to hexane but it does not have the environmental and health concerns associated with hexane. Effects of extraction temperature, extraction time, particles size of the seeds, and solvent to solid ratio towards the extraction yield were investigated. The oil yield varied with the changing of the parameters. Temperature between 85 ^{O}C to 105 ^{O}C, extraction time between 2 hours to 4.5 hours, solvent to solid ratio between 7.5:1 to 15:1 and seeds size between 0.5 mm to 2.0 mm were used during the investigation. The optimum parameters that give highest oil yield of 40.10 % were temperature at 100°C, 4 hour extraction time, particle sizes less than 0.50 mm and solvent to solid ratio of 15:1. The *M. oleifera* seeds were characterized before and after the extraction process by using Scanning Electron Microscopic (SEM), Fourier Transform Infrared (FTIR) and elemental analyzer tests. Physical and chemical characterizations of the extracted oil were analyzed. Oleic acid, palmitic acid and oxalic acid were detected with oleic acid having the dominant percentage of about 65.90%. *M. oleifera* oil extracted was yellow in color and has density of 0.905 g/mL. The refractive index and viscosity

values were 1.46705 and 65cP, respectively. Saponification value determined was 198.63 mg KOH/g oil. From the kinetics study, the kinetics second-order extraction model fitted well with the experimental data with correlation coefficient (R^2) almost 1.00. The existence of phytochemicals in the extracted *M. oleifera* oil was tested and the results showed that the flavonoids, saponins, phenol, tannins and terpenoids exist in the oil. From the investigation, it was found that heptane is an effective solvent for the extraction of *M. oleifera* oil using Soxhlet extraction method.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Moringa oleifera (*M. oleifera*) belongs to the Moringaceae and *Moringa* genus has been used for such a wide variety of purposes that it has been described as a ‘miracle tree’. It is grown in many parts of the world as a ‘vegetable tree’, with the roots, leaves, flowers and fruit all being used for food. Since *M. oleifera* leaves contain more calcium than milk, more iron than spinach, more Vitamin C than oranges and more potassium than banana, this tree’s portion has been taken to overcome malnutrition issue especially among infants and nursing mother (Zhao and Zhang, 2013c). The leaves are probably the most widely used part, being compared to spinach in appearance and nutritional quality (Papillo 2007). *M. oleifera* seeds kernel (Figure 1.1) is contain of oil which is called “*Ben oil*”. The seed kernels contain approximately 35-40% of oil according to Ruttarattanamongkol et al. (2014), 33-41% by Zhao and Zhang (2013a) or 39% of oil by da Silva et al. (2010). This oil composed of approximately 13-18% saturated and 82-87% unsaturated fatty acid make it resistance to oxidative rancidity (Anwar et al., 2007). Moreover, it has linoleic acid, behenic acid and high degree of saturation (Tsaknis and Lalas, 2002).

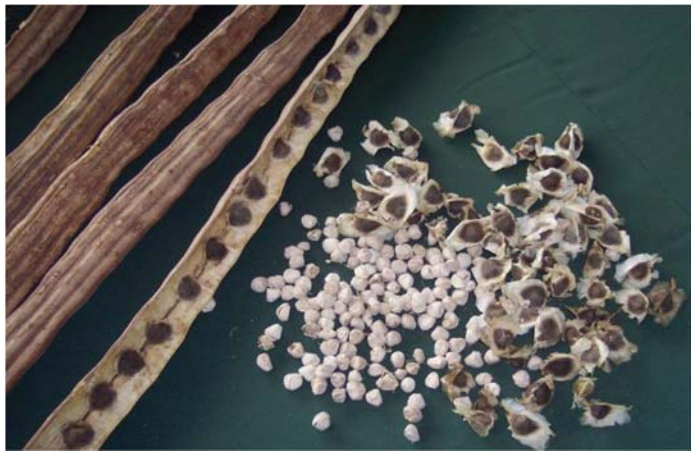
Figure 1.1 Seeds and kernels of *M. oleifera*

Besides sunflower, corn, palm or olive oil, *M. oleifera* oil has been proposed as alternative source to be used in making biodiesel. It can react with alcohol to produce biodiesel which later can be used as energy sources in transportation. Another uses of this oil is it acts as the lubricant in watch making and precision equipment (Mani et al., 2007). *M. oleifera* oil was widely used for medical purpose too. It is reported contain large quantity of tocopherols, an organic chemical compound which has vitamin E activity, rich in vitamin A and C plus it is a good antioxidant. For treatment of rheumatism and gout, this oil could be effective in advance. Furthermore, it enhances cardiovascular system as it helps in the purification of blood. According to Foidl et al. (2001), the oil externally applied for curing skin diseases due to the antimicrobial, antiseptic and anti-inflammatory properties content in it (Ruttarattanamongkol et al., 2014).

In cosmetic industries, *M. oleifera* oil is useful in producing hair care products, soap, perfumes and cosmetic preparation. Since the oil consists high amount of oleic acid (more than 70%) as reported by Mani et al. (2007), hence, it is edible and can be used for food consumption. In Haiti, it is commercially used as ingredient in culinary and salad oil. It is a good choice for frying purpose as it is very stable with the presence high oleic acid. Other than that, from the oil extraction process, the press cake obtained as residue has positively charged protein molecules. These molecules have coagulant properties which can be utilized in purification and wastewater treatments.

Production of vegetable oil either from its fruits, leaves or seeds is usually through the solvent extraction due to high oil yield obtained which is approximately more than 99 wt.% (Zhao and Zhang, 2013b). Examples of commonly used solvents are hexane (Kostić et al., 2014; Lohani et al., 2015; Silva et al., 2016), petroleum ether (Sayyar et al., 2009; Tian et al., 2013; Fakayode and Ajav, 2016), acetone (Mani et al., 2007; De Oliveira et al., 2013; Palafox et al., 2012) and alcohol like ethanol (Toda et al., 2016; Santos et al., 2015; Dagostin et al., 2015) and methanol (Mani et al., 2007). Hexane is widely used due to its efficiency and easy to recover (Akaranta and Anusiem, 1996). Petroleum ether with boiling point in the ranging from 30-70°C has saturation paraffin such as pentane and hexane that make it suitable for extracting oil (Mani et al., 2007). Plus, they are chemically inert, high selective dissolving power and very volatilite. However, sulfur and nitrogenous compounds must be removed from petroleum ether (Mani et al., 2007). The disadvantage of using solvent extraction method is the solvent residues may remain in the oil that can be dangerous to human health and environment. Therefore, selection of a suitable extraction technique is very important for the standardization of products as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvents. Furthermore, the optimization of various parameters is also a critical factor to improve the quality and efficiency the yield of product.

1.2 Problem Statement

Since almost every part of *M. oleifera* tree such as seeds, leaves and roots have benefits, there are quite a number of research were done in order to study the characteristics, properties, yield and uses of the products obtained. The current research focuses more on the extraction of oil from the *M. oleifera* seeds. From previous research done, the authors concerned about the method used for the extraction process in order to achieve high yield of the oil. Commonly, there are two favourable extraction methods used which is Soxhlet extraction and supercritical fluid extraction.

Currently, the demand of vegetable oil in the world was quite high. The common oil in the market was coming from various kind of plant such as palm kernel, sunflower seeds, sesame seeds and corn. Therefore, *M. oleifera* seeds were proposed as another source for oil extraction. Based on the results from recent studies, it can be seen that, most of the researchers used Soxhlet extraction method with similar solvent which is hexane but, the percentage of *Ben's* oil extracted is different. In addition, the use of hexane as solvent was detriemental to the environment and human health. Other type of solvents such as petroleum ether, chloroform, methanol and acetone was also commonly used in oil extraction. However, they are not preferable since low yield of about less than 34 % of oil was produced (Mani et al., 2007). Hence, use of heptane as the solvent in extraction of oil had been proposed in this study. Heptane may be a good choice to replace hexane as the extraction solvent as it does not have the environmental and health issue associated with hexane (Conkerton et al., 1995). Moreover, literature review on using heptane as the extraction solvent has never been reported.

Therefore, this research focuses on the characterization of oil before and after extraction. In addition, further study is done on the extraction process chosen which could verify the possible parameters that can affect the yield of the *M. oleifera* oil. Soxhlet extraction method is straightforward and inexpensive (Luque-Garcıa and De Castro, 2004). Since hexane is typically used in the Soxhlet extraction, heptane is used as the solvent instead, and the yield of the oil is determined in this experiment. Besides, in order to maximize the extraction of *M. oleifers* oil, this study is purposely done to determine either by varying the variables such as extraction time, temperature, ratio solvent in solid and particle size could give effects to the yield of oil.

Once the oil is extracted, the characterization of extracted oil is done to identify the physical and chemical properties of the oil. The oil extracted from the *M. oleifera* seeds is compared with the olive oil for its properties, compositions and edibility to be used as food consumption. Olive oil is chosen to be compared with the extracted *M. oleifera* oil as it is well known as one of the most beneficial and widely used edible oil nowadays. Finally, the kinetics and thermodynamis studies for the extraction process are done.

1.3 Research Objectives

The objectives of the research are:

1. To study the extraction of *M. oleifera* oil using heptane as solvent in Soxhlet extraction process.

2. To characterize the *M. oleifera* seeds used in the extraction study and determine the physical and chemical characteristics of the oil extracted from *M. oleifera* seed kernels.

3. To study the extraction of oil kinetics and thermodynamics from *M. oleifera* seeds.

1.4 Scope of Study

Basically, this study can be divided into three phases which are:

- A study on the *M. olifeira* oil extraction process by using Soxhlet extraction method and heptane as the extraction solvent

- Characterization of the *M. oleifera* seeds before and after oil extraction and characterization of the extracted oil

- Kinetic and thermodynamic studies of *M. oleifera* oil extraction

1.5 Organization of Thesis

This thesis covers five chapters and each chapter describes the in sequence of this research.

Chapter 1 explains about the introduction of the *M. oleifera* and the benefits of that valuable plant. This chapter also presents the problem statement, objective of the studies, and finally the organization of the thesis.

Chapter 2 refers to literature review that covers the literature of *M. oleifera* oil, various methods of oil extraction and kinetics and mechanisms of solid liquid extraction. This chapter also elaborates on the kinetics and mechanisms of the extraction process, and also the physical and chemical characteristics of *M. oleifera* seeds oil.

Chapter 3 covers the materials and methods used throughout the research. The chapter describes the experimental procedures in details for characterization of *M. oleifera* seeds, Soxhlet extraction process, and followed by characterization of extracted *M. oleifera* oil and kinetics and thermodynamics studies of the extraction process.

Chapter 4 covers results and discussion and presents all the acquired results and explained all the findings followed by discussion about results.

Chapter 5 refers to conclusions and recommendations for future research. The findings reported in Chapter 4 are used as the as the reference to write the conclusions. Recommendations for prospect studies are presented due to their significance with current research.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Description of *M. oleifera*

Moringa oleifera (*M. oleifera*) belongs to the Moringaceae and *Moringa* genus, the best known and most widely distributed species. There are a few known varieties namely Jaffna, Chauakacheri Murunga, Chem, Kadu, Palmurungai and Periyakulam 1 (Tsaknis et al., 1999). *M. oleifera* is the scientific name for the *Moringa* tree which is believed originated in Northern India. This species is from the *Moringaceae* family and grows in Asia, Africa, South America, the Caribbean and Oceania. In Malaysia, *M. oleifera,* commonly known as *kacang merunggai* (Endut et al., 2016). Some other common names of *Moringa* are *drumstick* tree, *(Horse) radish* tree, *Morango* and Benzolive (Abdulkarim et al., 2005). The tree of *M. oleifera* (Figure 2.1(a)) carries some physical characteristics such as it is filled with long and delicate-looking branches, which are covered with small and oval-shaped dark green leaves (Ghazali and Yasin, 2016). The height of the tree can reach a maximum of 7-12 m and a diameter of 20-40 cm at chest height (Foidl et al., 2001). The leaves are 20-70 cm long, grayish-downy when young, long petiole with 8-10 pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, all 1-2 cm long; with glands at the bases of the petioles and pinnae (Figure 2.1 (b)). The flowers (Figure 2.1 (c)), which are pleasantly fragrant, and 2.5 cm wide are white or cream colored and yellow-dotted at the base. *M. oleifera* fruits (pod-like capsules) borne singly or in pairs, air light-green, slim tender, firm and thinly woody (Padayachee and Baijnath, 2012). The seed pods can reach well 20-60 cm in length and when they are dry they open into 3 parts. Each pod contains between 12 and 35 seeds (Figure 2.1 (d)) (Abdulkarim et al., 2005). *M. oleifera* seeds (Figure 2.1 (e)) are circular-shaped, 7 to 8 mm in diameter, with 4 papery wings and are yellow-grey (Padayachee and Baijnath, 2012). Each tree can produce between 15,000 and 25,000 seeds/year. The average of seed weight of approximately 0.3 g and about 70-75% of the weight are composed from the kernel(Mani et al., 2007). *M. oleifera* seed *kernel* is white in color (Figure 2.1 (f)) and contains a significant amount of oil which is commonly known as "Ben Oil" (Daba, 2016; Yadav, 2013).

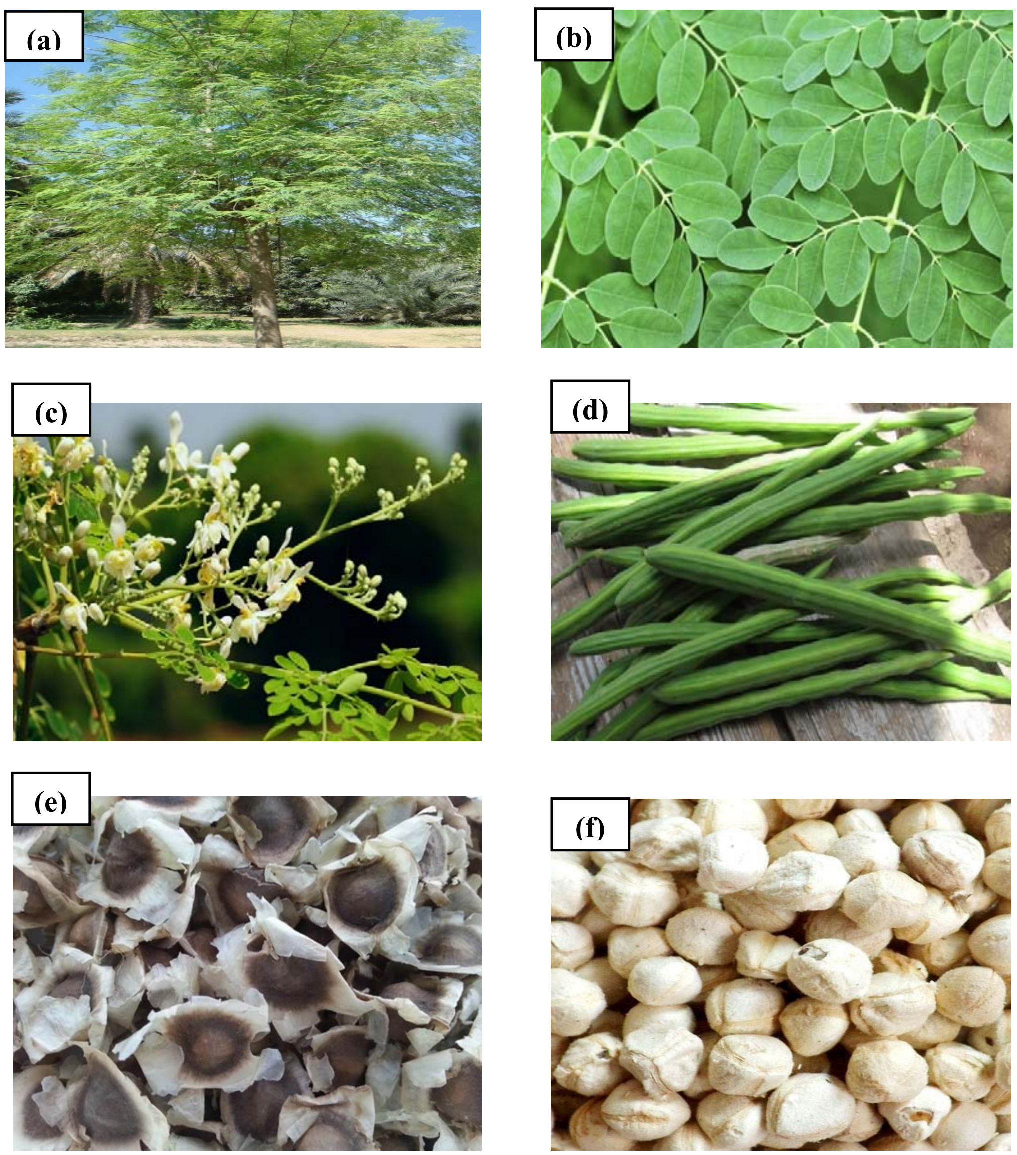

Figure 2.1: Part of *M. oleifera* (a) tree, (b) leaves (c) flowers (d) fruits/pods (e) kernels and (f) seeds (Parrotta, 2009; Price, 2007).