ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING



Extraction, Kinetics Study and Characterization of Moringa *Stenopetala* Seed Oil

By Tilahun Gisila

A Thesis Submitted To

The School of Chemical and Bio Engineering, Addis Ababa Institute of Technology,

Addis Ababa University

Presented in Partial Fulfillment of the Requirement for the Degree of Master of Science in Chemical Engineering (Process Engineering Stream)

Addis Ababa University

Addis Ababa, Ethiopia

July, 2018

ADDIS ABABA UNIVERSITY

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This is to certify that the thesis prepared by *Tilahun Gisila*, entitled: *Extraction, Kinetics Study and Characterization of Moringa Stenopetala Seed Oil* and submitted in partial fulfillment of the requirement for the degree of Master of Science in Chemical Engineering complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Dr. Shegaw Ahmed (Assistant Professor)	_____	_____
Advisor	**Signature**	**Date**
Prof. Belay Woldeyes	_____	_____
Internal Examiner	**Signature**	**Date**
Prof. Eduordo Ojito	_____	_____
External Examiner	**Signature**	**Date**

DECLARATION

I declare that this thesis entitled "*Extraction Kinetics study and Characterization of Moringa stenopetala seed oil"* has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Information taken from published work of others has been acknowledged in the text and a list of references is given. The research work was done under the guidance of *Dr. Shegaw Ahmed* instructor in School of Chemical and Bio-Engineering, Addis Ababa Institute of Technology, Addis Ababa University.

Name: Tilahun Gisila

Signature _____

ABSTRACT

In this thesis the effect of particle size, temperature and extraction time of solvent extraction of *M. stenopetala* seed kernels, using hexane as the extracting solvent and Soxhlet extractor as the extracting medium was studied. Fatty acid composition of the *M. stenopetala* seed kernel oil was determined by using Gas Chromatography Mass Spectrometer (GC-MS) analysis. The moisture content, ash content and fat content of *M. stenopetala* seed were found to be 5.04 %, 4.48%, and 40.9%, respectively. The Physico-chemical properties of the extracted oil, which include density, specific gravity, viscosity, acid value, saponification value, iodine value and Easter value for *M. stenopetala* oil was found to be 0.906g/cm, 0.9056, 19mPa.s, 3.74mgKOH/g, 179.52mgKOH/g, 67.5gI/100g oil and 177.2mgKOH/g oil respectively. The saturated fatty acid content was found to be 27 %, with palmitic acid dominating, and then followed by, stearic, arachidic acids and behenic acid. The oil contains a high level of monounsaturated fatty acids, up to an average of 70.83. The optimum process variables combination to find the highest oil yield was particle size of 0.68mm, temperature at 74.12^{o}C and extraction time of 6.93 hours. The kinetics of extraction process is developed by second order kinetics equation and the extraction rate constant, k and the initial extraction rate, h, was determined for 70, 77.5 and 85^{o}C. Finally *M. stenopetala* seeds are a promising resource for food and Cosmetics applications, due to their content of monounsaturated fatty acids with a high monounsaturated to saturated fatty acids ratio.

Key Words: *Moringa stenopetala*, Extraction, Kinetics, Characterization,

ACKNOWLEDGMENT

First of all, I would like to praise the Almighty God for his wisdom and guidance throughout my life and help in the entire situation and challenge that I face in this work, which was really very tiresome.

I would like to express my deepest thank for my advisor Dr. Shegaw Ahmed for his sustainable appreciable guidance, tireless advising, for sharing his knowledge, skill and encouragement and patience starting with the conception of the research idea till the end of this research work. I'm honored to have worked under your supervision.

I would like to express my gratitude to the AAIT Chemical engineering staff, DBU chemical engineering staff and LIDI laboratory technicians in general and to Ato Hintsasilase Sefu in particular for his determined help during my experimental work.

My Thanks also go to Ato Tewodrose Gizaw for his help in sample collection, Ato Melese for his help in identification of Moringa *stenopetala* from Moringa *oleifera* and agricultural locations of Moringa *Stenopetala*, and Endayhu Gebeyu for his help in making things easier during my laboratory work in Debre Berhan University.

My deepest gratitude also goes to my friends especially Kitaw Abrham, Tewodros Geremew and Alemshet Desalgn for their valuable help and advice in the research laboratory work and sharing information throughout the academic period.

Finally my deepest gratitude goes to family members especially my mother Emebet Fikade, my father Ato Gisila Abebe and my brother Tesfaye Gisila who have been always besides me, helping me psychologically, emotionally, physically and materially to overcome all the obstacles.

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ACRONYMS

AAIT	Addis Ababa Institute of Technology
AEOE	Aqueous Enzymatic Oil Extraction
A.V	Acid value
ANOVA	Analysis of Variance
AOA	Antioxidant activity
AOAC	Association of official analytical chemist
CCD	Central Composite Design
DBU	Debre Berhan University
DF	Degree of Freedom
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agriculture Organization
FDA	Food Drug Administration
FFA	Free fatty acid
FTIR	Fourier Transform Infrared Radiation
GC-MS	Gas chromatography- mass spectrometry
I.V	Iodine vale
ILCA	International Livestock Center for Africa
LIDI	Leather Industry Development Institute
MAAEE	Microwave-Assisted Aqueous Enzymatic Extraction
MAE	Microwave-Assisted Extraction
MUFA	Monounsaturated fatty acid
NSAID	Non-Steroidal Anti-Inflammatory Drug
PUFA	Polyunsaturated Fatty Acids
S.V	Saponification value
SFA	Saturated fatty acids
SFE	Supercritical Fluid extraction
SFE	Supercritical fluid extraction
Sp.gr	Specific gravity
SV	Saponification value
WHO	World Health Organization

1. Introduction

1.1. Background

Plants have always been vital for mankind irrespective of the era and area all over the globe since the beginning of life. These were, are and will remain ever beneficial from nutritional, social, cultural, religious, environmental and human's health etc. The global trade in herbals is increasing rapidly and in hundreds of billions in US$ (Mahmood et al., 2010). There are many underutilized plants in Ethiopia which have the potential to bring a lot of prosperity. Moringa is one of the underutilized resources of Ethiopia. Its tree could easily and cheaply be cultivated and grown in Ethiopia.

M. stenopetala, which is most common in Ethiopia and Kenya, has the second highest content compared with the other species (Glaerum, 2012). *M. stenopetala* is often referred to as the African Moringa Tree because it is native only to Ethiopia and northern Kenya. Though it does grow in many other parts of the world tropics, it is not as widely known as its close relative, Moringa *oleifera* (Lalas & Tsaknis, 2003). *M. stenopetala* has large edible leaves and seeds and is more drought- but less freeze-resistant than M. *oleifera*. Freezes may cause it to die back to ground level, where new sprouts may be produced. *M. stenopetala* has also lushest green foliage and continues to grow during exceptionally long dry seasons. It develops into a round shrub-like tree (Lalas & Tsaknis, 2003) and has been grown as an ornamental in private gardens in Kenya, reaching a height of 10-12 m and a trunk diameter of at least 2-3 times as that of *M. oleifera* in Sudan.

As the Indian *M. oleifera*, *M. stenopetala* is now introduced in many tropical countries for its popular uses but its origins are the southern regions of Ethiopia and Northern Kenya. Commonly known as cabbage-tree in English, *M. stenopetala* is widely used by many Ethiopian communities use it as a source of diet. In this regard, one of the greatest socioeconomic impacts of the plant lies in its ability to grow in poor soil and achieving harvest in a short amount of time. This quality of the plant has also been noticed in recent years by many international aid agencies as funding was made available for a large-scale Moringa farming in areas vulnerable to frequent droughts (Habtemariam & Varghese, 2015).

In Ethiopia, *M. stenopetala* grows wild altitude range of about 1100-1600 meters (Mekonnen and Gessese, 1998). The *M. stenopetala* tree, locally called shiferaw (Amharic) or aleko (Konso,), grows widely in southern Ethiopia, mainly in the Keffa, Gamo Gofa, Bale, Sidamo,

Borana and Debub Omo zones, and in Konso and Dherashe areas. In the Arba Minch and Wollayta areas, the local people cook the leaves of the *M. stenopetala* tree and eat them with their traditional kurkufa (a cereal dish made with maize and sorghum) (Abuye et al., 2003). The people of Konso use the tree not only for food but also as a medicine and they cultivate it in large areas around their villages (Ejigu et al, 2010). Moringa leaves have been taken to combat malnutrition, especially among infants and nursing mothers, because they contain more calcium than milk, more iron than spinach, more Vitamin C than oranges and more potassium than a banana (Fahey, 2005). The leaves and roots of *M. stenopetala* are used as a cure for malaria, stomach problems and diabetes (Habtemariam & Varghese, 2015) and the seeds are used in some areas to clear muddy water.

Moringa oil is highly valued in the cosmetic industry for its unique property. Moringa oil is light and spreads easily on the skin. It is best for massage and aromatherapy applications. Moringa oil is used in ant ageing creams, hair care products, soaps and Liquid body wash, aromatherapy oils, massage oil, face creams, perfumes and deodorants (Mulugeta & Fekadu, 2014). This would make it a potential feedstock for the production of oil for different end use and industrial application, since it does not compete with food production. As far as my knowledge no work has been previously investigated sufficient research on the uses of the seeds of *M. stenopetala* for food and cosmetics application in Ethiopia.

This work embraces the extraction and characterization of *M. Stenopetala* seed oil by using solvent extraction technology employing hexane as an extract solvent, it is being the most commonly used technology in the cosmetics, food processing areas. Solvent extraction is chemical oil extraction method which is the most popular method of extraction of oil because of its high percentage of oil recovery from seeds.

1.2. Statement of the Problem

The increasing importance of oils as food, pharmaceutical and cosmetics has opened up wide opportunities for global marketing and this bring an increase in cost and shortage on common oil seeds. Ethiopia is endowed with a diversity of flora. Most of which has remained unexploited. Its application in antioxidant, aromatherapy, perfumery, soap and other related industries are limited due to lack of adequate research on the chemical and biological potential of its raw materials.

An increase in the world’s population leads to searching for an alternative oilseed with equivalent or superior quality to the market. To satisfy the need of a continuously increasing population of the world, in addition to mass production of known oilseed, it is wise to take a look at another minor oilseed like Moringa. Currently, Ethiopia is importing oil from abroad for chemical, cosmetics and pharmaceutical uses although there is the potential for oil resources used as a raw material in different chemical industries. Literature indicates that in the previous time's extracts of natural materials were used but currently synthetic ingredients are often used in the cosmetic products. Some of these synthetic additives could be dangerous for the consumer health.

Shortage for the utilization of locally available raw materials and cultivating them for production of valuable product from them has multiple effects on the Scio-economic development of once country. Starting from job creation to local community, to reduce lack of currency to import products and protecting the bio-diversity by cultivating and processing this valuable medicinal tree in bulk. Additionally, moringa seed is underutilized oil source in Ethiopia due to lack of awareness for harvesting and processing. Even though, it has large application for the production of cosmetics and other valuable products. Environmental problem, such as global warming, due to excess carbon dioxide emission to the environment caused by deforestation and dependency of chemical products on the petrochemical based raw material is another problem that is facing the world now a day.

1.3. Objective of the Research

1.3.1. General objective

The general objective of this research was to extract oil from *Moring stenopetala* seed using solvent extraction method, study the extraction process variables, kinetics of the extraction process and characterize the Physico-chemical properties of the extracted oil.

1.3.2. Specific Objectives

The specific objectives are:-

- To extract *Moringa stenopetala* seed oil and characterize the Physico-chemical properties of the extracted oil.
- To investigate the effects of extraction process variables (temperature, particle size and extraction time) on oil yield and determine the optimal operating condition.
- To study the fatty acid composition of *Moringa stenopetala* seed oil and to compare with other relative vegetable oils.
- To study the extraction process kinetics and develop a model equation that, represent the extraction process.

1.4. Significance of the Study

This research has benefits to many stakeholders in the economy and utilization of these oils can have a great economic impact on local communities and significantly contribute to local farmer's economy. Oils and fats are natural source have extensive applications in our modern industrial world. Global industrialization and the increasing demand for environmentally acceptable materials has led to the investigation and exploitation of more vegetable oils as a renewable feed stock in the preparation of oleo chemicals in order to meet the growing needs of human society.

The *M. stenopetala* seeds are used to obtain high oil yield for multiple applications in the food and cosmetics industries as result of a rich source of fatty acids. Besides the industrial uses such as fine lubricant and perfumery, the fatty acids profile of the oil with its very high content of oleic acid make its oil with high potential for further industrial application which has been shown to be beneficial to the skin. This oil is of excellent quality similar to the olive oil, and is slow to become rancid It gave high oil yield, which has good antioxidant capacity

with potential for industrial, nutritional and Cosmetics applications. The healing properties of moringa seed oil have been reported by ancient cultures. Moringa oil has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner. Moringa seeds oil has been used in skin preparations and ointments since Egyptians times.

In general the significance of this research can be seen from different perspectives as follows

- Provide a means to exploit and use valuable local resources.
- Provide a new alternative industrial oilseed crop, for production of nutritional and cosmetics products.
- Gives the awareness for the utilization of available natural resources in the country for the production of important products locally.
- Utilization of alternative vegetable oil can support on job creation for the local community, to cultivate, to process, to reduce import substitution and support the green development of the country and
- The extraction of oil from its seeds could be of high economic benefit to the communities where the tree is cultivated.

1.5. Scope of the Study

The thesis work generally covers collection and preparation of *M. stenopetala* seed, extraction of the oil with specified parameters by using solvent extraction method, characterize the physical and chemical properties of the extracted oil, study the effect of particle size temperature and extraction time on oil yield and the optimum extraction process variables and developing kinetics model for the extraction process was studied.

2. Literature review

2.1. General description

Moringa of Moringaceae family there are 13 species (namely, *M. arborea*, indigenous to Kenya; *M. rivae* indigenous to Kenya and Ethiopia; *M. borziana*, indigenous to Somalia and Kenya; *M. pygmaea* indigenous to Somalia; *M. longituba* indigenous to Kenya, Ethiopia and Somalia; *M. stenopetala* indigenous to Kenya and Ethiopia; *M. ruspoliana* indigenous to Ethiopia; *M. ovalifolia* indigenous to Namibia and Angola; *M. drouhardii*, *M. hildebrandi* indigenous to Madagascar; *M. peregrine* indigenous to Red sea and Horn of Africa, *M. concanensis*, *Moringa oleifera* indigenous to sub-Himalayan tracts of Northern India (Leone et al., 2015). Among which *M. oleifera* has so far become the most used and studied species due to the distribution in different area of the world. *M. oleifera* is a tropical multipurpose tree that naturally grows in India, but now distributes worldwide in the tropics and subtropics (Lalas & Tsaknis, 2002). *M. oleifera* found including in our country Ethiopia, Saharan Africa and South-America. The rest species of Moringa, on the other hand, have not been studied in detail and their potential uses have not been fully understood. *M. stenopetala* is domesticated in East African lowlands and indigenous to southern Ethiopia. Many different ecotypes and varieties of *M. stenopetala* are found in Ethiopia (Seifu, 2012).

Within the first year of growth, moringa has been shown to grow up to 4 meters and can bear fruit within the same first year (Sutherland, 1996). The tree can grow almost in all type of climate and soil, however it grows well in semi-arid tropics and sandy-loam soil (Kumar et al, 2017). The trees range in height from 5 to 10m and sometimes can be even 15m and it can grow even in hot dry lands or destitute soils and are little affected by drought. Moringa is called the "miracle tree"" because the plant can provide as a food supplement for fortification, energy drinks, creams, cosmetics, shampoos, etc. (Mulugeta & Fekadu, 2014). According to the study, the rate to absorb carbon dioxide (CO_2) of Moringa tree to is fifty times (50x) higher when compared to the Japanese cedar tree and also twenty times (20x) higher than that of general vegetation (Kumar et al., 2017) Incorporation of such type of tree species in agroforestry system in different parts of the country will mitigate the impacts of climate change. Many other ecosystem services provide tree like, Improvement of soil fertility, act as a barrier for Air, Water, Soil erosion, Biodiversity and habitat conservation are very essential for equilibrium between human and nature (Sutherland, 1996).

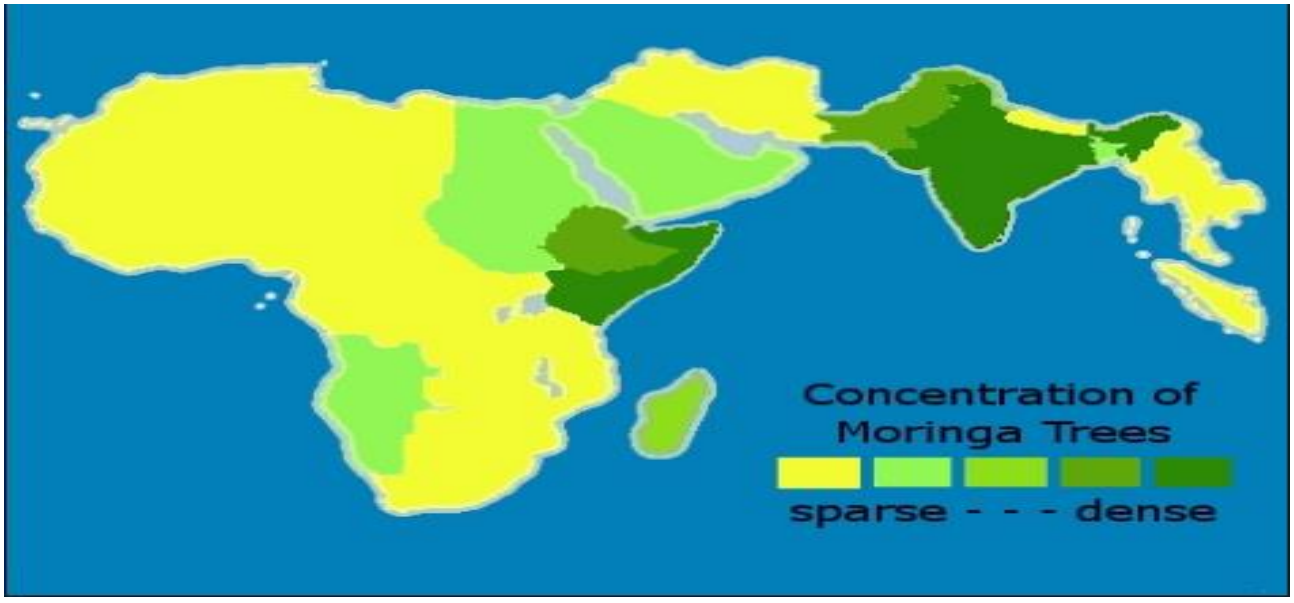
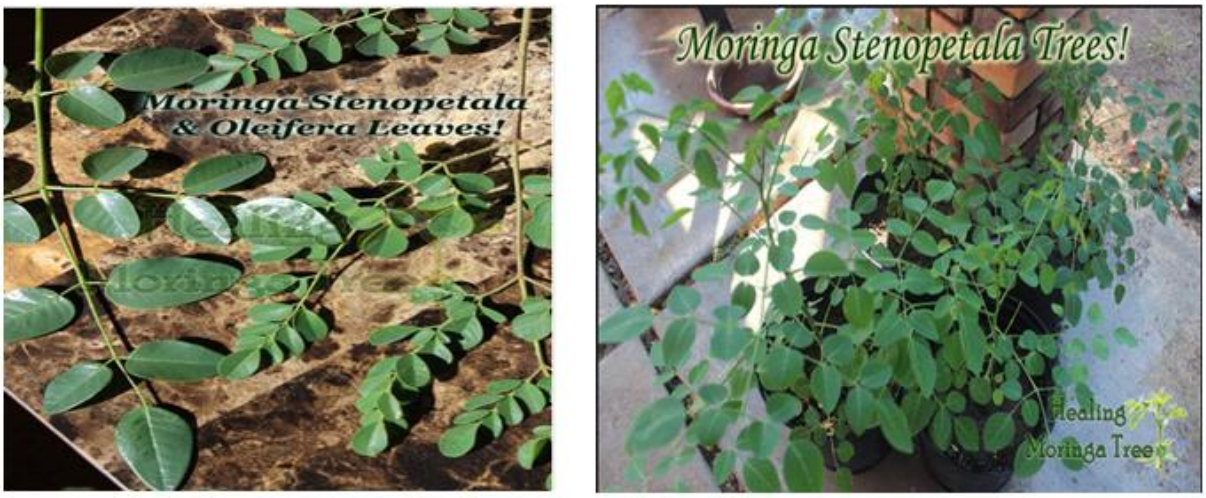


Figure 2.1 Moringa tree concentration and number of species in Africa and part of Asia

Moringa stenopetala belongs to the family Moringaceae and is often referred to as the African Moringa Tree because it is native only to Ethiopia and northern Kenya. It is a native or endemic multi-purpose tree species in East Africa which is grown in Southern Ethiopia, Northern Kenya and Eastern Somalia (Denton and Grubben, 2004). This tree is one of the most frequently cultivated indigenous species for its palatable leaves in the semiarid areas of Konso, Derashe and Arba Minch Zuria districts of the Southern Rift Valley of Ethiopia and locally called as "aleko" or "shiferaw". It is also cultivated from the lower Omo Valley to the north and in the neighboring regions of South Omo, Gamo Gofa and Borena. It is a unique food tree in drought prone areas and with its high social, environmental and economic value. It has been recently distributed to Wello, Shoa, Harargie and Sidamo for demonstration purpose by the Soil and Water Conservation of the Ministry of Agriculture. The Forestry Research Center has also tried an alley cropping in Fontenina (Wello), Dhera (Arsi) and a windbreak trial at Ziwai, Awassa College of Agriculture and International Livestock Center for Africa (ILCA) planted the tree in Ziwai. They all performed well at establishment phase in these semi-arid areas under rain fed conditions (Jiru et al., 2006).

Although it does grow in many other parts of the world tropics, it is not as widely known as its close relative, Moringa oleifera. The Ethiopian Moringa, Moringa stenopetala is incredibly similar to the Indian Moringa M. oleifera in many respects. In both cases, they are called "The Miracle Tree" owing to their multipurpose applications including as a source of food,

medicine, edible oils, Cosmetics, biofuel and applications for water sanitations (Anwar, Zafar, & Rashid, 2006; Habtemariam & Varghese, 2015).



(a) (b)

Figure 2.2 (a) M. stenopetala and M. oleifera Leaves (b) M. stenopetala Leaves

M. stenopetala has the following features: It is a tree 6-10 m tall; trunk up to 60 cm in diameter at breast height; crown strongly branched; sometimes with several trunks, thick at base; bark white to pale grey or silvery, wood soft, branches with leafy scarce 6-10 mm across; young stems and leaves densely soft pubescent (Beyene, 2005). It grows abundantly in Southwestern Ethiopia where the leaves are eaten as a vegetable. The species is known by different vernacular names such as "Shiferaw" in Amharic, "Aleko" in Konso, Wollayta and Gamugna and “Cabbage tree” in English. It grows widely at an altitude range of 1000 to 1800 m. *M. stenopetala* is a multipurpose plant.

The leaves are one of the best vegetable foods that can be found in the locality (Aviara et al., 2017).The raw leaves of *M. stenopetala* contain per 100 g dry matter: energy 295 kcal, protein 9.0 g, fat 5.8 g, carbohydrate 51.8 g, crude fiber 20.8 g, Ca 793 mg, P 65.6 mg, Zn 0.53 mg and ascorbic acid 28 mg (Abuye et al., 2003). The various parts of *M. stenopetala* are extensively employed in the Ethiopian and Kenyan traditional medicine for treating a range of illnesses such as, diabetes, hypertension, stomach pain, malaria, leishmaniasis, leprosy, epilepsy, diarrhea, asthma, colds, as an anthelmintic, emetic and wound healing (Habtemariam & Varghese, 2015).Now a day moringa tree popularized among the farmers across the world including Ethiopia and adopted by them on their field as a tree component in agroforestry system. The various uses and a wide range of adoptability make it as an ideal

crop for sustainable food production, survival of life and climate resilience. However there are several constraints which hinder the actual economics of these miracle trees like, lack of markets, lack of appropriate knowledge about cultivation practices, lack of planting material and competition for land with other food crops (Kumar et al., 2017). To overcome from these constraints a lot of research activities are necessary on the cultivation and utilization of the tree constituent for valuable products.

The study that evaluated the antioxidant effect and nutritional content of four types (*M. oleifera*, *M. peregrina*, *M. stenopetala* and *M. drouhardii*) showed that all have a high content of antioxidants (Yang et al., 2006). Moringa stenopetala, which is most common in Ethiopia and Kenya, has the second highest content compared with the other species (Yang et al., 2006). It is stated that all four types have an enormous potential to contribute to improved diet and health, where Moringa stenopetala is the most important economic species (Yang et al., 2006).

2.2. *Moringa stenopetala* seed characteristics

The *M. stenopetala* fruits are trilobed capsules, and are frequently referred to as pods. Immature pods are green and in some varieties have some reddish color. Pods are pendulous, brown, triangular, splitting length wise in to 3 parts when dry, 30-120 cm long and 1.8 cm. wide. The moringa stenopetala seeds are round with a white to brawn and Moringa Olifera seed hulls are generally brown to black. The hulls itself has three white wings that run from top to bottom at 120^{o} intervals. The average weight per seed is 0.3 g for M. Olifera and 0.42 g for M stenopetala and the kernel to hull mass ratio is 75:25 (Foidl et al., 2001). M. oleifera seeds are globular, about 1 cm in diameter. They are three-angled, with an average weight of about 0.3 g, 3-winged with wings produced at the base of the seed to the apex 2–2.5 cm long, 0.4–0.7 cm wide; the kernel is responsible for 70%–75% of the weight of the seed(Leone et al., 2017).



(a) (b)

Figure 2.3 *Moringa stenopetala* (a) Tree with pods (b) Seeds

The seeds of M. stenopetala are packed full of fixed oils (over 30% by weight) which are characterized by the predominant oleic acid composition (Ejigu et al., 2010; Lalas & Tsaknis, 2003). The seeds are also sources of the cationic proteins which are responsible for the flocculant activity and/or water purification potential of the plant.

M. stenopetala is a promising tree for nutrition, water purification and herbal medicine and it has many beneficial qualities, similar to those found in M. oleifera. There is however, still a great need for further detailed chemical and pharmacological evaluation of M. stenopetala. In the future, this tree could have the potential to surpass M. oleifera as an important multipurpose crop (Padayachee & Baijnath, 2012).

2.3. Plant Seed Oil

At the beginning of human civilization, animal fats like butter produced from milk of horses, goats, sheep and cattle were probably used instead of vegetable oils before the discovery of oil pressing and extraction from olives or seeds afterwards (Olson, 2010). Ancient Egyptians firstly produced infusions of medicinal or aromatic plants in vegetable oils as solvents for therapeutic, nutritional, aesthetic and spiritual purposes. They used vegetable oils in the formulation of cosmetics providing emollient, moisturizers and grooming, or acting as solvents and vehicles to carry other agents (Edinson et al., 2017).

The developments in organic chemistry and fundamental knowledge of Physics and Chemistry have provided solutions to some problems which were previously encountered in vegetable oil based products. Unlike fossil fuels which are nonrenewable and limited in supply, seed oils are renewable (Derksen et al., 1996); they can be produced on the farm by the producer to satisfy his demand. Consequently, vegetable oils are being investigated as potential replacement for fossil fuels (Lligadas et al., 2013). As natural products, seed oils have been commonly used for significant skin application from ancient times to the present. For about the last 100 years, synthetic substitutes have become available and have been used as a substitute of natural seed oils. It is now part of a growing trend to replace these synthetics and return to the use of natural oils in the cosmetic and pharmaceutical industries (Kleima et al., 2008).

Oil seed crops are major sources of lipids for human nutrition as well as for several industrial purposes. They are defined as those seeds that contain considerably large amounts of oil. The most commonly known oil seeds are; groundnut, soybean, palm kernel, cotton seed, olive, sunflower seed, rape seed, sesame seed, linseed and safflower seed among others (Samuel, 2015).

Vegetable oil processing industry involves the extraction and processing of oils and fats from vegetable sources like oil seed, fruits and nuts. Most used oilseeds include sunflower, soybean, groundnuts, coconut, palm, cottonseed, olive, corn, rapeseed, and many others (Mallyele s. V. and Kahemela I.F., 1990). Plant seeds have been used as sources of vegetable oil for food, nutraceuticals, aromatherapies, fuels, soap, body and hair oils, detergents and paint and industrial lubricants. Some of the plant seeds which have been conventionally exploited commercially for this purpose include soya beans, cotton seed, groundnut, corn, palm seeds and sunflower (Ochigbo and Paiko, 2011). Seeds of plants are a good source of food, because they contain nutrients necessary for plant’s initial growth, including many healthy fats, such as omega fats. In fact, the majority of foods consumed by human beings are seed-based foods. Seeds are typically high in unsaturated fats and, in moderation, are considered a healthy food, although not all seeds are edible (Emmanuel, 2012). Vegetable fats and oils are lipid materials derived from plants. Physically, oils are liquids while fats are solids at room temperature. Chemically, both fats and oils are composed of triglycerides. Fats are made up of saturated fatty acids while oils are up of mostly unsaturated fatty acids.

Although many different parts of plants may yield oil, in commercial practice, oil is extracted primarily from seeds of plants which grow in many different parts of the world.

2.3.1. Moringa seed oil

A lot of work has been carried out on the extraction and analysis of Moring seed oils by a number of workers, primarily on *M. oleifera* because of distributed large area of the world and extensive demands for oils both for human consumption and for industrial applications; consequently there is an increasing need to search for oils from non-conventional sources to supplement the available ones and also to meet specific applications. Previous studies on moringa have focused majorly on its use medicinally and on the nutritional uses of the tree parts specially the leaves as well as on the use of the seed in clarification during waste-water treatment (Folkard et al, 1996). The oil extracted from moringa seed is known as ben or behen oil due to the high behenic acid content relative to other oil crops. *M. stenopetala* can be used as a food, a cosmetic and a lubricant. Seed biomass remaining after the oil extraction can be used as a fertilizer or a flocculating agent for water purification. The oil extracted from Moringa seeds is regarded as having a good commercial interest due to its physical, chemical and pharmacological characteristics (Fahey, 2005). Moringa oil has been mentioned as very useful oil in the medicinal books of Greece and Rome. Even today, this oil is used for a number of industrial applications. It has great significant to use for the skin and hair. Its properties make it suitable for both human consumption and commercial purposes. Indeed, Moringa oil could be a good substitute for olive oil in the diet as well as for non-food applications, like biodiesel, cosmetics, and a lubricant for fine machinery. Moreover, after oil extraction, the seed cake can be used in waste water treatment as a natural coagulant or as an organic fertilizer to improve agricultural productivity (Yongabi, 2012).

2.3.2. Current research and literature gap analysis

Moringa Olifera seeds contain between 35-40 % (w/w) of vegetable oil (Lalas & Tsaknis, 2002) and *M. stenopetala* seed contains 44.9% (w/w) of oil (Lalas & Tsaknis, 2003), The oil from Moringa stenopetala seeds variety of Marigat from the island Kokwa was extracted using 3 different procedures including cold press, extraction with n-hexane and extraction with a mixture of chloroform and methanol (1:1) and high oil yield was obtained from n-hexane extraction. In this thesis the extraction of M. stenopetala seeds variety of Shewa Robit from Ethiopian is studied. The effect of extraction temperature below and near to the boiling

point was investigated in the previous study but in this study the effect of extraction temperature above the boiling point of hexane on oil yield. The effect of particle size on the oil yield was not considered in the previous studies even though it greatly affect the oil yield in solvent extraction method but in this work the effect of three particle size ranges are considered. The fatty acid composition of *M. stenopetala* seed oil of the Ethiopian variety is investigated and compared with the fatty acid composition of *M. oleifera* and olive oil. This oil resembles olive oil in its fatty acid composition and is oleic acid-rich, which makes it suitable for edible purposes. Moreover, other applications have been pointed out as preparation of cosmetics, mechanical lubricant, and lately for potential biodiesel fuel elaboration. The extraction kinetics of different oil seeds extraction process is studied to now the characteristics of extraction and time factor on the extraction process. In this thesis the kinetics of extraction process and the model equation that represent lab scale solvent extraction of *M. stenopetala* seed kernel oil is investigated.

The prices of vegetable oil have also increased dramatically in the last few decades and this will affect the economic viability of biodiesel industry. According to (Ejigu et al., 2010) the use of Moringa seed oil as a feedstock for producing biodiesel is considered but the use of this nutritional and pharmaceutical oil to biodiesel production is not commendable and economically feasible, furthermore, the use of such valuable oil to produce biodiesel is not feasible in the long term due to the use of this oil for food pharmaceuticals and cosmetics applications.

2.4. Extraction Technology

The main point of extraction is the separation of the oil from the oil-bearing material with subsequent purification of the raw oil, but looking at the oil production as a whole not only the oil processing is important for the production of high-quality oil, but also the harvest, the pretreatment of the harvested material, and the storage conditions until processing have to be taken into consideration. The aim of the extraction method is to optimize the oil yield with simultaneous maintenance of the oil quality (Gupta, 2012).

There are three main techniques that have been identified for extraction of oil: mechanical extraction, chemical or solvent extraction, and enzymatic extraction. Besides, Accelerated solvent extraction (ASE), Supercritical fluid extraction (SFE) as well as Microwave-assisted extraction (MAE) method is frequently used. It has been observed that mechanical pressing

and solvent extraction are the most commonly used methods for commercial oil extraction (Atabani et al., 2013).

2.4.1. Mechanical oil extraction

Prior to the discovery of any other oil extraction method oil was extracted mechanically or cold pressed. It is the simple process of heating the plant material to low temperatures and then physically pressing the oil out. Today mechanical expression is used mainly for citrus peels and is unpopular due to the low extraction yield. Mechanical press oil extraction is the most conventional technique. A manual ram press or an engine driven screw press can be used (Jahirul, et al, 2013). It has been found that engine driven screw press can extract 68–80% of the available oil while the ram presses only achieved 60–65% (Bhuiya et al., 2015).

According to literature, oil extraction efficiencies calculated from data reported in more recent studies are found to generally correspond to these ranges, although the efficiency range of engine driven screw presses can be broadened to 70–80% (Atabani et al., 2012, 2013; Bhuiya et al., 2015). The oil extracted by mechanical presses needs further treatment of filtration and degumming in order to produce a purer raw material (Atabani et al., 2013; Lokanatham & K, 2013). An additional problem associated with conventional mechanical presses is that the design of mechanical extractor is suited for some seeds, and therefore, the oil yield is affected if that mechanical extractor is used for other seeds (Atabani et al., 2012, 2013; Bhuiya et al., 2015; Lokanatham & K, 2013). It has been also found that pretreatment of seeds before applying mechanical extractor increases the amount of oil recovery (Atabani et al., 2013).

2.4.2. Solvent extraction

The solvent extraction is chemical oil extraction method which is the most popular method of extraction of oil because of its high percentage of oil recovery from seeds. Solvent extraction bridges the gap between mechanical extraction which produces oil with high turbidity metal and water content and supercritical fluid extraction which is very costly to build and maintain its facilities. Solvent extraction is the process in which the oil is removed from a solid by means of a liquid solvent, it is also known as leaching (Bhuiya,et al., 2016).

Solvents are compounds that are generally liquid at room temperature and atmospheric pressure; they are able to dissolve other substances without chemically changing them. The

liquid mixture formed on dissolving a substance (solute) in a solvent is termed a solution. The molecules of the solution components interact with one another. Solutions are obtained by mixing liquid, solid, or gaseous components with liquids, the liquid always being termed the solvent. When performed at low temperature, solvent extraction has another advantage over screw-pressing because it gives a better quality oil (Yahaya, et al., 2016). Solvent extraction is basically a mass transfer operation involving diffusion of a suitable solvent into oil-bearing cells of the raw material, resulting in an oil-solvent solution (miscella). The oil is then distilled and the solvent evaporated leaving the oil behind. This process leaves minimum oil residual in the cake compared to mechanical.

Soxhlet extraction is required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled (Pandey & Tripathi, 2014). Extraction of oil using solvents is the most effective method for oil recovery of almost 98%, especially with materials having low oil content.

Common solvent extraction uses a pure organic or mixed organics to extract the valuable extracts from the plant material. Typical solvents include ethyl acetate, diethyl ether, methanol, ethanol, petroleum ether, isopropyl alcohol, carbon tetrachloride and hexane. The chemical extraction using n-hexane method results in the highest oil yield which makes it the most commonly used solvent. N-hexane is selected due to its low viscosity, high volatility, simple structure and percentage of saturation etc. Other factors such as economics high solvency, low boiling point low toxicity, and low cost also considered (Atabani et al., 2012). Hexane is often used as a solvent for oil extraction due to its lower boiling point for easy separation after extraction, its non-polar nature which makes it suitable for extracting vegetable oils which are generally non-polar and its comparatively low toxicity when compared to other solvents. There are, however, some disadvantages associated with the solvent extraction technique. Solvent residues often contaminate the product; therefore, with solvent extraction effective separation of the extracted oil from the solvent is necessary to remove any solvent which may contaminate the oils.

2.4.3. Factors that affect solvent Extraction

It has been observed that there are many factors affecting the rate of solvent extraction such as particle size, the type of solvent used, temperature, time and agitation speed (Atabani et al., 2012, 2013). The oil yield from Moringa seeds depends on the nature of the solvent, the temperature of extraction, seed particle size, contact (residence) time between the solvent and the seed and pre-treatment conditions (Sayyar, Abidin, Yunus, & Muhammad, 2009).

a. Particle size

Particle size influences the extraction rate in a number of ways. The smaller the size, the greater is the interfacial area between the solid and liquid, and therefore the higher is the rate of transfer of material and the smaller is the distance the solute must diffuse within the solid as already indicated. On the other hand, the surface may not be so effectively used with a very fine material if the circulation of the liquid is impeded, and separation of the particles from the liquid and drainage of the solid residue are made more difficult (Richardson et al., 2002).

b. Solvent type

The solvent has to be selected in such a way that it would be a good selective solvent and its viscosity would be sufficiently low to circulate freely. Generally, a relatively pure solvent will be used initially, although as the extraction proceeds the concentration of solute will increase and the rate of extraction will progressively decrease, first because the concentration gradient will be reduced, and secondly because the solution will generally become more viscous. The most commonly used solvents for food processing are water, aqueous solutions of acids and nontoxic salts, commercial hexane, and in some cases other alkanes, ethanol and to a lesser extent the other lower alcohols, methylene chloride, methyl ethyl ketone, and acetone.

According to Sayyar, et al., (2009) oil extracted Jatropha seed by n-hexane and petroleum ether and found that the extraction yield with n-hexane to be about 1.3% more than that of petroleum ether (47.3% and 46.0% wt. respectively) under similar conditions. The authors recognized n-hexane as a more preferable solvent for extraction of Jatropha seed oil as compared to petroleum ether. In the extraction of olive oil using organic solvents like hexane, ethanol, petroleum ether, isopropyl alcohol and carbon tetrachloride by a Soxhlet extractor,

(Banat,et al., 2013) did also obtain the highest oil yield (12.7%) by n-hexane. However, it has been observed that this method consumes much more time compared to other techniques. The solvent extraction is only economical attractive at a large-scale of production (Atabani et al., 2012).

c. Temperature

Temperature generally affects both the equilibrium and mass transfer rate of the extraction process. In the former, higher temperature results in greater solubility of compounds in the solvent, resulting in a larger *K* value (equilibrium constant). In the latter, the higher the temperature, the higher will be the *D (*diffusion coefficient*)*, hence increasing the rate of extraction. In conventional solid-liquid (solvent) extraction processes, the temperature is limited by the boiling point of the solvent. It is also important to bear in mind that increasing the extraction temperature may also potentially degrade bioactive compounds. Thus, an optimized balance has to be determined when selecting the extraction temperature.

Oil solubility in solvent increases with extraction temperature, High temperature also has a positive effect on viscosity and diffusivity of oil. Viscosity decreases while diffusivity increases as the extraction temperature increases, resulting in shorter extraction times. The energy required for solvent recovery decreases when the higher operating temperature is used for extraction. However, high temperatures may cause deterioration and denaturation of some oil and meal components. Hence, temperature selection is based on the type of oil and required specifications of the final product. It is expected that residual oil in the meal to be less than 1 percent after commercial solvent extraction.

d. Extraction Time

In general, a prolonged extraction time results in an increased yield of the oil until equilibrium is reached. Thereafter, the concentration of compound will not increase further but there will have greater liability for degradation. Prolonged extraction time is also not desirable from an economic standpoint of labor and energy requirements. Therefore it is essential to find an optimum extraction time.

2.4.4. Accelerated solvent extraction

Accelerated solvent extraction (ASE) is also referred to as pressurized solvent extraction (PSE) is another modern oil extraction technique which uses organic or aqueous solvents at elevated temperatures and pressures (Bhuiya et al., 2015). It has been observed that high temperature accelerates the extraction rate, while elevated pressure prevents boiling at temperatures above the normal boiling point of the solvent.

2.4.5. Enzymatic oil extraction

Aqueous enzymatic oil extraction (AEOE) method is a promising technique for extraction of oil from plant materials (Rosenthal et al., 2001; Shah et al., 2005). In AEOE, enzymes should be used to extract oil from crushed seeds (Mahanta & Shrivastava, 2018). Aqueous enzymatic oil extraction can also be used in combination with other methods of oil extraction.

2.4.6. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) also called microwave extraction, is a new extraction technique, which combines microwave and traditional solvent extraction (Hao et al., 2002). MAE has been regarded as an important alternative in extraction techniques which have several advantages over other extraction processes, such as reduction of costs, shorter time, less solvent, higher extraction rate, and better products with lower cost, reduce energy consumption and CO_2 emissions (Cardoso et al., 2013; Hao et al., 2002). In microwave-assisted aqueous enzymatic extraction (MAAEE) of pumpkin seed oil by using mixtures of cellulose, pectinase and proteinase (w/w/w) (Jiao et al., 2014) obtained the highest oil recovery of 64.17%. The authors also reported that there were no significant differences in physicochemical properties of MAAEE and soxhlet extracted oils, and thus, MAAEE is a promising and environmentally friendly technique for pumpkin seed oil extraction..

2.4.7. Supercritical fluid extraction (SFE)

SFE is used on a large scale for the extraction of some food grade oils and pharmaceutical Products from plants. It is comparatively rapid because of the low viscosities and high diffusivities associated with supercritical fluids. The extraction can be selective to some extent by controlling the density of the medium and the extracted material is easily recovered

by simply depressurizing, allowing the supercritical fluid to return to the gas phase and evaporate leaving no or little solvent residues (Mohamed, Abdulamir, & Abas, 2008).

Supercritical fluid extraction (SFE) of oils is a modern technique, currently being applied in the process industry. Supercritical carbon dioxide can also be used as a more environmentally friendly solvent for dry cleaning as compared to more traditional solvents such as hydrocarbons and percholroethylene. Supercritical carbon dioxide is used as the extraction solvent for the creation of essential oils and other herbal distillation. Its main advantages over solvents such as hexane and acetone in this process are that it is non-toxic and non-flammable. Furthermore, separation of the reaction components from the starting material is much simpler than with traditional organic solvents, merely by allowing it to evaporate into the air or recycling it by condensation into a cold recovery vessel.

Table 2.1 Advantages and Disadvantages of Extraction Technologies

Method	Advantages	Disadvantages
Mechanical press	❖ Virgin oil is more sought after ❖ the potential for solvent contamination ❖ Relatively inexpensive after initial capital costs ❖ Minor consumable costs ❖ Whole seeds or kernels can be processed ❖ No environmental problem	❖ Generally ineffective in Moringa oil extraction ❖ Time and labor intensive ❖ Relatively low yield and high oil loss ❖ High dependence on kernel moisture content ❖ Filtration/ degumming required ❖ Low and inconsistent oil production
n-Hexane	❖ Repeatable and reproducible results and process ❖ High oil yields ❖ Relatively simple and quick ❖ Suitable for bulk oil extraction ❖ Low capital investment ❖ No especial equipment required ❖ Hexane can be recovered and reused, reducing cost significantly	❖ Less sought after than virgin oil ❖ High potential for solvent contamination ❖ Safety issues and environmental concerns ❖ Very costly if the hexane cannot be recovered ❖ High hexane requirement ❖ Only kernel can be processed
Accelerated solvent extraction(ASE)	❖ Automatic technique ❖ Condition can be optimized ❖ More efficient and Clean process ❖ Relatively less solvent consumption ❖ Less time and labor incentives ❖ High oil yield	❖ Very high initial cost ❖ High preparation required ❖ Special equipment and skill required ❖ Potential for solvent contamination ❖ Only kernel can be processed
Supercritical Fluid extraction(SFE)	❖ Nontoxic solvent like CO_2 is used ❖ Fast extraction ❖ Less Separation cost	❖ Expensive initial cost

The results from the mechanical and chemical extraction methods clearly indicate that the hexane method is superior in terms of producing higher oil yields. It was also observed that the chemical method is more repeatable, relative ease of preparation and no requirement for

extensive training. Mechanical extraction using the screw press can produce oil from appropriately prepared product, but overall this method is ineffective, with relatively low yields for a great deal of effort. Chemical extraction using hexane as a solvent was found to be very effective, but due to a limited supply of hexane and the lack of a hexane recovery system, it was not possible to take full advantage of the effectiveness of the method. Moreover, as evidenced by the bulk extraction results, the hexane method proved to be a vastly more time and labor efficient process than mechanical extraction in the given circumstances (Bhuiya et al., 2015; Jahirul et al., 2013).

2.5. Vegetable Oil Composition and Application

2.5.1. Fatty acids composition in vegetable oil

Fats and oils are the most abundant lipids in nature. They provide energy for living organisms, insulate body organs, and transport fat soluble vitamins through the blood. They are insoluble in water but soluble in most organic solvents. Fatty acids are chains of covalently linked carbon atoms, bearing hydrogen atoms which terminate in a carboxyl group that is responsible for their properties as acids. Fatty acids are a component of fats (triglycerides) where they are combined with glycerin. Vegetable oils are the main source of fatty acid, which are predominantly triglycerides (95% - 98%), the remaining (5% - 2%) consists of complex mixtures of minor compounds in a wide range of chemical classes (Sirkoska et al., 2004) including: fatty alcohols, waxes, esters, hydrocarbons, volatiles, pigments, phenolic compounds, glyceride compounds, phospholipids and triterpenic acids (Cert et al., 2000).Fats and oils are called triglycerides (or triacylcylgerols) because they are esters composed of three fatty acid units joined to glycerol, a trihydroxy alcohol. The following figure shows the chemical structure and formation of triglycerides.

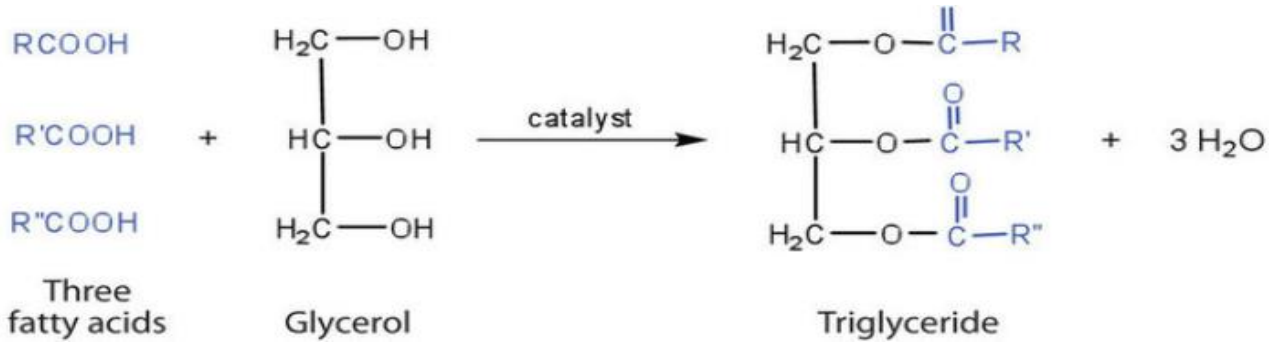


Figure 2.4 Chemical Structure of Triglyceride

If all three OH groups on the glycerol molecule are esterified with the same fatty acid, the resulting ester is called a simple triglyceride. Although simple triglycerides have been synthesized in the laboratory, they rarely occur in nature. Instead, a typical triglyceride obtained from naturally occurring fats and oils contains two or three different fatty acid components and is thus termed a mixed triglyceride.

These fatty acids are structurally diverse and often have unusual and interesting structures and, although they can have from 8 to over 80 carbon atoms, they are typically comprised of an even number of carbon atoms. In addition, the alkyl chains can contain a variety of functional groups and also have from 0 to 6 carbon-carbon double bonds that predominantly possess cis-geometry, although, fatty acids having trans-geometry are also known. Because of their structural diversity, fatty acids are extensively used as feedstock for food applications and the oleo chemicals industry for the manufacture of soaps, detergents, lubricants, coatings, and cosmetics among other specialty products (Kenar et al, 2017). Of the many fatty acids, only 20-25 are widely distributed in nature, and are of commercial significance. These fatty acids range between 10 and 22 carbons in length and are obtained in large quantities from the major domesticated commodity plant oils and animal fats.

Based on the degree of unsaturation the most common fatty acids have been subdivided into three broad classes; saturated fatty acids (SFA) in which all the carbon atoms are singly bonded, monounsaturated fatty acids (MUFA) these groups have one double bond and polyunsaturated fatty acids (PUFA) groups with two or more double bonds.

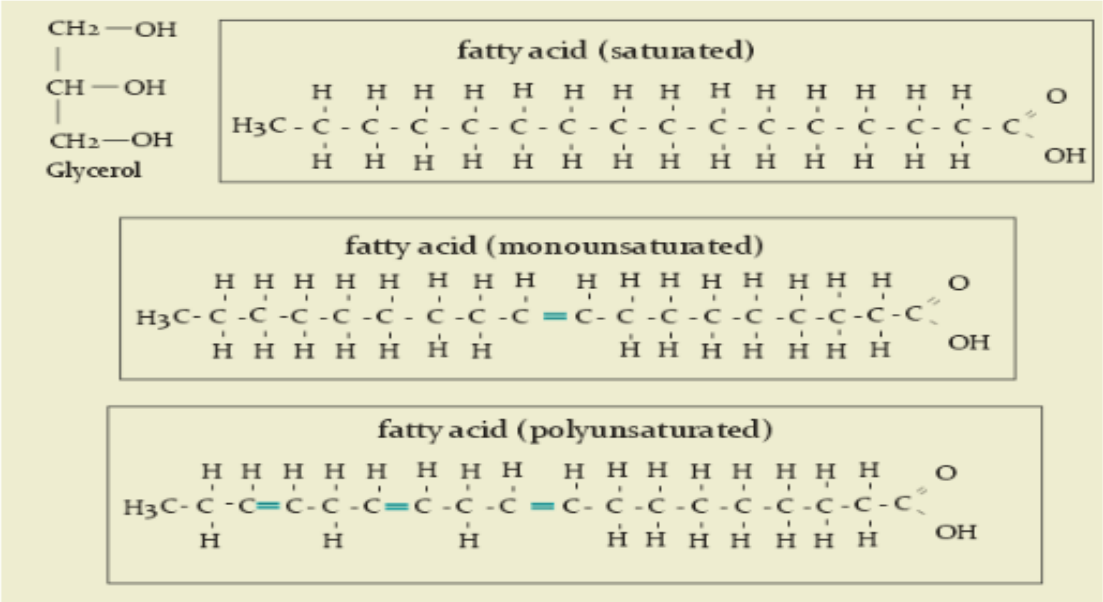


Figure 2.5 The structure of different components of triglycerides

In general, these fatty acids have an even number of carbon atoms and have un-branched structures. The double bonds of naturally occurring unsaturated fatty acids are very often of the cis orientation. A cis configuration means that the hydrogen atoms attached to the double bonds are on the same side. If the hydrogen atoms are on opposite sides, the configuration is termed trans. Mono and Polyunsaturated fatty acids can be classified in to Omega -3, Omega -6 and Omega -9 based on the location of the double bond in the chain. The fatty acid group classification is shown in figure 2.6.

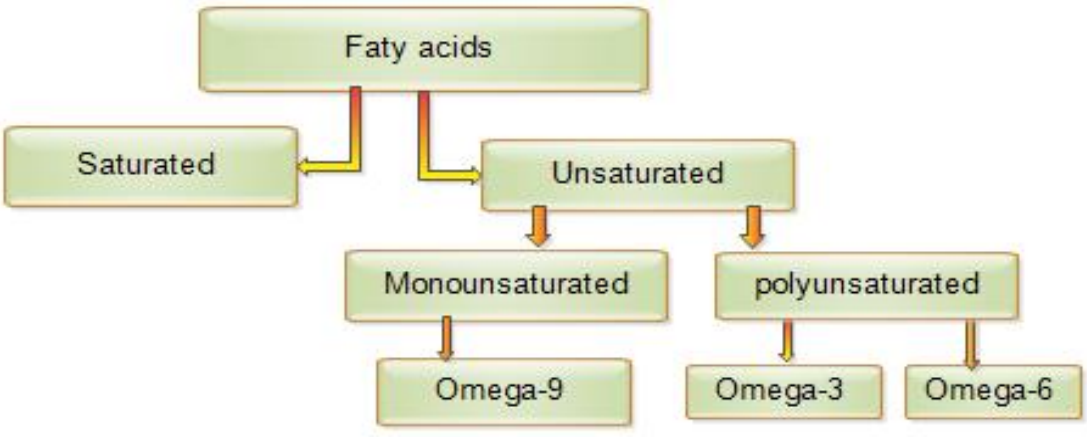


Figure 2.6 Fatty acids classification

Fatty acids and derivatives are critical components of cosmetic and personal care products. Their functions encompass aspects of product stabilization, function, and esthetics. Although

fatty acids and their salts were most likely the first cleansers and emulsion stabilizers to be used in what are now considered cosmetic and personal care products (Kelm & Wickett, 2017).

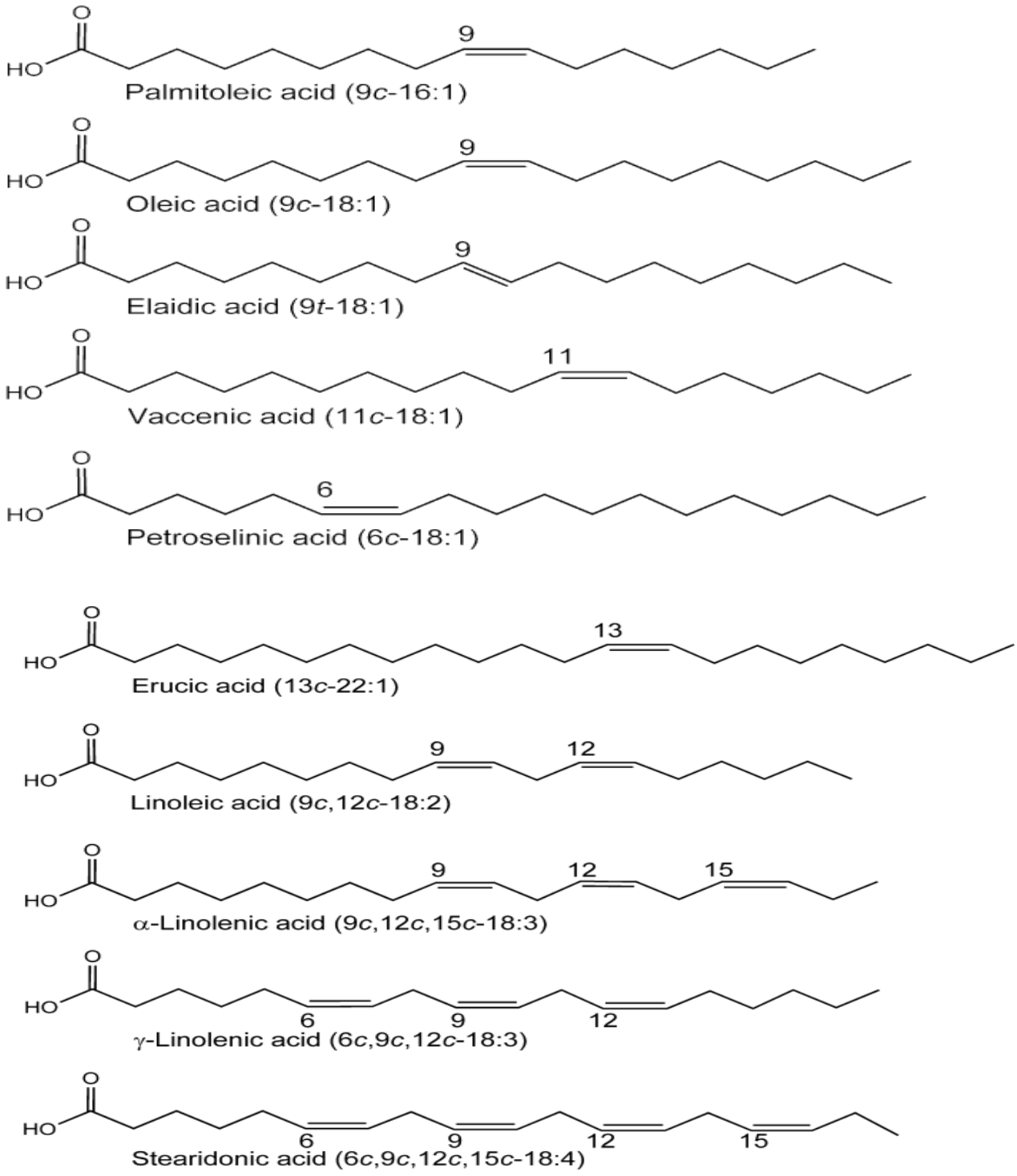


Figure 2.7 Structure of Some Monounsaturated and Polyunsaturated Fatty Acids

2.5.2. Minor Components in Vegetable Oils

Vegetable oils contain a wide range of minor components besides triacylglycerols, the major components of most oils. Sterols, tocopherols, carotenoids, phenolic compounds, chlorophyll, free fatty acids, and other minor derivatives, as well as trace metal ions are the most recognized of these compounds. Minor components (less than 5%) in vegetable oils, the presence cannot be ignored due to their interesting biological properties and nutritional values for pharmaceutical and nutraceutical industries, particularly in virgin pharmaceutical and nutraceutical industries. The tocopherols are interesting, as they are primarily responsible for the stability of the polyunsaturated fatty acids of vegetable oils. Tocopherols and carotenoids are important for human nutrition as they provide Vitamins E and A activities (Ferrari et al., 1996; Purcaro et al., 2016). Miner components of vegetable oil can be divided into two types: glycerolipids such as mono and diglycerides, phospholipids, and nonglycerolipids including sterols, tocopherols, free fatty acids, vitamins, pigments, proteins, phenolic compounds, water, etc. (Aluyor et al., 2009; Chen et al., 2011).

2.5.3. Application of Vegetable Oils

The oils of oilseed crops are major agricultural commodities that are used primarily for nutritional applications, but in recent years there has been increasing use of these oils for production of biofuels and chemical feedstocks. Oil seed crops are major sources of lipids for human nutrition as well as for several industrial purposes. They are defined as those seeds that contain considerably large amounts of oil. The most commonly known oil seeds (conventional oil seed) are; groundnut, soybean, palm kernel, cotton seed, olive, sunflower seed, rape seed, sesame seed, linseed and safflower seed among others.

a. Food and feed

The largest proportion of plant oils is consumed as food and feed, and the oils used in these markets contain various proportions of the five common, nutritionally important FAs. These five fatty acids are palmitic (16:0), stearic (18:0), oleic (18:1ω 9), linoleic (18:2 ω 9,12) and α linolenic (18:3 ω 9,12,15) acids. The properties of oils depend greatly on their fatty acid composition, and certain compositions are desirable for specific end uses. For example, cooking oils generally contain a higher proportion of mono-unsaturated FAs (such as oleic

acid), which are more stable under high temperature, while margarine and spreads are often rich in saturated fatty acids (e.g. palmitic and stearic acids).

b. Production of biodiesel

In light of rising petroleum prices and environmental concerns, the use of plant oils as liquid fuel has seen a strong increase, especially in Europe where biodiesel is already a major fuel derived from oils such as rapeseed, sunflower or palm.

c. Industrial feedstocks

There are numerous uses and applications of plant seed oils for plant-derived industrial feedstocks.

Table 2.2 Products produced from Plant oil

Derivatives	Applications
Fatty acids and derivatives	Metallic soaps, detergents, soaps, cosmetics, alkyd resins, paints, textile, leather and paper industries, rubber, lubricants
Fatty acid methyl esters	Biodiesel, cosmetics, solvents, intermediates in the production of alcohols
Glycerol and derivatives	Cosmetics, toothpaste, pharmaceuticals, food, paints, plastics, synthetic resins, tobacco, explosives, cellulose processing
Fatty alcohols derivatives	Detergents, cosmetics, textile, leather, and paper industries, duplicator stencils, petroleum additives
Fatty amines and derivative	Surfactants, fabric softeners, mining, road building, biocides, textile and fiber industries, petroleum additives
Drying oils	Paints, varnish, linoleum

2.5.4. Application of Vegetable Oil for Cosmetics Additives

Food and Drug Administration (FDA) of the United States, which regulates cosmetics, defines cosmetics as "Intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions." Products such as skin creams, lotions, shampoos hair oils, perfumes, lipsticks, fingernail polishes, eye and facial make-up preparations, permanent waves, hair colors, toothpaste, deodorants, and any material intended for use as a component of a cosmetic product are included in this definition. Cosmetics refer to all of the products used to care for and clean the human skin and make it more beautiful. The intentions of using cosmetic products are to maintain the body in a good condition protect it from the effects of the environment and aging processes, change the appearance, and make the body smell nicer. Cosmetic products are widely used by every socioeconomic class of human beings to cleanse, perfume, protect, and change the appearance of skin (Sumit et al., 2012). In many cosmetic products, oil components form an important part of the formulation. Vegetable oils are a rich source of fatty acids and have been successfully used in cosmetic products. Because of their oiling, softening, smoothing and protective properties they are classified to the group of emollients. They make the skin look smooth and properly moistened. These oils can be derived from a variety of plants and plant parts. Olive oil, corn oil, avocado oil, safflower oil, castor oil, cocoa butter and others are some of the examples (zielińska, 2014).

In recent days, the interest in natural and organic cosmetics has increased among the consumers. This is due to the increase in wariness of the consumer to the chemical usage in cosmetic products. As the industry seeks for the natural alternatives, the demand for seed oil has increased dramatically (Afiq, Rahman et al., 2013).The beneficial effect of the oil used in cosmetics and other personal care products is due to the fatty acids composition of the oil (Vermaak et al., 2011).

In the modern era of personal care products, due to the enormous consumer awareness about healthcare concerns associated with traditional cosmetics and also as a result of a better scientific understanding of the skin physiology, a plethora of cosmetic products with innovative natural ingredients and formulations have been fashioned to meet the demands. These products with additional health purpose apart from the cosmetic application are referred as cosmeceuticals which refers to a hybrid functionality combining the principles of

cosmetics and drugs (Dorni & Amalraj, 2017). Now a day there is turn to return towards the use of herbal products and to adopt a more natural way of life. The seed oil is used as a source in personal care products due to its healing and nurturing properties. A hair lotion prepared with the oil is used to revitalize, strengthen and detangle hair and it is used as a body rub during dry winter months. It protects the skin by acting as a cleanser, moisturizer and emollient (Juliani et al., 2007). The oil of excellent quality similar to the olive oil, of Moringa seed oil finds wide application in cosmetic industry.

Seed oils used in cosmetics contain a range of fatty acids which contribute several beneficial properties in cosmetic and personal care products. Fatty acids are divided into saturated acids (e.g. palmitic; stearic; arachidic) and unsaturated acids (e.g. oleic; linoleic). The proportion of saturated and unsaturated fatty acid has an important role in the behavior of vegetable oils. For cosmetic products, linoleic acid is the most frequently used essential fatty acid. It prevents barrier and cornification disorders, lowers the trans epidermal water loss and increases skin moistness (Lautenschläger, 2003). Palmitic, oleic and stearic acids are synthesized in the body but linoleic acid is not and a deficiency will cause various signs. The skin dries out and becomes scaly, nails crack, and hair loss as well as trans epidermal water loss increases (Lautenschläger, 2003; Vermaak et al., 2011). These natural oils are of significant nutritional importance and are also desirable emollients for skin care applications. However, the drawback of the unsaturated oils is their high sensitivity to oxidation and many of them present limited shelf-life during storage as well as in application (Alander et al., 2006). In general, oxygen reacts with the double bonds present in lipids, following a free radical mechanism, known as autooxidation. This reaction is quite complex and depends on the lipid type used and the processing conditions. Consequently, the shelf life and the final use of any lipid depend on its resistance to oxidation or oxidative stability (Martinez and Saldana, 2016).

The linoleic acid is widely used in cosmetic products as it helps to heal dermatoses, sun burns and effectively treat acne vulgaris (Lautenschlager, 2003). Oleic acids, which is unsaturated fatty acids, exhibited the best permeation enhancing effect, while amongst the saturated fatty acids, palmitic acid had the most potent skin permeation enhancing effect (Kim et al., 2008). The high oleic acid content in seed oil can be a good source of effective percutaneous absorption enhancer by enhancing the penetration of tenoxicam. Tenoxicam is a non-steroidal anti-inflammatory drug (NSAID), an effective anti-inflammatory and analgesics, which has

been widely used in conditions such as chronic rheumatic disorders treatment (Larrucea et al., 2001). The unsaturated fatty acids most important in cosmetology and medicine include (Zielinska, 2014):

- Monounsaturated fatty acids , containing one double bond, such as palmitoleinic acid 16:1 (ω -7) , oleic acid 18:1 (ω -9) , erucic acid 22:1 (ω -9) , nervonic acid 24:1 (ω -9).
- Biunsaturated acids (diene) having two double bonds , such as 18:2 linoleic acid (ω-6)
- Triunsaturated acids (triene), containing three double bonds , such as α-linolenic acid 18:3 (ω-3 , ALA) acid, γ-linolenic acid 18:3 (ω -6 , GLA).

Moringa oil is utilizable in creams, lotions, balms, scrubs, body oils, and hair care formulations. Another interesting application of Moringa oil is in the production of expensive and natural perfumes and fragrances. Many commercially produced perfumes are created with scents that are synthesized using chemicals. However, a portion of the perfume market still employs more traditional and natural production practices to create their perfumes, using a technique known as effleurage. This procedure uses oils to capture the scents of natural plant materials, locking the scent molecules into the oil. Moringa oil's high oleic level, combined with its enduring shelf life, make it a popular choice for traditional perfume production. Moring seed oil is a rich source of vitamin A, and E, with strong antibacterial properties and it confers softness and smoothness to dry and tuff skin. It also possesses antihypertensive, antifungal and antiepileptic characteristics. The seed extract has been found to possess good antimicrobial activity against numerous bacterial and fungal species. Powdered seed act as a natural flocculent; able to clarify even the most turbid water. The powder joins with the solids in the water and sinks to the bottom. This treatment also removes 90- 99% of bacteria contained in water, water purification by flocculation, sedimentation and antibiosis (Mahmood et al., 2010).

The bactericidal and antifungal properties of fatty acids are well known. Fatty acids can inhibit the growth of numerous types of bacteria, as well as protozoans, viruses and fungi. According to (Mcgaw et al., 2002) unsaturated fatty acids showed strong bactericidal activity against Mycobacterium segment is in low concentrations, whereas saturated fatty acids, except for lauric and myristic acids, were not very effective. Palmitic, arachidonic and linoleic acids displayed strong antibacterial activity, among the saturated fatty acids and oleic and linoleic acids from the unsaturated fatty acids. Saturated fatty acids are effective against

microorganisms at lower chain lengths, while monounsaturated and polyunsaturated fatty acids with longer chain lengths are more effective.

Unsaturated fatty acids present in vegetable oils are characterized by high absorbability and antiallergic properties. Thanks to their beneficial and diverse effects on the skin they have found wide applications in many branches of industry, in particular in cosmetic industry and cosmetology, pharmacy and medicine. In cosmetic industry, vegetable oils are used mainly as the vehicle for other active ingredients, dissolved or dispersed in oil-water type emulsions. The most often used essential unsaturated fatty acids are those from the omega-3, omega-6 and omega-9 series. The most important among them are 18-carbon acids (C18): monounsaturated (omega-9) – oleic acid, biunsaturated (omega-6) linoleic acid, including cis-linoleic acid with conjugated double bonds at positions 3 and 6 (Zielinska, 2014).

Moringa oil is highly stable, penetrating, rich in anti-oxidants, it helps to improve the appearance and radiance of the skin and is a remarkably nutritive addition to facial creams, anti-wrinkle serums, anti-aging formulations, lip care products, hair products, make-up, and sun care preparations, shaving products, massage and other cosmetic formulations. It is balancing oil and is suitable for dry, oily and combination skin. Within hair care applications, Moringa oil is regarded as cleansing oil because it helps to rid the scalp and hair of dirt and foreign residue. Virgin Moringa oil is also known for its ability to act as a natural fixative as it helps stabilize volatile and flighty fragrances.

2.6. Characteristics of Seeds and Seed Oil

2.6.1. Proximate composition of *M. Stenopetala* Seeds

The nutritional composition of oil seeds can vary depending on the environment, climate, soil nutrition, biology, agronomic practices and stress factors (biotic and a biotic).

Moisture content

The moisture content of seeds is an important factor that affects the yield and quality of the oil extracted. Turbid oil is obtained from seeds with high moisture level; therefore, moisture adjustment of the seed is necessary before pressing. Thus, moisture increases the flow of oil through the pores of the press cake, hence reducing the amount of oil entrained in the cake and increasing the oil yield mostly in mechanical expression (Aremu et al., 2015).

Ash content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the analyte) can be distinguished from all the other components (the matrix) within a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components.

Oil yield

Oil yield refers to the amount of oil that can be derived from an oilseed or from algae. It is usually represented as a % of sample weight. Most oil seeds and nuts are heat-treated by roasting to liquefy the oil in the seed cells and facilitate its release during extraction. All oil seeds and nuts undergo this treatment except palm fruits for which "sterilization" replaces this operation. For large scale production, oil seeds are dried to moisture content around 10%. An increase in the heating temperature and heating time increases the free fatty acid value, peroxide value and the color intensity of the oil. Several parameters (particle size, flakes, thickness, extraction time, extraction temperature and moisture content of seed) affecting the solvent extraction of edible oil were investigated. Size reduction of the sample is one of the most important steps in oil content analysis.

2.7. Physico-chemical Characteristics of Oil

a. Specific gravity

The specific gravity (SG) indicates the FAs average molecular weight of oil (Frank Gunstone, 2011). It is the heaviness of a substance compared to that of water, and it is expressed without units. The SG is proportional to the FAs mean chain-length of the oil, as the FA chain-length is proportional to the FA molecular mass. As the temperature increases, the SG of the oil decreases (Lawson, 1995).

b. Viscosity

Viscosity is a measure of the resistance offered by a fluid to flow. Viscosity may be considered the integral of the interaction forces of molecules. When heat is applied to fluids, molecules can then slide over each other more easily making the liquid to become less viscous. Viscosity can be reported as kinematic viscosity or dynamic viscosity with the two values being related through density. The viscosity of a vegetable oil depends on its chemical composition and the temperature of measurement. Which correlated with density, refraction and surface tension (Frank D. Gunstone, 2011).

c. Acid value

The acidity of oil is given by the number of FAs derived from the hydrolysis of the triglycerides i.e. separation between FA and the glycerol in the triglyceride (Gurr, 1999). This alteration occurs under unsuitable conditions of treatment and preservation of the oil. The oil acidity, can therefore, indicates the purity of the oil. The oil acidity is expressed either as the percentage of free FAs or in terms of the number of milligrams of KOH required to neutralize one gram of sample (mg KOH/g) (Frank D. Gunstone, 2011). Numerically the acidity of ordinary fats and oils is approximate twice the percentage of free Fas (Aremu et al., 2015).

d. Iodine value

The Iodine Number gives a measure of the average degree of unsaturation of oils and fats: the higher the iodine value, the greater the number of C=C double bonds. By definition, Iodine value is expressed as the number of milligrams absorbed per gram of sample and it is an identity characteristic of oils, making it an excellent raw material for soaps and cosmetics industries or other application (Aremu et al., 2015). For the reviewed oils, iodine value ranges from 2.65 in almond seed oils to 153.00 in melon seed oil. The iodine value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of the oil to oxidation. Oils with iodine value less than 100 gI_2/100g of oil are non-drying oils; correspondingly, (Aremu et al., 2006) reported that the lower the iodine value the lesser the number of unsaturated bonds; thus the lower the susceptibility of such oil to oxidative rancidity. Oil and fats can be divided as follows based on the iodine value.

a. Drying fats and oils: oils with an iodine value of 130-200,

b. Semi drying fats and oils: oils with an iodine value of 100-130, and

c) Non-drying fats and oils: oils with an iodine value lower than 100.

Therefore, non-drying oils are not suitable for ink and paint production due to their non-drying characteristics but may be useful in the manufacture of cosmetics and can be regarded as liquid oil.

e. Saponification value

Saponification value (SV) is defined as the number of milligrams of potassium hydroxide required to saponify 1 g of oil (AOCS, 1993). It is an indicator of molecular weight or size as a function of the chain lengths of the constituent FAs (Agatemor, 2006). The saponification value of around 195 indicates that oil contains mainly FAs of high molecular mass. For instance, the saponification value of palm oil ranges from 196 to 205, that of olive oil from 185 to 196, linseed oil from 193 to 195, cotton seed from 193 to 195 and that of soya bean oil is around 193(Pearson, 1981). One the other hand oils having high saponification value (around 300) have mainly FAs of low molecular mass and are useful for soap making (Alabi,1993).

2.8. Kinetics Study of Solvent Extraction of Seed Oil

Solid liquid extraction is a mass transfer process because there is no reaction between the seed oil and the organic solvent. This process consisting of two stages: In first stage the major part of the solute gets extracted quickly because of the dissolution and scrubbing of the surface solute caused by higher driving force of the fresh solvent and in the second stage extraction rate is slowed down due to slow diffusion of the remainder solute (Saxena et al., 2015). The process is controlled by diffusion due to an oil concentration gradient in the solid phase. For this model, the main mechanism which controls the rate of seed oil extraction assumed to be the mass transfer of seed oil from the seed (solid) to bulk liquid *n*-hexane.

Applying diffusive model to explain mass transfer means solving Fick's Second Law. However, mass flow by diffusion requires knowledge of the concentration gradient inside the particles, which is difficult to determine. At the solid-liquid interface, mass flow by diffusion is equal to mass flow by convection. For these reasons, the model of mass transfer by convection has been used to represent kinetics of the vegetable oil extraction process (Saxena et al., 2015; Sayyar et al., 2009).

To describe the process a typical second order reaction kinetics for the solid-liquid extraction process has been proposed by most of the researchers (Meziane et al., 2006; Porte et al., 2005; Saxena et al., 2015; Sayyar et al., 2009). Considering that the process occurs at non steady state and there are no chemical reactions, the rate of variation of the oil concentration in a solvent or the rate of solute dissolution in the solvent can be described by the following second order kinetics equation is obtained.

$$\frac{dC_t}{d_t} = k(C_s - C_t)^2 \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. (2.1)$$

Where k= second order extraction rate constant (L g^{-1} min^{-1})

Cs= the extraction capacity (concentration of oil at saturation in (g L^{-1})

Ct= the concentration of oil in the solution at any time (g L^{-1}), t (min).

By considering the boundary condition t = 0 - t and Ct = 0 - Ct, the integrated rate law for a second-order extraction was obtained:

$$C_t = \frac{C_s^2 kt}{1 + C_s\, kt} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. \quad (2.2)$$

The rate constant can be written as follows

$$\frac{C_t}{t} = \frac{1}{\frac{1}{kC_s^2} + \frac{t}{C_s}} \dots\dots\dots\dots\dots\dots\dots\dots\dots .. \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. (2.3)$$

The linear form of the equation (2) would be

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s} . \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. \dots . (2.4)$$

Then, when t approaches 0, the initial extraction rate, h, can be written as:

$$h = kC_s^2 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots . (2.5)$$

By rearrangement of equation 4 the concentration of oil at any time can be obtained as follows

$$C_t = \frac{t}{\frac{1}{h} + \frac{t}{C_s}} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. (2.6)$$

The initial extraction rate, h, the extraction capacity, Cs and the second-order extraction constant, k, can be calculated experimentally by plotting t/Ct vs. t. The influence of temperature on the rate constant was determined by fitting k to Arrhenius equation,

$$k = A \exp\left(\frac{-E}{RT}\right) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots, \dots\dots\dots\dots (2.7)$$

$$\ln k = \ln(A) + (\frac{-E}{R}) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2.8)$$

Where k is the leaching rate constant (L g^{-1} min^{-1}), A is a temperature independent factor (L g^{-1} min^{-1}), E is the energy of leaching (kJ mol^{-1}), R is the gas constant (8.314 J mol^{-1} K^{-1}), and T is the suspension absolute temperature (K). From ln k versus 1/T plot we can get activation energy (E) and temperature independent factor (A).

3. Material and Methods

3.1. Materials

The main raw materials and chemicals used for this experiment were M. stenopetala seed, n-hexane, ethanol, phenolphthalein indicator, weij's solution, glacial acetic acid, diethyl ether, carbon tetrachloride, starch, potassium iodide, Potassium hydroxide, sodium thiosulphate and hydrochloric acid. *M. stenopetala* Seed samples were collected from shewa Robit; it is a town in north-central Ethiopia at 346.19 km distance from Addis Ababa. Located in the Semien Shewa Zone of the Amhara Region, this town has a longitude and latitude of 10°00′N 39°54′E Coordinates: with an elevation of 1280 meters above sea level. All the other chemicals were analytical reagent grade and bought from Neway Chemical and Rankem chemical stores in Addis Ababa.

3.2. Equipment

The main equipment's used are, soxhlet extractor, water bath, Electric heater, thimble, Digital weighting balance, Water bath, Beakers, Round bottom flask, vibro-viscometer , pH meter, density meter, oven, muffle furnace, sieve, glass bottles, thermometer, condenser, crusher, separating funnel, vacuum pump, oven, different size conical flasks, beakers, measuring cylinders, burette, micropipettes, GC/MS, FTIR analyzer equipment.

3.3. Methods

3.3.1. Preparation of *M. stenopetala* seed

The pods were collected from the tree and seeds were collected from the pods by removing the barks and two kg of seeds were collected. The seeds also had extra seed husk which was separated after sun drying for one day to facilitate the separation of husk and seed kernels. The seed kernel, which is oil rich part of the seed was measured with an analytical balance and obtained 1.46 kg of seed kernel. The bulk density, the ratio of seed kernel and husk and average mass of seed were determined.



(a) (b)

Figure 3.1 *Moringa Stenopetala* (a) seeds (b) seed kernels

The seed kernels were sun-dried to reduce the moisture content. The dried kernels were ground and sieved to get three average particle size groups, which are the average size of 0.34 mm (powder size), 0.77 mm (coarse size) and 1.2 mm. to make the solvent extraction easier and study the effect of particle size in the oil yield. Thereafter, the crushed seed were dried at 100 ^{o} C for further moisture removal until constant mass of seed kernel meal obtained.



(a) (b)

Figure 3.2 *Moringa stenopetala* Seed (a) Coarsely crushed (b) Powder

3.3.2. Extraction of *M. stenopetala* Seed Oil

Solvent extraction method was used to extract oil from *M. stenopetala* seeds kernels, using hexane as the extracting solvent and Soxhlet extractor as the extracting medium. This experiment was done in chemical engineering department laboratory of Debre Berhan University (DBU). The Soxhlet apparatus used for this solvent extraction (SE) where fitted

with 250 mL round-bottom flask and a condenser. The extraction was executed in a water bath; 150 mL of normal hexane was poured into round bottom flask. 20 gram of prepared *M. Stenopetala* seed was placed in the thimble and inserted in the center of the extractor. The extraction was carried out at different variable combination; particle size (0.34, 0.77, 1.2 mm), temperature (70, 77.5 and 85 ^{o} C) and time (4, 6, 8 hr.). When, the solvent boil vapor rises through the vertical tube into the condenser at the top. The liquid condensate drips into the thimble that, contain the sample in the center, which contains the oil to be extracted. The extract trickles through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. This was allowed for continuous extraction for three different extraction times (4, 6, 8 hr.). The oil was obtained after hexane is recovered by evaporation using a water bath at 80°C to remove the excess solvent from the extracted oil until constant mass of oil was obtained. Then the oil was kept in the refrigerator until test analyses were done.

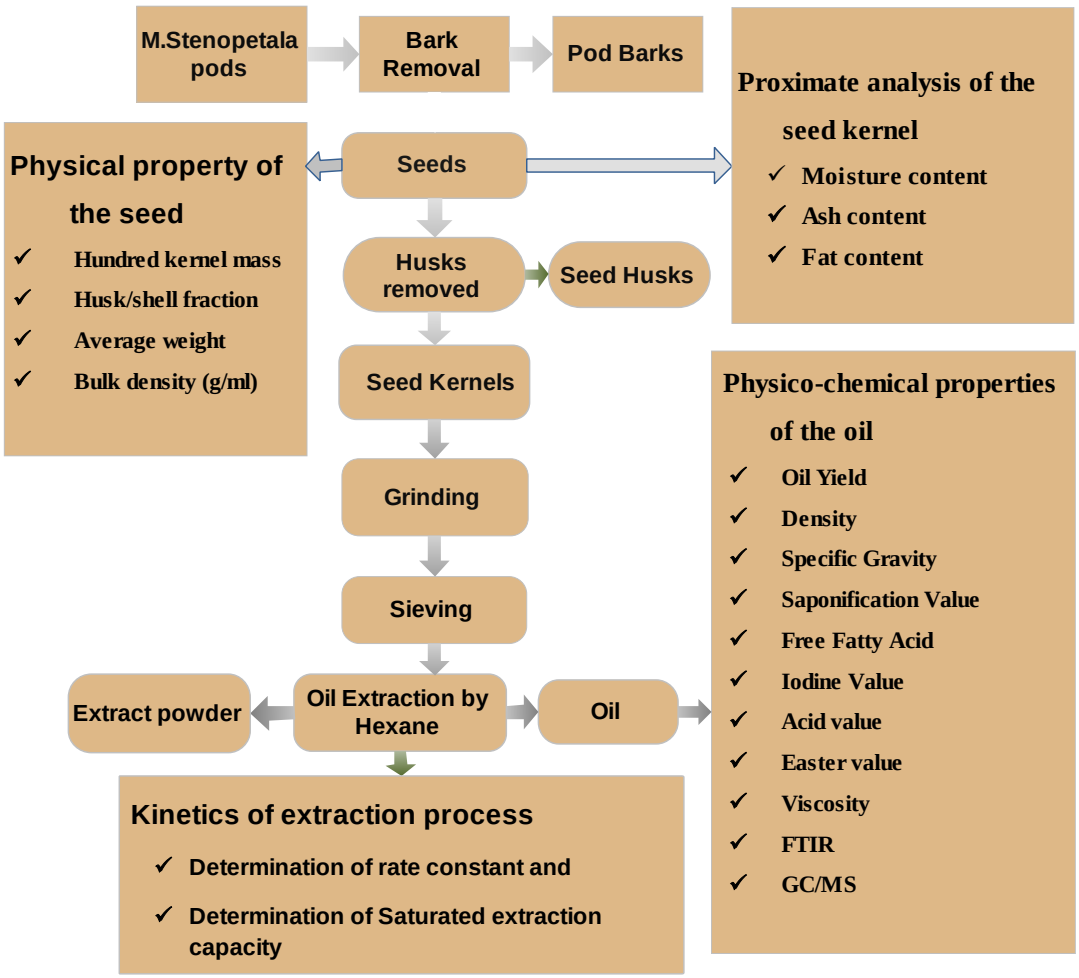


Figure 3.3 Frame work of the experment and tests

3.4. Proximate composition analysis of *Moringa Stenopetala* seed

3.4.1. Determination of moisture content

The moisture content of the seed was measured according to AOAC (1990) methods by drying in the oven at 105^{o}C until constant mass obtained and by using Ohus MB-45 Moisture Balances in AAIT laboratory. In case of moisture balance, one g of course seed sample was weighted and dried in the moisture balance for 10 minute at 100°C. Finally the average value was registered.

3.4.2. Determination of crude ash content

The ash content was analyzed by weighing the samples before and after burning at 550^{o}C for 4 hours in muffle furnace according to AOAC, 930.22 (2000). Crucible was cleaned and dried at 550^{o} C for 1 hour and weighed (m_1). A ground sample of 3 g was weighed (m_2). The sample was dried at 120^{o} C for 1 hour. Then the dried sample was carbonized over a blue flame and ignited in a muffle furnace at 550^{o} C until ash completed (4 hours). After ignition, the sample was cooled in the desiccator to ambient temperature and weighed (m_3). Finally total ash content was calculated as follows:

$$\%\ \text{Ash} = \left(\frac{m_3 - \text{m}_1}{m_2 - \text{m}_1} \right) \text{x}100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

Where: m_1 is mass of crucible (g),

m_2 is sampled mass with crucible (g) and

m_3 is final mass of sample with crucible (g)

3.4.3. Determination of crude fat content

The total fat content of the oil seeds was determined based on the AOAC, (2000) method number 935.38.

3.5. Determination of Physico-chemical Properties of the Oil

3.5.1. Determination of oil yield

The percentage of M. Stenopetala seed oil yield was calculated using the following eqaution.

$$\%\ \text{oil yeld} = \frac{\text{weight of oil}}{\text{weight of sample on adry basis}} x100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.2)$$

3.5.2. Determination of density and specific gravity

The density and specific gravity of the oil were determined using density meter. The oil was injected using a syringe to the density meter and the density meter was recorded for density and specific gravity of crude oil at 20^{o}C.

3.5.3. Determination Acid value

Standard alcoholic potassium hydroxide solution (0.1 N) was prepared by dissolving KOH (pellet) with 95 % ethanol. Furthermore, a mixture of 1 to 1 ratio (v/v) 95% ethanol and diethyl ether was prepared by mixing 100 mL diethyl ether and 100 mL of ethanol. *M. stenopetala* (0.5g) was accurately weighted and dissolved in 10 mL of 1 to 1 mixture of ethanol and diethyl ether. The solution was titrated with 0.1N ethanolic KOH solution in presence of 3 drops of phenolphthalein as an indicator and with continuous mixing until the end point (pink color) is seen. The volume of 0.1 N ethanolic KOH (V) for the sample titration was recorded. Afterward, the acid value was determined using the following equations:

$$\text{Acid Value} = \frac{56.1\ (\text{Vx N})}{\text{m}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (3.3)$$

Where V=Volume of the KOH solution used

N= Normality of KOH

m= Mass of oil sample

3.5.4. Determination of Saponification value

Saponification value represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify 1g oil under the condition specified. Saponification value was

calculated by European Pharmacopeia standard procedure. M. stenopetala (0.5g) was accurately weighed and dissolved in 10mL of 70% ethanol and then 10mL of 2.5N KOH solution was added. This procedure was performed together with a blank experiment which was also performed omitting the oil. The mixture was refluxed for two hours then cooled. The unreacted KOH was titrated with standard 0.5N hydrochloric acid by adding 2-3 drops of phenolphthalein indicator. After that, the saponification value was determined using the following

$$\text{Saponification Value} = \frac{56.1\ \text{XN}\ (\text{V1} - \text{V2})}{\text{m}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(3.4)$$

Where:

V_1 = Volume in ml. of 0.5 N Hydrochloric acid used for the blank,

V_2 = Volume in ml. of 0. Hydrochloric acid used for the sample

N= 0.5N actual concentration of HC

m = Mass in gram of the oil

3.5.5. Determination Ester value

The determination of the ester content is of great importance in the evaluation of many oils. Most esters, which occur as normal constituents of oils, are esters of monobasic acids. Ester value may be defined as the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of esters present in 1g of the oil materials. The ester value can be calculated as follow:

$$\text{Ester value} = \text{Saponification value} - \text{Acid Value} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(3.5)$$

3.5.6. Determination of Iodine value

For this research Wiji's method (AOAC 993.20) was applied to determine the iodine value of oil. A standard Wiji's solution was obtained from JIJE analytical testing service laboratory. To determine the iodine number, first a mixture of carbon tetrachloride and glacial acetic acid (each 40 mL) was prepared. Then 0.3g of oil was dissolved in 15mL glacial acetic acid mixture in a ground in glass stopper conical flask and 25 mL standardized Wiji's solution was added. Then the stopper was replaced at once and the flask was allowed to stand for 1

hour at atmospheric temperature in the dark. When the reaction was completed, 20 mL of 10% KI solution and 150 mL water was added. Finally the free iodine was titrated with 0.1N sodium thiosulphate until the color is pale yellow. A few drops of starch solution (1mL) were added and the titration was continued until the blue color is discharged. The volume of 0.1N thiosulphate was recorded. Similarly a blank determination was done for the same length of time and at the same temperature.

$$\text{Iodine value} = \frac{[(B - S) \text{ X N X } 12.6]}{\text{Weight of the sample}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.6)$$

Where:

B = 0.1 N Sodium Thiosulfate required (mL) by blank

S =0.1 N Sodium Thiosulfate required (mL) by sample

N = Normality of Sodium Thiosulfate solution

3.5.7. Determination of functional group by FTIR Analysis

FTIR Analysis was done in, chemistry department laboratory of Natural Science collage of AAU by using Perkin-Elmer Spectrum Model 65 FTIR Spectrophotometer. A small quantity of the sample oil was deposited with the use of a pipette between two well-polished KBr disks, creating a thin film. All spectra were recorded from 4000 to 400 cm^{-1} and processed with the computer software program Spectrum for Windows (Perkin-Elmer). Infrared spectroscopy is an important technique in organic chemistry. It is an easy way to identify the presence of certain functional groups in a molecule. Also, one can use the unique collection of absorption bands to confirm the identity of a pure compound or to detect the presence of specific impurities. Analysis by infrared spectroscopy is based on the fact that molecules have specific frequencies of internal vibrations. These frequencies occur in the infrared region of the electromagnetic spectrum: 4000 cm^{-1} to 400 cm^{-1}.

3.5.8. Gas Chromatography Mass Spectroscopy Analysis of *M. Stenopetala* seed oil

Analysis of Oil was done in Leather Industry Development Institute (LIDI) using Gas Chromatography -Mass Spectrometer (GCMS). The analysis was performed with Agilent GC system. Samples were injected by a sampler injector at an oven temperature of 325 to 350^oC

for a total run time of 38 minutes. The data, obtained using MS - Agilent Technologies EMS detector and processed using Chemstation software, were used to obtain a fatty acid composition of oils. The GC-MS is composed of two major building blocks: the chromatography is used for the separation of a mixture of chemicals into individual components. The combination of gas chromatography (GC) for separation and mass spectrometry (MS) for the purpose of detection and identification of the components of a mixture of compounds has become the definitive analytical tool for both commercials as well as in the research laboratories. Combining these two techniques, it is possible to both qualitatively and quantitatively evaluate a solution containing a number of chemicals. These two components, used together, allow a much finer degree of substance identification than either unit used separately (Garba et al, 2015).

3.6. Experimental Design

The design of the experiments was carried out in this work was based on the fact that oil yield is functionally related mainly to three factors: particle size, extraction time and extraction temperature. The experiments carried out were designed with the aid of Design Expert 7.0.0. By applying the Central Composite design (CCD) of Response Surface Methodology (RSM) with face centered central composite design (FCCCD) was employed to optimize *M. stenopetala* seed oil extraction. Average Particle size (A), Temperature (B) and Time (C) were the independent variables studied to optimize oil yield. Based on preliminary test values the independent variables were set. The levels used for the experimental factors considered are shown in Table 3.1. Using three levels of the three factors with four center point, According to the design expert eighteen (18) runs of experiments were carried out.

Table 3.1 Experimental factors and levels

Factors	Unit	-1	0	1
Particle size	Mm	0.34	0.77	1.20
Temperature	^{0} C	70.00	77.50	85.00
Extraction Time	hr.	4.00	6.00	8.00

3.6.1. Optimization of operating parameters using Response Surface Methodology

Response surface methodology (RSM) is a very effective mathematical and statistical technique to identify the optimal conditions as well as to determine the effect of process parameters and the interaction of input variables (Wang et al., 2012). It is considered as the faster and less laborious technique as it requires minimum experimental runs for optimizing multiple independent variables (Sharif et al., 2013). In Response Surface Methodology, several factors are simultaneously varied. The multivariate approach reduces the number of experiments, improves statistical interpretation possibilities, and evaluates the relative significance of several affecting factors even in the presence of complex interactions. It is employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariable equations simultaneously. There are several works have been carried out on optimization of vegetables by Response Surface Methodology.

3.6.2. Statistical analysis

The experimental plan was designed and the results obtained were analyzed using Design Expert version 7.0.0. An analysis of variance (ANOVA) and R^2 (coefficient of determination) statistic was used to check the adequacy of the developed model. F-test was used to test the variation of the data around the fitted model (lack of fit). The significance level was stated at 95%, with p-value 0.05. The optimal parameter conditions for the extraction of *M. stenopetala* seed oil obtained using the software's numerical optimization function.

3.7. Kinetics of the Extraction process of *Moringa stenopetala* seed

3.7.1. Extraction Process conditions

Based on the optimum parameters achieved from the parameter optimization process, the rate of extraction of *M. stenopetala* oil was determined at three different temperatures namely 70, 77.50 and 85^{o}C. The extraction was carried out in seven extraction cycles, from 30 min to 6 hrs. In each cycle, 15 g of 0.68mm average particle size seeds were extracted using 150 mL normal hexane with solid to solvent ratio of 1:6.6 (w/w). At the end of each cycle, the thimble was withdrawn from the setup and dried in the oven for 1 hour at 85^{o}C and the amount of the extracted oil was determined by subtracting the final dried thimble with powder mass from

initial thimble with seed powder mass. Based on the oil mass the concentration of oil in the solvent (Hexane) was calculated for each cycle of extraction.

3.7.2. Extraction Procedures

The following are the steps of the experimental procedures:

1. The temperature of the water bath was set at the desired extraction temperature.
2. The solvent storage flask was charged with the desired amount of hexane and immersed in the water bath and the setup was prepared.
3. A desired pre measured amount of crushed *M. stenopetala* seed sample was added to the extraction by putting in the thimble.
4. Stop watch was set at zero time and started when the first drop of solvent touched the solid sample.
5. The solid extract was withdrawn at a desired time interval
6. After the extracted sample had been taken the mass was measured and dried in the oven until constant mass obtained then the final mass was subtracted from the initial sample mass and the mass difference had been taken as the mass of oil extracted.
7. Step 5 and step 6 repeated to obtain the mass of oil extracted at each time interval.
8. The whole procedures will be repeated for the three extraction temperatures.

4. Results and Discussions

4.1. Physical Properties of *M. Stenopetala* Seeds

The result of the Physico-chemical properties of *M. Stenopetala* seed is given in Table 4.1. Based on this result the average kernel weight contains 72.3 % of the seed and this result is lower than the (79 %) seed kernel reported by Seifu, (2012). This difference may come due to the ripening time, moisture content and geographical variations on the collected sample. Knowing the amount and type of husk/hull can be significant for the design of dehulling equipment and for estimation of the separation cost of preparation and oil production. The amount of hull on oilseeds varies significantly for different oil crops. The percentage of hulls for cotton seed, sunflower seeds and soybean are 45, 25 and 7 %, respectively and hulls of oilseeds do not contain a significant amount of oil (less than 1 %) (Dunford, 2008). The hundred kernel seed weight, weight per seed and bulk density of the seed were 42.6g, 0.426g and 0.918 g/ml respectively. The Ethiopian Institute of Agricultural Research EIAR, (2003) reported that an average weight of a single *M. stenopetala* seed is 0.5 g. This average weight per seed difference may possibly come from the maturation of the seed, climate condition, soil type and geographical variations of the location where the samples are collected.

Table 4.1 Physical properties of *Moringa stenopetala* Seed

Seed Characteristics	Obtained Values	Literature value (Seifu,2012)
Hundred kernel mass (g)	42.6	58.1-62.2
Kernel fraction (% from seed mass)	72.3	75.0
Husk/shell fraction (% from seed mass)	27.7	25.0
Average weight (g/seed)	0.426	0.58- 0.62
Bulk density (g/ml)	0.918	0.89-0.96

4.2. Proximate composition of *Moringa stenopetala* Seed Kernel

Table 4.2, presents the proximate composition of *M. stenopetala seed* kernel. As shown from table 4.2, the moisture content of 5.04 % was obtained. This result is lower than 5.8-6.5 % reported by Seifu, (2012) and is in agreement with that of 5 % reported by Aynalem, (2008). The moisture content of oil seed can determine the storage condition and the effectiveness of downstream processing and also influence the quality of the oil (Dunford, 2008).

The average ash content of *M. stenopetala* seed kernel analyzed in the present study (4.48%) is comparable to 4.4 -5 % reported by Seifu, (2012),and higher than 4.43%reported for *M. oleifera* seed kernels from Brazil (Olivera et al.,1999) and 2.5% reported for *M. peregrina* from Saudi Arebia (Somali et al.,1984). The high ash content observed suggests that *M. stenopetala* seed kernel may contain essential mineral elements; however, this needs further investigation. The crude fat content of seed kernel obtained in the present study (40.9 %) is lower than the 44.9 % and 45.3% crude fat content that is reported for *M. stenopetala* (Lalas & Tsaknis, 2003) and (Aynalem, 2008), respectively. Comparatively an equivalent oil yield (41.4%) is reported by (Seifu, 2012). The fat content of *M. stenopetala* seed is higher than 33.6 and 27.5% fat reported for the common oil seeds flax seed and safflower seed respectively (Bozan and Temelis, 2008).Research finding from Malawi indicated that cooking oil with unique characteristics and good sensory quality can be extracted from M. stenopetala seeds ((Lalas and Tsaknis, 2003). Farming land type, climate condition, ripening stage, the harvesting time of the seeds may be the reasons for the difference in proximate analysis results of *M. stenopetala seed*. The high percentage of oil yield makes this seed a potential raw material for the oil industry.

Table 4.2 Proximate Composition of *Moringa stenopetala* Seed kernel

Characteristics	Obtained value	Literature	Reference
Oil Yield	40.35	41.4	(Seifu, 2012)
Moisture (%)	5.04	5.0	(Aynalem, 2008)
Ash Content (%)	4.48	4.4 -5.0	(Seifu, 2012)
Fat content (%)	40.9	44.9	(Lalas & Tsaknis, 2003)

4.3. Physico-Chemical properties of *M. stenopetala* seed oil

The results of the Physical and chemical properties of the extracted seed oil are shown in Table 4.3. As shown from table 4.3 the density of *M. stenopetala* seed oil was found to be 0.905 g/mL which is similar to that of 0.903 g/mL reported for *M. stenopetala* seed by (Ejigu et al., 2010). The viscosity of the oil was 19 and 27 mPa s at 30 and 20^{o}C respectively. The oil obtained by cold pressure extraction is higher in viscosity than that obtained by solvent-extraction (Lalas & Tsaknis, 2003).

Table 4.3 Physico-chemical properties of *Moringa stenopetala* seed oil

Properties	**Obtained Result**
Density (g/ml)	0.904 at 20 ^{0}C
Specific Gravity	0.9056
Saponification Value (mg KOH/g)	179.52
Free Fatty Acid (mg KOH/g oil)	1.87
Iodine Value (g I/100g oil)	67.50
Acid value (mg KOH/g oil)	3.74
Easter value (mg KOH/g oil)	177.78
Viscosity (mPa s) @ 30^{o}C	19.0

The saponification value of 179.52 mgKOH/g of oil was found in this study, which is comparable to that of 177.2 mgKOH/g oil for *M. stenopetala* seed oil reported by Lalas and Tsaknis, (2003) and less than the saponification value of virgin olive oil (190mgKOH/g) and *M. oleifera* seed (183mgKOH/g). The acid value was found to be 3.74mgKOH/g. According to Lalas and Tsaknis, (2003), AV of 3.66 mgKOH/g for cold pressed and 2.2 mgKOH/g for solvent extracted *M. stenopetala* seed oil was reported. The low-level acid values and FFAs are viewed as testimony to the freshness of the crude oil and that show that the oil is not degraded from processing stage (Ochigbo and Paiko, 2011). The low acid value in oil

indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. This could be attributed to the presence of natural antioxidants in the seeds such as vitamins C and A as well as other possible phytochemicals like flavonoids. Acid value is used as an indicator for edibility of oil. High acid value in oil showed that the oil may not be suitable for edibility. High acid value oil requires neutralization before consuming the oil. Free fatty acid content of the oil in the present study was 1.87 mg KOH/g oil and a lower free fatty acid content of 1.33mgKOH/g was reported for cold pressed *M. oleifera* oil by (Janaki, 2015).

The iodine value was 67.5gI/100g and the iodine value reported by S. Lalas et al (2003) is 65.8 g I2 /100 g of oil and iodine value of 69gI/100g was reported by (Ejigu et al., 2010). In this regard, *M. stenopetala* oils are relatively stable to oxidation when compared to, sunflower (125-140gI/100g), linseed (170-180gI/100g) and soybean oil (128-143gI/100g) (Gunstone, 2004). The iodine value is also lower compared to olive oil because the *M. stenopetala* oil is less unsaturated than the olive oil (Lalas & Tsaknis, 2003). The iodine value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of the oil to oxidation. Oils with iodine value less than 100 g I/100g of oil are non-drying oils; correspondingly, Aremu and Akintayo, (2007) reported that the lower the iodine value the lesser the number of unsaturated bonds; thus the lower the susceptibility of such oil to oxidative rancidity and *M. stenopetala* seed oil is grouped under non-drying oil.

4.3.1. Determination of functional group by FTIR Analysis

Figure 4.1 shows % Transmittance versus wave length (cm^{-1}) graph of FTIR analysis. Each peak represents specific functional group present in the *M. stenopetala* seed oil. The oil composition affects the exact position of the band and yields shifts when the proportion of the fatty acid changes. The *M. Stenopetala* seed oil spectra showed a peak at 3476 cm^{-1} for N–H, sharp peak at 3004cm^{-1} for C-H stretching vibration of the cis-double bond (= CH), sharp peaks at 2922 cm^{-1} for Symmetric and asymmetric stretching vibration of the alkanes, C-H stretching is found to be aldehydes and acids presence due to the appearance of absorption peak at 2853.35 cm^{-1}, O-H stretching indicates carboxylic acid the band at 2224.61cm^{-1} representing alkynes indicates C≡C stretching, a sharp peak at 1749 cm^{-1} for ester carbonyl functional group of the triglycerides(C=O), sharp peak at 1647 cm^{-1} present in C=C stretching presence of amides and alkenes. The peak at 1462 cm^{-1} and1449.50cm-1

indicates C-C stretching of aromatics groups and 1379 cm^{-1} for bending vibrations of CH_2 groups. The peaks below 1235 cm^{-1} represent the fingerprint regions. 1238 cm^{-1}, 1163 cm^{-1} represent stretching vibration of the C-O alkynes and ester groups, 723 cm^{-1} represents overlapping of the CH_2 rocking vibration and the out-of-plane vibration of cis-disubstituted olefins. The presence of different characteristic functional groups are identified, these are responsible for different kind of biological activities depending their pharmaceutical and therapeutic uses.

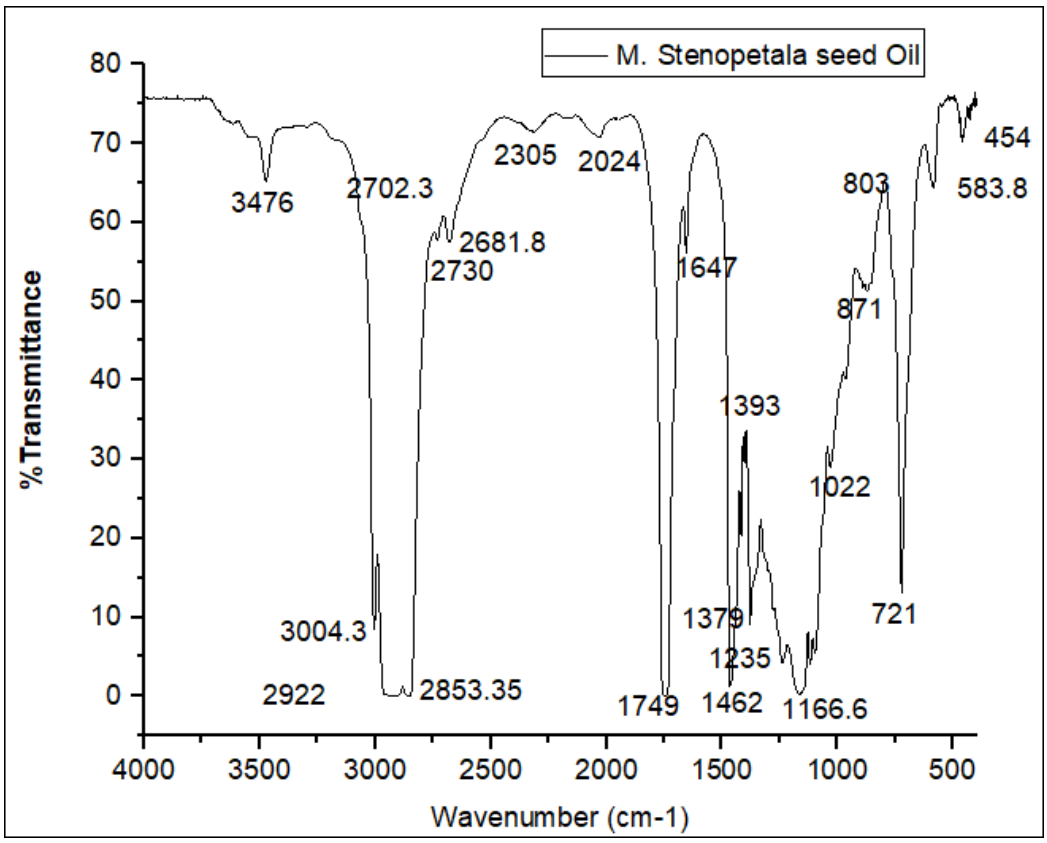


Figure 4.1 FTIR Result of *Moringa stenopetala* Seed Oil

4.3.2. Fatty acids composition of *Moringa stenopetala* seed oil

The result of fatty acid composition from GCMS analysis of *M. Stenopetala* seeds oil revealed that a total fatty acids profile (saturated and unsaturated fatty acids) comprising 12 components with greater than 0.1% composition. Table 4.4 show the fatty acids composition of *M. stenopetala* seed oil compared with the *M. oleifera* and virgin olive oil Fatty acid composition from the literature. As shown in table 4.4 the *M. stenopetala* seed oil contains a high level of monounsaturated fatty acids of 70.83%. Oleic acid was the major monounsaturated fatty acids, which is 67.67 % of the total fatty acid content. The oleic acid

content of *M. stenopetala* seed oil extracted by hexane is less than that of 74.6% reported by (Lalas & Tsaknis, 2003). According to Lalas, (2003) The oleic acid content of *M. stenopetala* seed oil was almost equal to the oleic acid content of virgin olive oil (74.4%). The soil type and climate condition of the cultivation area could be the cause for some differences in the fatty acid composition of the oil. Additional mono unsaturated fatty acids present in the oil were gondoic acid and palmitoleic acids. The saturated fatty acid content is 27 %, with palmitic acid dominating, and then followed by, stearic, arachidic acids and behenic acid. There is very low content of polyunsaturated fatty acids, which is approximately 0.9 % of the total fatty acids composition and the PUFAs present in the oil in this study are linoleic (C18:2 and linolenic (C18:3) acids with 0.707% and 0.18% composition respectively. The low level of polyunsaturated fatty acids (C18:2 and C18:3) and the relatively high content of C22:0 fatty acid (behenic acid) in *M. stenopetala* oil when related to other common vegetable oils, makes it a preferred oil in terms of the oxidative stability. The fatty acid composition of *M. stenopetala* oil is comparable to that of the *M. oleifera* though there are some differences as regards Stearic acids (C18:0), Oleic (C18:1) and Behenic acid (C22:0) composition. Figure 4.2 shows the main fatty acids found in *M. stenopetala* seed oil.

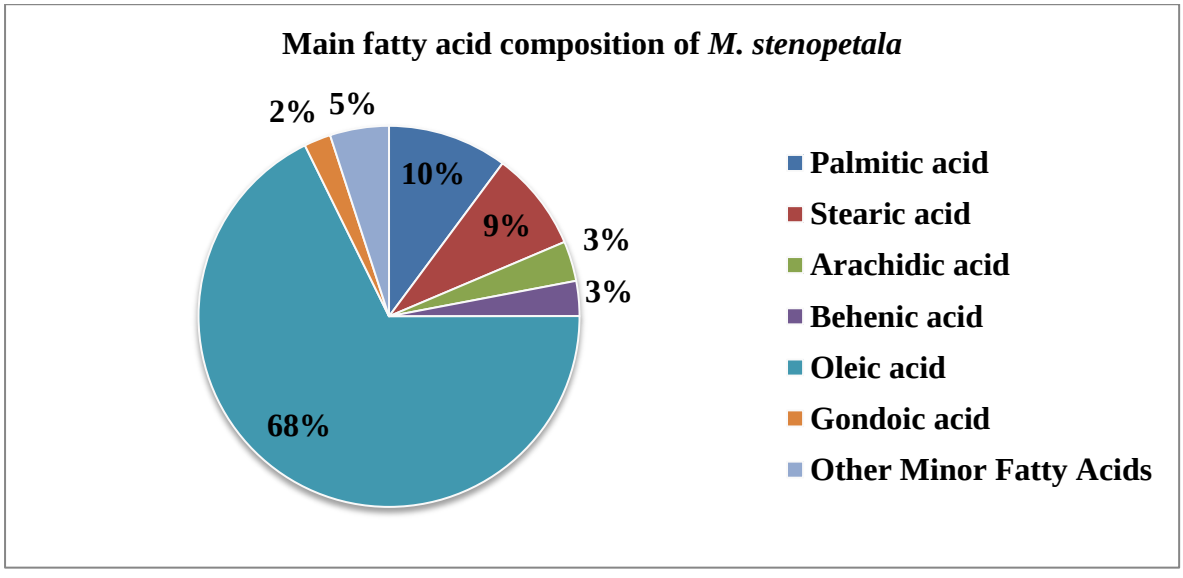


Figure 4.2 Main Fatty Acids Composition in *Moringa stenopetala* Seed Oil

According to its fatty acid composition, oils that contain high proportions of oleic fatty acid have been placed in the category of high oleic oil. The high level of oleic acid of *M. stenopetala* seed oil is comparable to olive oil. The comparative similarity in high oleic acid composition with other high oleic acid oils from sources such as olive oil (65.64%)(Fares et

al, 2016), high oleic sunflower seeds (79%), high oleic safflower seeds (78%), high oleic canola seeds (75%) and *M. oleifera* (73.56%) (Leone et al., 2017) promises well for the application of the oil as edible oil and production of cosmetics.

Table 4.4 Fatty acids composition result and comparison with *M. oleifera* and olive oil

Numerical symbol	Common Name	Composition Obtained	*M. oleifera* (Leone et al., 2017)	Virgin olive oil (Fares et al., 2016) Oueslati variety
C14:0	Myristic acid	0.64	0.11	-
C16:0	Palmitic acid	10.15	6.41	14.5
C17:0	Margaric acid	0.15	0.08	0.03
C18:0	Stearic acid	8.45	5.03	2.03
C20:0	Arachidic acid	3.39	3.32	0.42
C22:0	Behenic acid	2.96	6.04	-
C24:0	Lignoceric acid	1.07	0.54	-
16:1	Palmitoleic acid	0.89	1.41	1.54
18:1	Oleic acid	67.67	73.56	65.64
20:1	Gondoic acid	2.27	1.79	0.28
18:2	Linoleic acid (LA)	0.707	0.83	14.81
18:3	α-Linolenic acid	0.14	0.36	0.65

The high oleic acid content in *M. stenopetala* seed oil can be a good source of effective percutaneous absorption enhancer by enhancing the penetration of tenoxicam. Oleic acids, which is unsaturated fatty acids, exhibited the best permeation enhancing effect, while amongst the saturated fatty acids, palmitic acid had the most potent skin permeation enhancing effect (Kim et al., 2008).

4.4. Model Fitting with Response Surface Methodology

4.4.1. Interpretation of Statistical analysis

The effect of process variables (Particle size, temperature and time) on oil yield was investigated by response surface methodology. Both main and interaction effects of the variables towards oil yield was thoroughly studied. The results of the analysis of variance (ANOVA) are summarized in Table 4.5.

Table 4.5 Analysis of variance result

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	297.76	9	33.08	107.94	< 0.0001	Significant
A	12.19	1	12.19	39.76	0.0002	
B	6.71	1	6.71	21.88	0.0016	
C	41.37	1	41.37	134.98	< 0.0001	
A^2	75.79	1	75.79	247.26	< 0.0001	
B^2	11.54	1	11.54	37.65	0.0003	
C^2	2.39	1	2.39	7.79	0.0235	
AB	0.74	1	0.74	2.43	0.0438	
AC	2.38	1	2.38	7.75	0.0238	
BC	0.14	1	0.14	0.44	0.5253	
Residual	2.45	8	0.31			
Lack of Fit	2.22	5	0.44	5.66	0.0922	not significant
Pure error	0.23	3	0.078			
Cor total	300.21	17				

Note: A, B, C, representing experimental variables: Average particle size, temperature and time respectively.

The Model F-value of 107.94 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. For more justification If the coefficient estimate p-values less than 0.01 are very significant, p-values less than 0.05 but greater than 0.01 are significant, p-values less than 0.10 but greater than 0.05 are marginally significant and p-values greater than or equal to 0.10 are considered insignificant. In this case A, B, C, A^2, B^2 were very significant model terms and C^2, AC and AB were significant model terms. BC had P-Values greater than 0.1000 indicate the model terms was not significant.

The "Lack of Fit F-value" of 5.66 implies the Lack of Fit is not significant relative to the pure error. There is a 9.22% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good because we want the model to fit.

This shows that the average size of seeds, Extraction temperature, Extraction time, interaction between Particle size with extraction time, interaction between particle size and time, square of particle size, square of temperature and square of time, affects the yield of oil significantly.

Table 4.6 Central composite design factors and response

Run order	Particle size A (mm)	Temperature B (^{0}C)	Time C (hr.)	Responses Oil yield (%)	Predicted (%)	Residual
1	0.34	70.00	4.00	32.7	32.31	0.39
2	1.20	70.00	4.00	27.6	28.40	-0.40
3	0.34	85.00	4.00	30	30.32	-0.32
4	1.20	85.00	4.00	28	27.64	-0.037
5	0.34	70.00	8.00	35.64	35.55	0.089
6	1.20	70.00	8.00	34.2	33.82	0.38
7	0.34	85.00	8.00	33.5	33.04	0.46
8	1.20	85.00	8.00	32.2	32.53	-0.33
9	0.34	77.50	6.00	35.2	35.81	-0.61
10	1.20	77.50	6.00	34	33.60	0.40
11	0.77	70.00	6.00	38.3	38.75	-0.45
12	0.77	85.00	6.00	37.35	37.11	0.24
13	0.77	77.50	4.00	37.4	37.02	0.38
14	0.77	77.50	8.00	40.5	41.09	-0.59
15	0.77	77.50	6.00	40.15	39.99	0.16
16	0.77	77.50	6.00	40.2	39.99	0.21
17	0.77	77.50	6.00	39.7	39.99	-0.29
18	0.77	77.50	6.00	40.35	39.99	0.36

The actual highest yield 40.5% was obtained at 0.77 mm average particle size, 77.5^{o}C extraction temperature and 8 hour extraction time. The lowest value of 27.6 % was obtained at 1.2 mm average particle size 70^{o}C temperature and 4 hour extraction time.

Table 4.7 Comparison of model fitting for oil yield

Source	R^2	Adjusted- R^2	Predicted-R^2	
Linear	0.2007	0.0295	-0.4099	
2FI	0.2116	-0.2184	-4.471	
Quadratic	0.9918	0.9826	0.9113	Suggested
Cubic	0.9988	0.9950	-	Aliased

4.4.2. Development of regression model equation

The model equation that correlates the response (oil yield) to the extraction process variables in terms of actual value after excluding the insignificant terms was given below. The quality of the model developed could be evaluated from their coefficients of correlation. Fitting of the data to various models (linear, two factorial, quadratic and cubic) and analysis of variance showed that the solvent extraction of *M. stenopetala* seed oil is most properly described with a quadratic polynomial model. As shown in table 4.7 the model selection for the extraction showed that the Adjusted R^2 of the quadratic model (0.9826) is higher than that of linear (0.0295) and two factorial (-0.2184) models. The cubic model was found to be aliased. Therefore the second-order polynomial model is used to express the oil yield (Y) as a function of independent variables is presented in terms of coded levels.

Final equation in terms of coded factors can be written in the following form

$$Oil\ yield(\%) = 39.99 - 1.10xA - 0.82xB + 2.03xC - 5.29xA^2 - 2.06xB^2 - 0.94xC^2 + 0.31xAxB + 0.55xAxC \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.1)$$

Where A=Particle size

B=Temperature and

C= Extraction Time

4.4.3. Model adequacy check

As shown in table 4.7 the coefficient of determination (R^2) and adjusted coefficient of determination (R^2adj) is 0.9918 and 0.9826, respectively which indicated that the estimated model fits the experimental data properly. The value of R-squared for the developed correlation is 0.9918. It implies that 99.18% of the total variation in the percentage of yield is attributed to the experimental variables studied. Lee et al. (2010) suggested that for a good fit of a model, R^2 should be at least 0.80. The R^2 for these response variables was higher than 0.80, indicating that the regression models explained the actual response. The "Pred R-Squared" of 0.9113was also in reasonable agreement with the "Adj R-Squared" of 0.9826, the diffrence is less than 0.1 then the model is fitting the data and can reliably be used to interpolate. Adequacy Precision measures the signal to noise ratio. A ratio greater than 4 is desirable i.e. the model has a strong enough signal to be used for optimization. For this case the ratio was 33.036 which were much greater than 4 and it indicates an adequate signal. This model can be used to navigate the design space.

Figure 4.3 presents the normal plot of residuals and experimental versus predicted oil yield. From the predicted versus actual plot a linear distribution is observed which is an indicative that the model is a well-fitting. The values predicted were close to the observed values of oil yield.

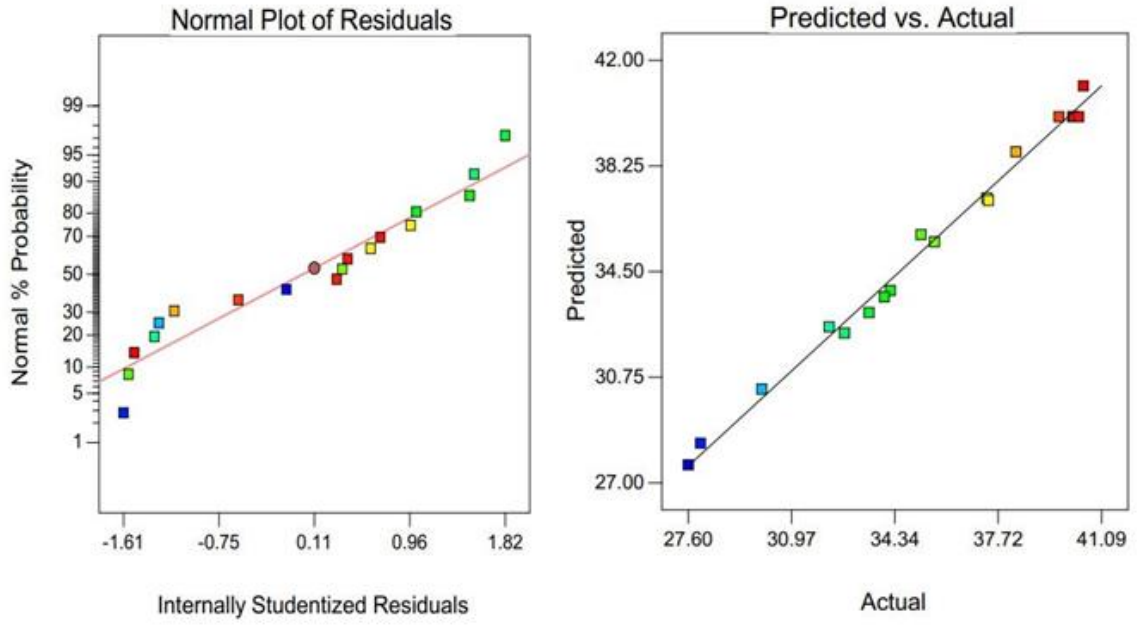


Figure 4.3 Diagnostic plots for adequacy of proposed model

4.4.4. The effect of individual factors on oil yield

In this study, effects of process variables (Particle Size Temperature and extraction time) and their interaction effects on the response oil yield, was determined. Figure 4.4 presents the effect of independent factors on the oil yield obtained from *M. stenopetala* seed extraction.

Effect of particle size: The effect of particle size of meal on extraction yield at extraction time of 6 hour and Temperature of 77.5^{o}C was shown in Figure 4.4 (a) the extraction was carried out using three different average meal sizes namely 0.18 - 0.5mm, 0.71 - 0.85mm and 1 - 1.4mm. The highest percentage of oil yield was obtained from the intermediate size particle (0.5- 0.75 mm) which is 40.35%.

Lower oil yield was found from the larger particles larger than 0.77 mm compared to the smaller size particles. The reason is that larger particles have smaller contact surface areas and more resistant to solvent entrance and oil diffusion distance to the external surface. Therefore, less amount of oil will be transferred from inside the larger particles to the surrounding solution in comparison with the smaller ones. However, when the particle is too small i.e., below 0.5 mm, less oil can be extracted even though the contact surface area for small particle is supposed to be significantly higher than the larger particles. This may be due to the agglomeration or formation of paste like structure of the fine particles which reduces the effective surface area available for the free flow of solvent to solid. This problem is more apparent when oily seeds are in use since the oily particles stick together easily to form paste material which prevents free interactions between solid and solvent. It can be concluded that the medium particle size meal between 0.5 and 0.75 mm (can be referred to as coarse size seeds) are more suitable for solid liquid extraction of *M. stenopetala* seed. As shown in Figure 4.4(a) the oil yield increases from 35.2% to 40.35% as the average particle size increases from 0.34mm to 0.75mm and the oil yield decreases from 40.35% to 33.6% when the average particle size increases from 0.75 mm to 1.2 mm.

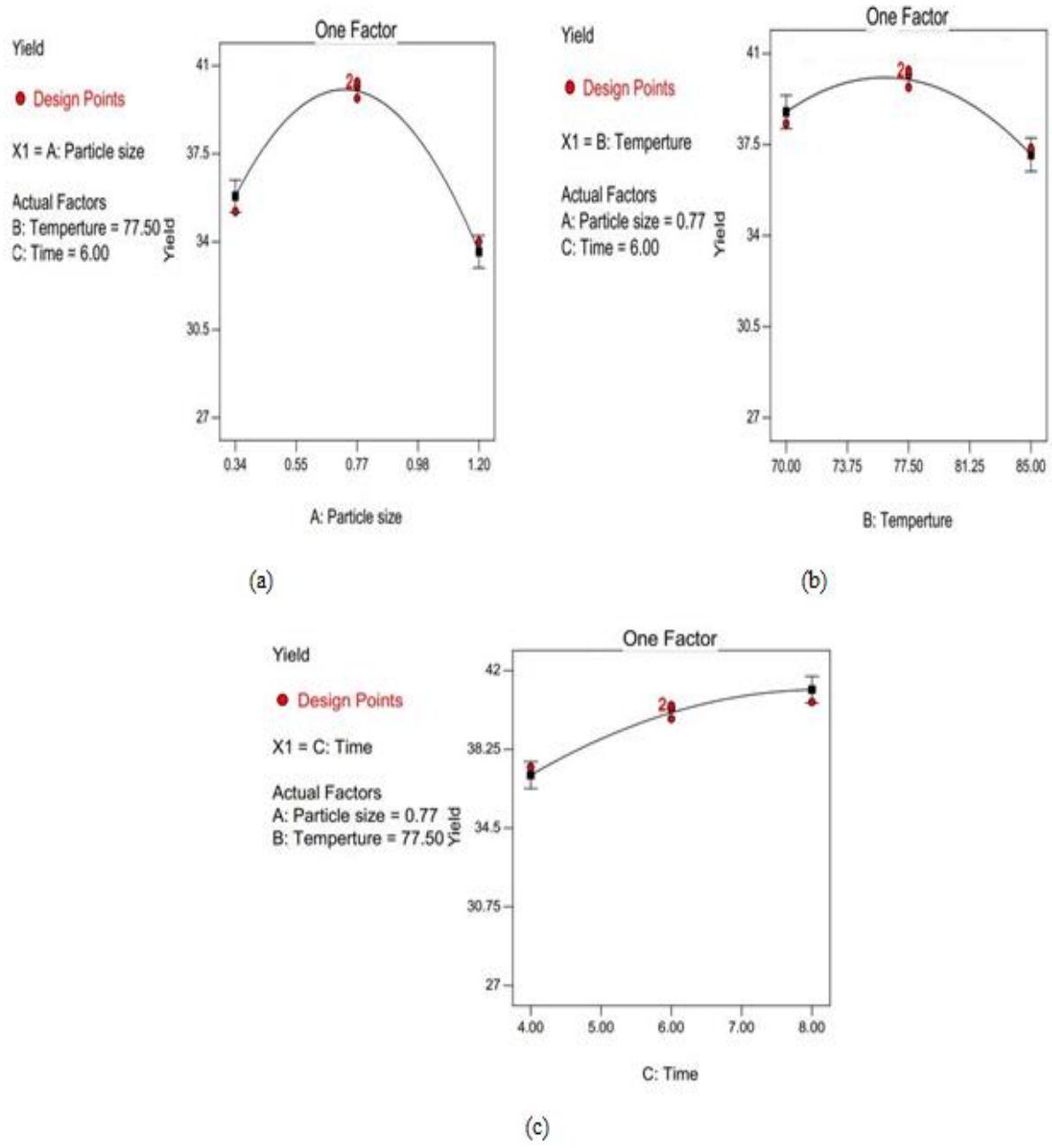


Figure 4.4 The effect of (a) Particle size (b) Temperature (c) Time on oil yield

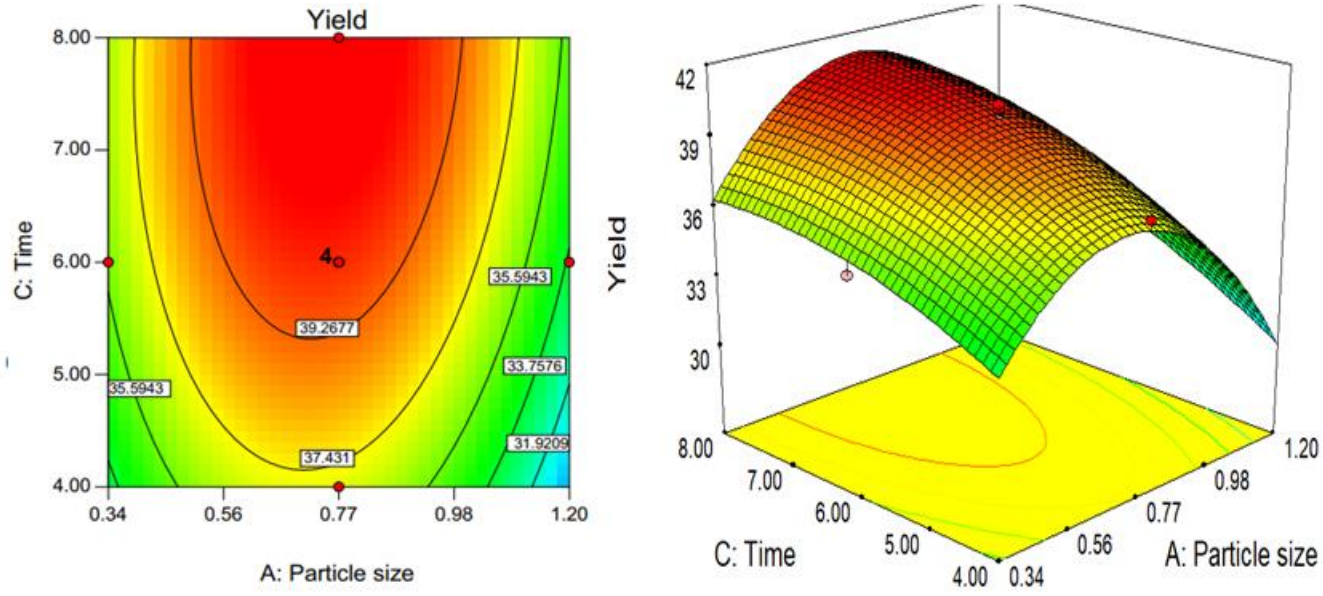
Effect of extraction temperature: The effect of extraction temperature on the amount of extracted oil is shown in Figure 4.4(b). The amount of extracted oil increased around 2% by increasing the extraction temperature from 70^{o}C to 77.5^{o}C and decreased around 3% when the Temperature increased further from 77.5^{o}C to 85^{o}C. Extraction at above the boiling point gives higher oil yield as a result of decreasing in viscosity of the solvent favorable to its flowing through meal and the solubility of the oil in the solid increases but as the temperature increase further the evaporation rate of the solvent increases, which may reduce the contact

time between the solvent and solid and may be the cause for lower oil yield at temperature above 80^{o}C

Effect of Extraction time: The extraction was carried out at three different extraction time (4, 6, 8 hours). Figure 4.2(c) shows the total amount of oil extracted from moringa seeds at different extraction times. The oil yield did not have significant change after 6 h. Because most of the oil is extracted the first four hours and only a small increase of yield is obtained from 4 - 6 hour of extraction although maximum extracted oil is achieved after 6 h with more than 40%.

4.4.5. The effect interaction between process variables

The interactive effect of the process variables on the yield of the Moringa seed oil shown on Table 4.5, from the analysis of variance (ANOVA), the interaction between the factors particle size (A) and time (C) was significant with p-value of 0.0238, and the intraction effect between Prticle size (A) and temperature (B) was marginaly significant because p-value was 0.0438 and intraction effect between tempereture (B) and time (C) was not significant because p- value of 0.5253 was greater than 0.05.



a) b)

Figure 4.5 Interaction effect of particle size and time on oil yield (a) Contour plot (b) 3D response Surface plot

The process variables were found to have significant interaction effects except extraction time with temperature interaction. Figure 4-5 and Figure 4- 6 shows the interaction between particle size and temperature and particle size and time on percentage of oil yield respectively. From the two interaction effects shown in the figures at lower and higher particle size the oil yield was low and had small increase as the time increases (figure4.5). At the center point of particle size the oil yield is maximum which shows that when the particle is too small i.e., below 0.5mm, less oil is extracted even though the contact surface area for small particle is supposed to be significantly higher than the larger particles. This may be due to the agglomeration or formation of paste like structure of the fine particles which reduces the effective surface area available for the free flow of solvent to solid. Lower oil yield was obtained from the larger particles larger than 0.77 mm compared to the smaller size particles. The reason is possibly larger particles have smaller contact surface areas and more resistant to solvent entrance and oil diffusion distance to the external surface. According to Sayyar et al., (2009) The highest percentage of oil yield (47.3%) was obtained from the intermediate particle size (0.5 - 0.75 mm) of Jatropha seed oil extraction by hexane. Oil extraction yield is higher as particle size decreased and contact time increased maximum yield (425 g/kg) was attained with the smallest particle size (0.59 mm) and longest contact time (5 hrs.)was reported by (Rodríguez-Miranda et al., 2014) for pumpkin seed oil extraction.

From this study the interaction of particle size and temperature higher oil yield (38.62%) is obtained at middle of particle size (0.77mm) and temperature of little higher than the center point temperature and lower oil yield is obtained at lower (70^{o}C) and higher (85^{o}C). The reason is probably that as the temperature of the solvent increased above the boiling point the viscosity of the solvent (hexane) decreased and the solubility of oil in the solid increase but when the temperature further increased the evaporation rate increased and the contact time between the solvent and the solid meal decreased and that may be the cause for reduction of oil yield at temperature increased above 80^{o}C. Therefore optimum temperature needs to be suggested for optimal oil yield and energy minimum usage.

According to Oniya et al., (2017) the oil yield was observed to decrease with higher temperature (75^{o}C) particular at the temperature higher than the boiling point of the solvent. This is because at this temperature higher than the boiling temperature of the solvent, evaporation of the solvent will take place as a result of the increased temperature. The

combined effect of these two variables (particle size and temperature) at high level experimental process was obviously resulting in a decrease in oil yield.

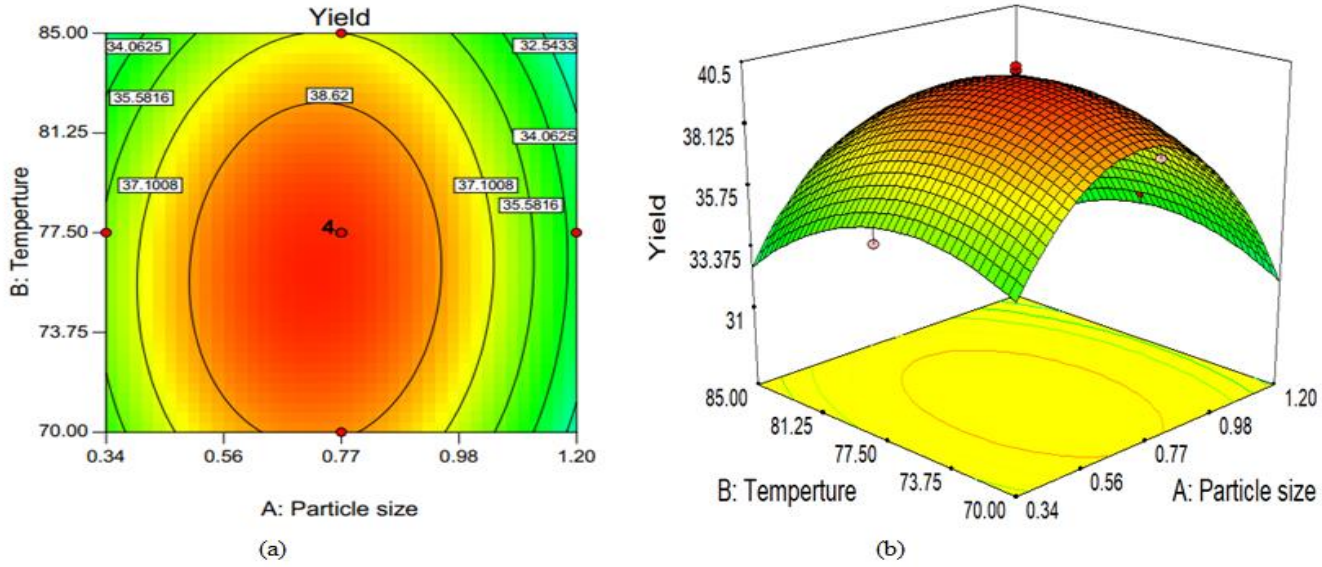


Figure 4.6 Interaction effect of particle size and temperature on oil yield (a) Contour plot (b) 3D surface plot

4.4.6. Optimal process variable for the extraction of *M. stenopetala* seed

After the design evaluation, statistical analysis and diagnostics graphs, i.e. tools for making assured the models provide a good estimate of the true response surface. The results above shown that the three extraction process variables (particle size, extraction temperature, extraction time and the two interaction, the particle size with temperature (AB) and particle size with time (AC) significantly affect the yield of oil. Therefore, the next step was to optimize the process variables, in order to obtain the highest yield (%) of oil using the regression model developed in equation (4.1). This function searches for combination of independent parameter levels that simultaneously satisfy the requirement for response (yield) in the design. So from the design expert, numerical optimization was used in order to obtain the optimum conditions for the lab scale solvent extraction, the predicted combination of parameters were particle size of 0.68mm, temperature at 74.12^{o}C and extraction time of 6.93 hour. Under these conditions, the model predicted oil yield of 40.688% with a desirability value of 1.00. To validate the optimum conditions predicted by the model using desirability ramp, duplicate experiments were conducted using the optimized extraction process conditions and mean oil yield value of 40.195 % was obtained. The results are closely related with the data obtained from optimization analysis using desirability functions. Therefore, this

research shows that hexane can definitely be used for extraction of *M. stenopetala* seed and optimum yield oil can be obtained from the extraction process based on the above determined process variables. According to Sayyar et al, (2009) hexane gives better oil yield compared to petroleum ether in Jatropha seed oil extraction.

4.5. Kinetics Model for Extraction of *M. stenopetala* Seed Oil

4.5.1. Oil concentration and extraction time effect

The effect of time has been evaluated by kinetic study of oil extraction process. As shown in Figure 4.7 the extraction rate is fast at the beginning of the extraction which is the washing stage, but gets slow gradually in the diffusion stage. The reason is that when the meal is exposed to the fresh solvent, the free oil on the surface of seeds is solubilized and oil gets extracted quickly inducing a fast increase in the extraction rate. Furthermore, since the oil concentration is low in the solvent at the beginning of the extraction process, the oil diffuses quickly from the meal to the liquid phase due to the concentration gradient between the solvent and the solid. As the time passing by, the concentration of oil increases in the solvent resulting in a decrease in the diffusion rate.

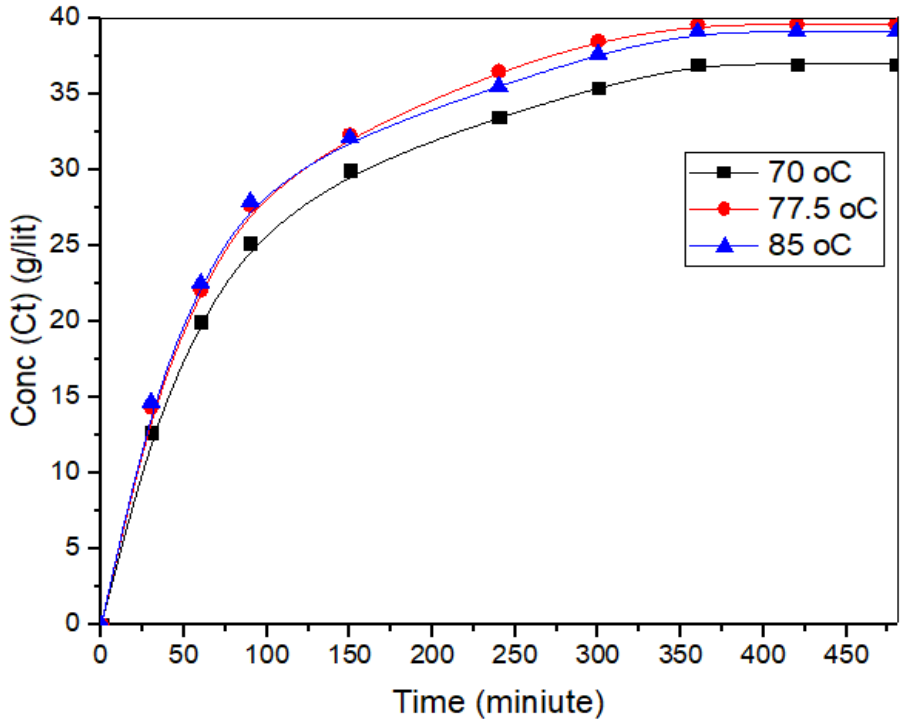


Figure 4.7 Concentration of extracted oil versus time at different temperature

When the maximum amount of extractable oil is obtained, the oil concentration level remains invariable even by extending the reaction time due to limited oil content in the sample run out. As shown on figure 4.7 the plot shows that, as the extraction temperature increases from 70^{o}C to 77.5^{o}C show increase on the oil concentration due to the thermodynamic effect of temperature on solubility of oil inside the solid and the decrease in viscosity of the solvent, hexane. When further increase the temperature to 85^{o}C the yield did not have a significant change. The final concentration also increased with temperature increased.

4.5.2. Rate constant determination and model equation development

According to equation (4.2) t/C_t represent the y- axis, t represents the x-axis, $1/kC^2_s$ represents y-intercept and $1/C_s$ represents the slope of t/Ct versus t plot in figure 4.8. The above values of y- intercept and slop was substituted in to equation (4.2), then the extraction rate constant (k), concentration of oil at saturation (C_s) and from equation (4.3), the initial extraction rate (h) was determined for each temperature. The results are presented on table 4.8 as follow.

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s} \quad \ldots\ldots (4.2)$$

$$h = kC_s^2 \quad \ldots\ldots (4.3)$$

Based on equation (4.2) the y-intercept and slopes are determined for each temperature profile from the liner fitted graph of t versus t/C_t of figure 4.8. The intercept and slope values of the linear fit graph for each temperature presented on table 4.8 as follow.

Table 4.8 The Intercept and Slope of Linear Fit Graph for each Temperature

Temperature (^{o}C)	Y- intercept	slope	R^2
70	1.51696	0.02353	0.99873
77.5	1.30214	0.02221	0.998869
85	1.26171	0.02267	0.99907

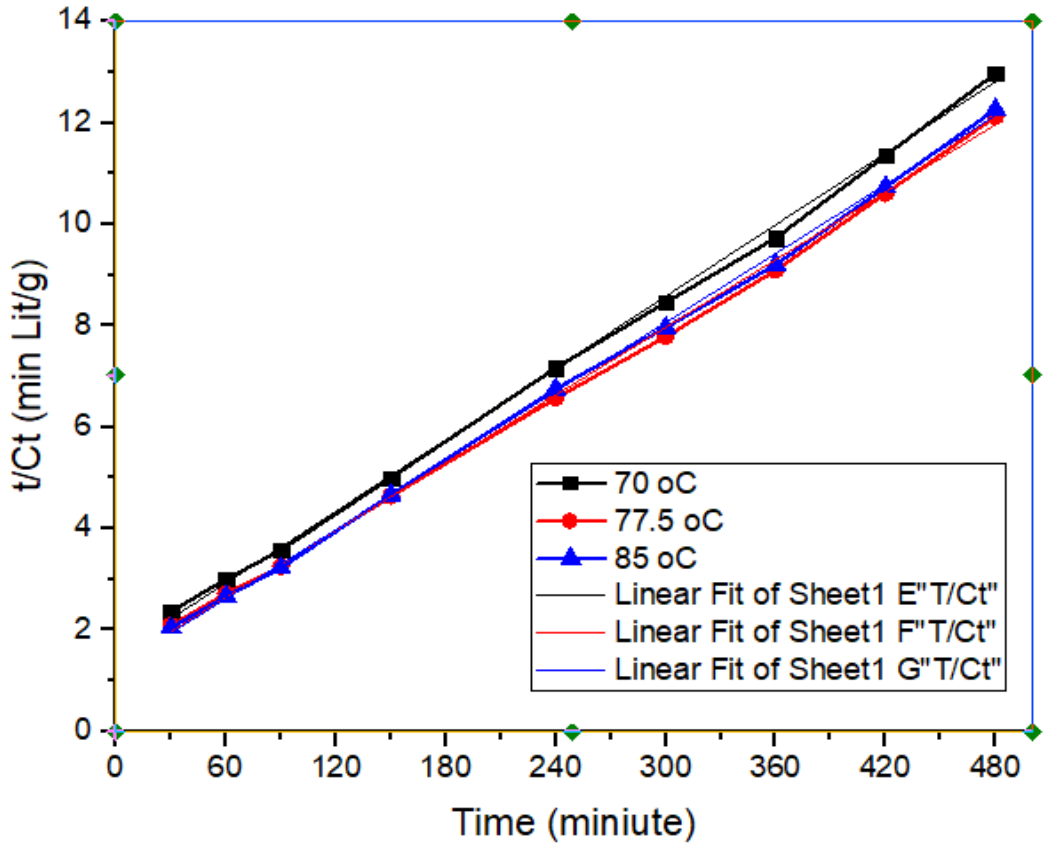


Figure 4.8 Second order extraction kinetics of *Moringa stenopetala Seed* oil extraction

The saturation extraction capacity, Cs, the extraction rate constant, k, the initial extraction rate, h and the coefficient of determination, R^2, were determined according to the linear curves.

Table 4.9 The Extraction Rate Constant and Saturated Concentration of Oil Values

Temperature (^oC)	C_s (g/L)	h (g/L min)	k(L/g min)
70	42.50	0.659	$3.65x10^{-4}$
77.5	45.07	0.768	3.7910^{-4}
85	44.11	0.793	$4.08x10^{-4}$

As shown in table 4.9, the saturated extraction capacity, Cs increased from 42.5- 45g L^{-1} when the temperature increased from 70 – 77.5^{o}C and shows small decrement as the temperature further increase to 85^{o}C. In the other way the extraction rate constant, k and the initial extraction rate, h, was increased their values from 3.65×10^{-4} to 4.08×10^{-4} L g^{-1} min^{-1}

and 0.659 to 0.793 g L^{-1} min^{-1} respectively as the temperature increased from 70 to 85 ^oC. The saturated extraction capacity, Cs, the extraction rate constant, k and the initial extraction rate, h, increased with temperature. The extraction temperature has a direct effect on all the three parameters (rate constant, saturated extraction capacity and initial extraction rate). Figure 4.8 indicates a good agreement of the second order extraction model with the experimental results with R square value of greater than 0.990. This agreement confirms the assumptions that solid liquid extraction of *M. stenopetala* seeds takes place in two subsequent (washing and diffusion) stages can be represented by second order kinetics model. The kinetics model can be represented by the following equation.

$$C_t = \frac{C_s^2 K t}{1 + C_s K t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.4)$$

By substituting the rate constant and saturated extraction capacity in equation (4.4) for 70, 77.5 and 85 k temperature the following Kinetics model equations were developed.

Kinetics model equation developed for extraction temperature of 70^o C

$$C_t = \frac{0.659t}{1 + 1.55x10^{-2}t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.4a)$$

Kinetics model equation developed for extraction temperature of 77.5^{o}C

$$C_t = \frac{0.767t}{1 + 1.71x10^{-2}t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.4b)$$

Kinetics model equation developed for extraction temperature of 85^{o}C

$$C_t = \frac{0.7935t}{1 + 1.80x10^{-2}t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.4c)$$

The concentration value obtained from the kinetics model equation is comparable to the extraction concentration data as shown in appendix B of table B-3, B-4 and B-5 for 70, 77.5 and 85^{o}C respectively.

4.5.3. Relationship between rate constant and temperature

The second order extraction rate constant, k increases with temperature as shown in table 4.9 and the changes can be described by the Arrhenius Equation of (Eq.4.7). The relationship

between k and T can be defined by the linearized Arrhenius Equation (Eq. 4.5). Figure 4.8 shows a linear plot of Arrhenius equation.

$$k = A \exp\left(\frac{-E}{RT}\right) \quad \ldots \quad (4.4)$$

$$\ln k = \ln(A) + \left(\frac{-E}{R}\right)\frac{1}{T} \quad \ldots \quad (4.5)$$

From equation 4.5, by using the data given in table 4.9 ln k versus 1/T plotted as shown in figure 4.9. The temperature independent factor (A) and activation energy values were obtained from the linear fitted plot of equation 4.5. From the equation ln(A) and (–E/R) represent intercept and slope of the linearized curve of ln(k) versus1/T(k) respectively.

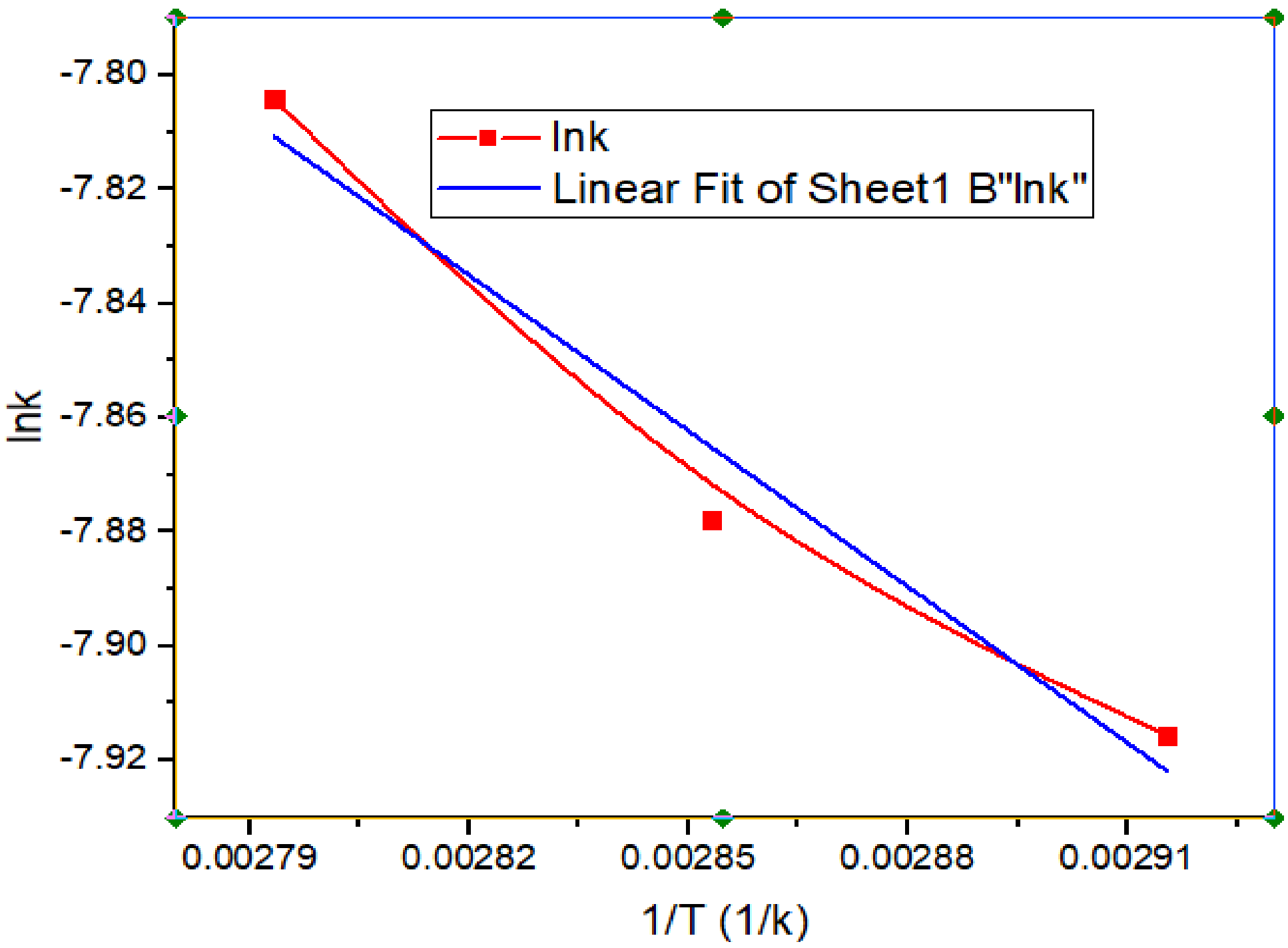


Figure 4.9 Linear relationship between second order extraction constant lnk and temperature for extraction of *Moringa stenopetala* seed oil.

Based on figure 4.8, intercept or the value of ln (A) is -5. 267 and slope or the value of (-E/R) is -909.71 with R-square value of 0.92353. When the value of the gas constant, 8.314 J mol^{-1} k^{-1} substituted and the value of A is determined, the temperature independent factor (A) and activation energy were found to be 0.005145 L g^{-1} min^{-1} and 7,563 J mol^{-1} respectively. According to Sayyar et al., (2009) the value of A and activation energy were 0.0087 L g^{-1}

min^{-1} and 8021.9 $Jmol^{-1}$ respectively for Jatropha seed extraction, which is comparable to the result found in this work. Based to the results achieved from Equation 4.5, it can be conclude that solid liquid extraction of *M. stenopetala* seed can be represented by second order kinetics model and the process is endothermic because the activation energy is positive.

5. Conclusions and Recommendations

5.1. Conclusions

This study focused on the extraction of Moringa seed oil by using solvent extraction technology employing hexane as an extract solvent, characterization of *M. Stenopetala* seed and oil and the extraction process kinetics of *M. stenopetala* seed oil. The extraction was carried out at different variable combination; particle size (0.34, 0.77, 1.2 mm), temperature (70, 77.5 and 85^{o}C) and time (4, 6, 8 hr.). Response surface methodology with central composite design was used to determine optimum conditions of the extraction process. The analysis of variance showed that the three factors; particle size, temperature and time were significant factors. The optimum process parameter combination to find the highest oil yield was particle size of 0.68mm, temperature at 74.12^{o}C and extraction time of 6.93 hour. Under these conditions, the model predicted oil yield was 40.688%.

The Physico-chemical properties of the extracted oil, which include density, specific gravity, viscosity, acid value, saponification value, iodine value and Easter value for *M. stenopetala* oil was found to be 0.906g/cm, 0.9056, 19mPa.s, 3.74mgKOH/g, 179.52mgKOH/g, 67.5gI/100g oil and 177.2mgKOH/g oil respectively.

The saturated fatty acid content was 27 %, with palmitic acid dominating, and then followed by, stearic, arachidic acids and behenic acid. The oil contains a high level of monounsaturated fatty acids, up to an average of 70.83%. Oleic acid was predominate monounsaturated fatty acids, which was 67.67 % of the total fatty acid content. The oleic acid content of *M. stenopetala* seed oil was almost equal to the oleic acid content of virgin olive oil. The soil type and climate condition of the cultivation area could be the cause for some differences in the fatty acid composition of the oil. Other mono unsaturated fatty acids present in the oil were gondoic acid and palmitoleic acids. There was very low content of polyunsaturated fatty acids, which was approximately only 0.9 % of the total fatty acids composition and the PUFAs were linoleic and linolenic acids with 0.707% and 0.18% composition respectively. According to its fatty acid composition, oils that contain high proportions of oleic fatty acid have been placed in the category of high oleic oil. The high level of oleic acid of *M. stenopetala* seed oil was comparable to olive oil.

The kinetics of extraction of oil from crushed *M. stenopetala* seeds were based on the assumption of a mechanism of a second order model that was developed to predict the rate constant of extraction, the saturated capacity of the extraction, and the initial extraction rate. The extraction rate increases with temperature. From the result of kinetics study, as the extraction temperature increases from 70^{o}C to 77.5^{o}C show increase on the oil concentration due to the thermodynamic effect of temperature on solubility of oil inside the solid and the decrease in viscosity of the solvent, hexane. When further increase the temperature to 85^{o}C the yield did not have a significant change. The final concentration also increases with temperatures. Activation energy of the extraction was evaluated to be 7,563 J mol^{-1}.

5.2. Recommendations for Further Works

Further investigations are recommended on the preprocessing processing and Commercialization of *M. stenopetala* seed and oil. A specific area recommended for further research works such as:

- Study on the production potential, productivity and collection mechanism of *M. stenopetala* seed and understand the behavior and to explore their commercial application
- Even if Moring kernel oil has been used worldwide in food and formulation of cosmetics product, in Ethiopia the oil is not commercially produced in some extent the leave powder is available in the market. Further study needed bout the formulation of specific cosmetics product for import substitution and promoting natural cosmetics products.
- In this research the extraction of oil was performed by solvent extraction method further research can be carried out on other extraction technologies.
- The characterization of the oil from the seeds of *M. stenopetala* showed that this oil could be utilized successfully as a source of edible oil for human consumption. It contains high monounsaturated to saturated fatty acids ratio, and might be an acceptable substitute for highly monounsaturated oils such as olive oil in diets. But the oil has displeasing odor and further study required in the refining, removal of this odor and fulfilling the standards of edible oil quality.
- Further studies are needed with this plant to dissociate, characterize and illustrate the compounds percent in every part of the tree.

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APPENDICES

Appendix A: Fatty Acid Composition and chemical properties of different type of seed oils

Table A-0.1 Fatty Acid composition of some vegetable oils

FAs [%]	SAF	GRP	SIL	HMP	SFL	WHG	PMS	SES	RB	ALM	RPS	PNT	OL	COC
C6:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.52
C8:0	nd	0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	7.6
C10:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.01	nd	nd	5.5
C12:0	nd	0.01	0.01	nd	0.02	0.07	nd	nd	nd	0.09	nd	nd	nd	47.7
C14:0	0.10	0.05	0.09	0.07	0.09	nd	0.17	nd	0.39	0.07	nd	0.04	nd	19.9
C15:0	nd	0.01	0.02	nd	nd	0.04	nd	nd	nd	nd	0.02	nd	nd	nd
C16:0	6.7	6.6	7.9	6.4	6.2	17.4	13.1	9.7	20.0	6.8	4.6	7.5	16.5	nd
C17:0	0.04	0.06	0.06	0.05	0.02	0.03	0.13	nd	nd	0.05	0.04	0.07	nd	nd
C18:0	2.4	3.5	4.5	2.6	2.8	0.7	5.7	6.5	2.1	2.3	1.7	2.1	2.3	2.7
C20:0	nd	0.16	2.6	nd	0.21	nd	0.47	0.63	nd	0.09	nd	1.01	0.43	nd
C22:0	nd	nd	nd	nd	nd	nd	nd	0.14	nd	nd	nd	nd	0.15	nd
C16:1 (n-7)	0.08	0.08	0.05	0.11	0.12	0.21	0.12	0.11	0.19	0.53	0.21	0.07	1.8	nd
C17:1 (n-7)	nd	nd	0.03	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:1cis (n-9)	11.5	14.3	20.4	11.5	28.0	12.7	24.9	41.5	42.7	67.2	63.3	71.1	66.4	6.2
C18:1trans (n-9)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.14	nd	nd	nd
C20:1(n-9)	nd	0.40	0.15	16.5	0.18	7.91	1.08	0.32	1.11	0.16	9.1	nd	0.30	nd
C18:2cis (n-6)	79.0	74.7	63.3	59.4	62.2	59.7	54.2	40.9	33.1	22.8	19.6	18.2	16.4	1.6
C18:3 (n-3)	0.15	0.15	0.88	0.36	0.16	1.2	0.12	0.21	0.45	nd	1.2	nd	1.6	nd
C18:3 (n-6)	nd	nd	nd	3.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SFAs	9.3	10.4	15.1	9.2	9.4	18.2	19.6	16.9	22.5	9.3	6.3	10.7	19.4	92.1
MUFAs	11.6	14.8	20.7	28.1	28.3	20.9	26.1	42.0	44.0	67.9	72.8	71.1	68.2	6.2
PUFAs	79.1	74.9	64.2	62.8	62.4	61.0	54.3	41.2	33.6	22.8	20.9	18.2	18.0	1.6
n-3 PUFAs	0.2	0.2	0.9	0.4	0.2	1.2	0.1	0.2	0.5	0.0	1.2	0.0	1.6	0.0
n-6 PUFAs	79.0	74.7	63.3	62.4	62.2	59.7	54.2	40.9	33.1	22.8	19.6	18.2	16.4	1.6

Data are expressed as percentages of total fatty acid methyl esters (FAMEs); nd means that FAs was not determined. Abbreviations of the samples mean: SAF- safflower, GRP- grape, SIL-*silybum marianum*, HMP-hemp, SFL-sunflower, WHG-wheat germ, PMS pumpkin seed, SES- sesame, RB- rice bran, ALM- almond, RPS- rapeseed, PNT- peanut, OL- olive, and COC- coconut oils.

Source (Orsavova et al., 2015)

Table A-0.2 Saponification, Iodine value and unsaponifiable matter of some commodity oils

	SE* (g oil/mol KOH)	SV (mg KOH/g oil)	IV (100 × g iodine/g oil)	Unsaponifiable matter (wt%)
butter	242–267	210–232	26–40	<0.5
castor	300–319	176–187	81–91	
coconut	212–226	248–265	6–11	<1.5
corn	288–300	187–195	107–128	1–3
cottonseed	283–297	189–198	100–115	<2
fish**	292–312	180–192	142–176	<2
groundnut (peanut)	286–300	187–196	86–107	<1
lard	276–292	192–203	45–70	<0.2
linseed	286–298	188–196	170–203	<2
olive	286–305	184–196	75–94	<1.5
palm	268–295	190–209	50–55	<1.3
palm kernel	221–244	230–254	14–21	<1
rape***	291–308	182–193	110–126	<0.2
sesame	288–300	187–195	104–120	<2
soybean	288–297	189–195	124–139	<1.5
sunflower	289–298	188–194	118–145	<2
tallow	281–295	190–200	33–47	<0.5

Source: Bailey's Industrial Oil and Fat Products six edition

Table A-0.3 Infrared Spectroscopy of functional groups

Alkanes	
Indentification of absorbance	Wavenumbers, cm^{-1}
sp^3 C-H stretch	2960-2850 (<3000)
Alkenes	
sp^2 C-H stretch	3100-3020 cm^{-1} (>3000)
C=C stretch	1680-1620 cm^{-1} (often weak)
Alkynes	
sp C-H stretch	3333-3267 cm^{-1}
C-C triple bond stretch	2260-2100 cm^{-1} (often weak)
Nitriles	
C-N triple bond stretch	2260-2240 cm^{-1} (aliphatic) 2240-2222 cm^{-1} (conjugated)
Alcohols	
O-H stretch	3600-3200 cm^{-1} (strong and broad)
C-O stretch	1150-1050 cm^{-1}
Aromatic Compounds	
sp^2 C-H stretch	3100-3020 cm^{-1} (>3000)
C=C stretch	~ 1585 cm^{-1}
Aromatic overtones of ring bends	2000-1667 cm^{-1}
Aromatic out-of-plane ring bends	~ 900-700 cm^{-1} (see p. 519)

Amines	
N-H stretch	3500-3350 cm^{-1}
C-N stretch (often weak and hard to find)	1250-1020 cm^{-1} (aliphatic) 1342-1266 cm^{-1} (aromatic)
Aldehydes	
C-H stretch of -CHO	2830-2695 cm^{-1}
C=O stretch	1740-1720 cm^{-1}
Ketones	
C=O stretch	1750-1710 cm^{-1} (1715 is typical for aliphatic ketones) 1685-1665 cm^{-1} (conjugated)
Carboxylic acids	
O-H of $-CO_2H$	3600-2500 cm^{-1} (centered at ~ 3000)
C=O stretch	1720-1710 cm^{-1} (aliphatic), 1700-1680 cm^{-1} (conjugated)
Esters	
C=O stretch	1750-1735 cm^{-1} (aliphatic), 1720-1715 cm^{-1} (conjugated)
C-O stretch	1300-1000 cm^{-1}

Appendix B: Experimental Results

Table B 1 Moisture content of *Moringa stenopetala* seed

m_1 (g)	m_2(g)	m_1-m_2(g)	Average Moisture content
5	4.755	0.245	$\frac{0.252}{5} * 100 = 5.04\%$
5	4.739	0.261	
5	4.75	0.25	
Average		0.252	

Table B- 1 Ash content of Moringa stenopetala seed

Crucible mass m_1 (g)	Sample +Crucible mass m_2(g)	Ash +Crucible mass m_3(g)	m_3-m_1(g)	m_2-m_1(g)	Ash content (%)
21.642	24.642	21.771	0.129	3	4.332
21.615	24.615	21.782	0.167	3	4.634
21.552	24.552	21.688	0.136	3	4.483
Average					4.483

B-2: Extraction result data for kinetics model equation Development

Table B- 2 Concentration versus time data at 70^{o}C

Extraction data at 70 ^{0}C					
Time	**m oil extracted**	**mi-mi+1 (g)**	**Conc.(Ct)**	**Conc.(Ct) value from kinetics model**	**T/Ct (min L/g)**
0	16.4	0	0	0	0
30	14.8	1.9	12.667	13.49	2.368358727
60	13.7	0.9	18.667	20.48	3.214228317
90	12.8	0.8	24.333	24.76	3.698680804
150	11.75	1.05	31	29.73	4.838709677
240	11.1	0.52	34.4667	33.51	6.963242782
300	10.7	0.3	36.4667	35.0	8.226683522
360	10.5	0.2	37.8	36.05	9.523809524
420	10.5	0	37.8	36.85	11.11111111
480	10.5	0	37.8	37.48	12.6984127

Table B- 3 Concentration versus time data at 77.5^{o}C

Extraction data at 77.5^{o}C					
Time(min)	**m oil extracted**	**mi-mi+1**	**Ct (g/ lit)**	**Conc. (Ct) value from kinetics model equation**	**T/Ct (min L/g)**
0	16.4	0	0		0
30	14.3	2.1	14.33	15.20819564	2.093510119
60	13	1.3	22.0667	22.71470879	2.719029125
90	12	1	27.667	27.18786924	3.252972856
150	11.3	0.7	32.33	32.27208976	4.639653573
240	10.9	0.4	36.533	36.06583072	6.569403006
300	10.6	0.3	38.533	37.53670473	7.785534477
360	10.4	0.2	40	38.58580212	9.090909091
420	10.4	0	40	39.37179174	10.60606061
480	10.4	0	40	39.98262381	12.12121212

Table B- 4 Concentration versus Time data at 85^{o}C

Extraction data at 85^{o}C					
Time	**mass oil extracted**	**mi-mi+1 (g)**	**Conc.(Ct)**	**Conc.(Ct) value from kinetics model**	**T/Ct (min l/g)**
0	16.4	0	0	0	0
30	14.1	2.3	15.33333333	15.45779221	1.956521739
60	13.1	1	22	22.88942308	2.727272727
90	12.3	0.81	27.4	27.25763359	3.284671533
150	11.7	0.6	31.4	32.16891892	4.777070064
240	11.2	0.51	34.8	35.79699248	6.896551724
300	10.7	0.5	38.13333333	37.1953125	7.867132867
360	10.4	0.3	40.13333333	38.18983957	8.970099668
420	10.4	0	40.13333333	38.93341121	10.46511628
480	10.4	0	40.13333333	39.51037344	11.96013289

Table B- 5 Experimental design and oil yield value

	Std	Run	Block	Factor 1 A:Particle size mm	Factor 2 B:Temperture 0C	Factor 3 C:Time hr	Response 1 Yield
	1	13	Block 1	0.34	70.00	4.00	32.7
	2	5	Block 1	1.20	70.00	4.00	28
	3	8	Block 1	0.34	85.00	4.00	30
	4	14	Block 1	1.20	85.00	4.00	27.6
	5	4	Block 1	0.34	70.00	8.00	35.64
	6	17	Block 1	1.20	70.00	8.00	34.2
	7	9	Block 1	0.34	85.00	8.00	33.5
	8	6	Block 1	1.20	85.00	8.00	32.2
	9	18	Block 1	0.34	77.50	6.00	35.2
	10	3	Block 1	1.20	77.50	6.00	34
	11	7	Block 1	0.77	70.00	6.00	38.3
	12	15	Block 1	0.77	85.00	6.00	37.35
	13	1	Block 1	0.77	77.50	4.00	37.4
	14	2	Block 1	0.77	77.50	8.00	40.5
	15	10	Block 1	0.77	77.50	6.00	40.15
	16	12	Block 1	0.77	77.50	6.00	40.2
	17	11	Block 1	0.77	77.50	6.00	39.7
	18	16	Block 1	0.77	77.50	6.00	40.35

Appendix C: Main Calculation in interpreting the experimental result

C1: M. Stenopetala seed oil yield Calculations

$$\%\ \text{oil yeld} = \frac{\text{weight of oil}}{\text{weight of sample on adry basis}} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Weight of sample =20g

Wight of Oil=6.54g

$\%\ \text{oil yeld} = \frac{6.54}{20} * 100$=32.7%, this result is only for the first run in Table B-6

C2: Acid Value Calculation

$$\text{Acid Value} = \frac{5.61\ \text{X (volume of ml of 0.1 N KOH)}}{\text{Wight of sample in gram}} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (2)$$

Volume of KOH=0.33 ml

Eight of sample = 0.5 g

$$\text{Acid Value} = \frac{5.61\ \text{X (0.33ml} *\ \text{0.1 N KOH)}}{\text{0.5 g}} = 3.736$$

C3: Saponification Value Calculation

$$\text{Saponification Value} = \frac{56.1\ \text{X N(V1} - \text{V2)}}{2 * \text{M}} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots..(3)$$

Where:

V_1 = Volume in ml. of 0.5 N Hydrochloric acid used for the blank,

V_2 = Volume in ml. of 0. Hydrochloric acid used for the sample

N= 0.5N actual concentration of HC

M = Mass in gram of the oil

V_1=41.1ml, V_2 =37.91ml, M=0.5ml

$$\text{Saponification Value} = \frac{56.1\ \text{X}\ 0.5(41.1 - 37.91)}{0.5} = 179 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots..(3a)$$

C4: IV Calculation

$$\text{Iodine value} = \frac{[(\text{B} - \text{S})\ \text{X N X}\ 12.6]}{\text{Weight of the sample}} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (4)$$

N =0.1, Weight of sample=0.3g, B-S=16.07

$$\text{Iodine value} = \frac{[16.07\ \text{X}\ 0.1\text{X}\ 12.6]}{0.3} = 67.50 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (4)$$

C5: Oil Concentration Calculation

$$\text{Concentration(Ct)} = \frac{\text{mass of oil}}{Volume\ of\ Hexane} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots.(5)$$

Mass of sample was obtained by subtracting final mass from initial mass of oil For example; concentrations of the first run of extraction at 70°C from table B-3

Mass of oil=1.9 g

Volume of Hexane =150ml=0.15lit

$\text{Concentration(Ct)} = \frac{1.9}{0.15} = 12.667\text{g/Lit}$

C6: Kinetics equation Calculations

The following calculation is for extraction equation at 70°C. The same procedure followed for 77.5 and 85°C.

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{1}{C_s} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (6)$$

$$Slope = \frac{1}{C_s} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. (6a)$$

$$0.02353 = \frac{1}{C_s}, From\ this\ \boldsymbol{C_s = 42.50}$$

$$Y - intercept = \frac{1}{kC_s^2} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. (6b)$$

$$1.51696 = \frac{1}{kC_s^2}, From\ this\ \boldsymbol{K = 3.65x10^{-4}}$$

$$C_t = \frac{C_s^2 kt}{1 + C_s Kt} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots . (7)$$

$$C_t = \frac{42.5^2 kt}{1 + 42.5x3.65x10^{-4}t} = C_t = \frac{1806.25x3.65x10^{-4}t}{1 + 1.55x10^{-2}t} \dots\dots\dots\dots\dots\dots\dots\dots\dots (7a)$$

Appendix D: Laboratory Equipment's and Samples Photo



D1: Soxhlet Apparatus set up



D2: Extracted oil in the solvent



D3: Hexane recovery set up



D4: M. stenopetala seed



D5: AV Test result color after Titration



D6: SV test color change after Titration



D7: GC-MS Analyzer Instrument



D8: FTIR Instrument