EXTRACTION AND CHARACTERIZATION OF MORINGA OLEIFERA KERNEL OIL AND ENCAPSULATION BY COAXIAL ELECTROSPINNING

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by

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DEDICATION

To Almighty God for His Love and Protection over me and my family.

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TABLE OF CONTENT

CHAPTER 4 - PHYSICOCHEMICAL PROPERTIES, OXIDATIVE STABILITY, ANTIOXIDANT ACTIVITY OF MORINGA OLEIFERA KERNEL OIL

CHAPTER 5 - THERMAL ANALYSIS AND STABILITY STUDY

CHAPTER 6 - MICROENCAPSULATION OF MORINGA OLEIFERA KERNEL OIL

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBRVIATION

ΔH	Enthalpy
AFM	Atomic Force Microscopy
CP	Cold Pressing
DSC	Differential Scanning Calorimetry
EAAE	Enzymes Assisted Aqueous Extraction
EACP	Enzymes Assisted Cold Pressing
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
Mo	Moringa oleifera
SE	Soxhlet Extraction
Tc	Crystallization Temperature
Tc	Thermal Cycle
Tg	Glass Transition
TGA	Thermogravimetric Analysis
Tm	Melting Temperature
UV-Spec	Ultra-Violent Spectroscopy
WCA	Water Contact Angle
α – tocopherol	Alpha tocopherol
γ – tocopherol	Gamma tocopherol
δ – tocopherol	Delta tocopherol

PENGEKSTRAKAN DAN PENCIRIAN BAGI MINYAK MORINGA OLEIFERA DAN PENGKAPSULAN MELALUI KAEDAH "COAXIAL ELECTROSPINNING"

ABSTRAK

Moringa oleifera *(Mo*) tergolong dalam keluarga Moringaceae dengan 14 spesis yang berasal dari Amerika Selatan, Afrika dan Kepulauan India. Kernel *Mo* boleh digunakan minyak, perubatan, makanan tambahan dan purifikasi air. Dalam kajian ini, empat jenis kaedah pengekstrakan yang berbeza iaitu penekanan sejuk (CP), penekanan sejuk dibantu dengan enzim (EACP), pengekstrakan akues dibantu dengan enzim (EAAE) dan pengekstrakan dengan menggunakan bahan kimia (SE) telah digunakan untuk menghasilkan minyak daripada kernel Moringa oleifera. Hasil dan peratusan perolehan semula minyak adalah 8% dan 23% (CP), 12% dan 36% (EACP), 23% dan 70% (EAAE) dan 33% and 99% (SE). Pengaruh kaedah pengekstrakan yang berbeza terhadap hasil minyak dan mikrostruktur kepingan *Mo* telah dikaji. Ciri-ciri fizikokimia dan antioksida, aktiviti oksidatif dan kestabilan simpanan minyak yang diekstrak juga telah dinilai. Profil asid lemak kesemua minyak yang diekstrak yang dianalisa dengan menggunakan Gas Kromatografi Spektroskopi Mass (GCMS) menunjukkan kandungan asid lemak mono tidak tepu (MUFA) adalah sebanyak 69 hingga 80%. Asid oleik didapati dominan dalam minyak kernel *Mo*, menunjukkan kestabilan yang tinggi terhadap pengoksidaan. Analisis haba minyak juga dikaji dengan menggunakan Kalorimetri Pengimbasan Berbeza (DSC). Hasil kajian menunjukkan bahawa kaedah pengekstrakan yang berbeza mempunyai kesan yang signifikan terhadap ciri-ciri lebur minyak kernel *Mo*. Berdasarkan ciri-ciri fizikokimia, kestabilan oksidatif dan komposisi asid lemak, minyak CP dipilih untuk dianalisa

selanjutnya dengan mengkaji sifat-sifat fitokimia, kesan degradasi terma terhadap kandungan tokoferol menggunakan Kromatografi Cecair Berprestasi Tinggi (HPLC). Kemudian, minyak CP diperkayakan dengan α, γ dan δ tokoferol dan kandungan tokoferol kemudiannya dikira selepas setiap rawatan haba (julat suhu 150-210ºC, kitaran haba 0, 1, 3 dan 5 kali selama 10 minit). Hasil menunjukkan bahawa penambahan δ-tokoferol mengurangkan degradasi terma dengan berkesan berbanding dengan α dan γ tokoferol. Bagi meminimumkan kemerosotan kualiti minyak kernel *Mo* disebabkan oleh rasa tengik minyak serta memperluaskan penggunaannya di dalam industri, mikroenkapsulasi dilakukan dengan menggunakan kaedah "coaxsial elektrospinning". Pembentukan bahan dinding yang optimum diperolehi pada 40 (w/v)% larutan zein prolamine asid asetik, iaitu gabungan aliran terbaik untuk elektrospinning bagi larutan zein prolamine asid asetik dan minyak kernel *Mo* (CP) yang masing-masingnya dicapai pada 0.7mL/jam dan 0.1 mL/jam. Ciri-ciri nanofiber yang dihasilkan diimbas dengan menggunakan Mikroskop Pengimbasan Elektron Pelepasan Medan (FE-SEM), Mikroskopi Transmisi Elektron (TEM), Mikroskopi Tekanan Atom (AFM), Spektroskopi Inframerah Transformasi Fourier (FTIR), Sedut Sentuh Air (WCA), Kalorimetri Pengimbasan Berbeza (DSC) dan Analisis Thermogravimetrik (TGA) yang masing-masing menentukan histologi, morfologi teras, topografi, interaksi kimia, ujian hidrofobik, ciri-ciri haba dan analisis haba. FE-SEM dan TEM mengesan nanofiber yang dihasilkan adalah berbentuk tiub tanpa pembentukan manik dan menunjukkan minyak berjaya di enkapsulasikan di dalam nanofiber. ATR-FTIR menunjukkan bahawa tiada interaksi kimia antara teras dan bahan dinding, nanofiber dari sampel A(tanpa minyak) dan B(dengan minyak) pada 0.5 dan 0.1mL/ jam alir polimer dan minyak, menunjukkan kehidrofobikan dengan 122 dan 119°, tetapi ikatan pada sampel C (0.7mL/jam) dan D (0.9mL/jam)aliran

polimer dan minyak 0.1mL/jam menunjukkan hidrofililik, sementara itu TGA menunjukkan bahawa tiada kemerosotan haba pada minyak yang telah dienkapsulasit. Kecekapan enkapsulasi nanofiber yang dihasilkan adalah 84 – 92 %. Kesimpulannya, kaedah pengekstrakan CP adalah kaedah terbaik untuk mengekstrak minyak dalam bentuk asalnya berbanding kaedah pengekstrakan lain yang melibatkan penggunaan bahan kimia, enzim dan haba. Selain itu, kaedah "coaxial elektrospinning" berjaya menghasilkan nanofiber yang boleh memerangi kerosakan minyak dan seterusnya dapat meluaskan pengguaan produk ini di dalam industri lain.

EXTRACTION AND CHARACTERIZATION OF MORINGA OLEIFERA KERNEL OIL AND ENCAPSULATION BY COAXIAL ELECTROSPINNING

ABSTRACT

Moringa oleifera (*Mo*) belong to the family Moringaceae with 14species, originated from South America, Africa and Indian Subcontinent. The kernel can be used for oil production, medicine, food supplement and water purification. In this study, four different types of extraction methods; namely cold pressing (CP), enzyme assisted cold pressing (EACP), enzyme assisted aqueous extraction (EAAE) and solvent extraction (SE) were used to produce oil from *Mo* kernel. The oil yield and oil recovery obtained were 8% and 23% for CP, 12% and 36% for EACP, 23% and 70% for EAAE and 23% and 99% for SE, respectively. The influence of different extraction methods on the microstructure of *Mo* kernel and flak*e* were investigated. The physicochemical properties, antioxidant properties, oxidative activities and storage stability of the extracted oil were assessed. The fatty acid profile of the extracted oil was analysed using Gas Chromatography Mass Spectroscopy (GCMS), which reveal the monounsaturated fatty acid (MUFA) content range from 69 to 80%. Oleic acid found to be dominant in the *Mo* kernel oil, indicating its high stability towards oxidation. Thermal analysis of oil was also investigated using Differential Scanning Calorimetry (DSC). The result shows that different extraction method has significant effect on melting behaviour of *Mo* kernel oil. Based on the physicochemical properties, oxidative stability and fatty acid composition, CP oil was selected to be analyzed further by testing for its antioxidant properties and the effect of thermal degradation towards tocopherol content using High Performance Liquid Chromatography (HPLC).

The CP oil was enriched with α, γ and δ tocopherol and the tocopherol content was quantified after each thermal degradation (temperature range of 150 – 210 ºC, thermal cycles of 0, 1, 3 and 5 times for 10 mins). The result shows that δ-tocopherol enrichment reduce thermal degradation effectively compared to α and γ tocopherol. With the aim to minimize *Mo* kernel oil deterioration due to rancidity, as well as to extend its usage in the industries, microencapsulation was carried out by coaxial electrospinning method. The optimal wall material formation was obtained at 40 (w/v)% zein prolamine-acetic acid solution, with the best combination of flow rate for zein prolamine –acetic acid solution and CP extracted *Mo* kernel oil was achieved at 0.7 mL/hour and 0.1 mL/hour respectively. The characteristic of the nanofiber produced were investigated using Field Emission Scanning Microscopy (FE-SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM), Fourier Transform Infrared (FTIR) spectroscopy, Water contact angle (WCA), Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) to determine the histology, core morphology, topography, chemical interaction, hydrophobicity, thermal behaviour and thermal analysis. The FE-SEM and TEM shows that the nanofiber produced were tubular structure without beads formation and the oil were successfully encapsulated in the nanofiber. The ATR-FTIR reveals that there is no chemical interaction between the core and the wall material, while the nanofiber from sample A (without oil) and B (with oil) at 0.5 and 0.1 mL/hour flowrates of polymer and oil, reflects hydrophobicity with 122 and 119º, but sample C (0.7mL/hour) and D (0.9mL/hour) flowrates of polymer and 0.1 mL/hour oil reveals hydrophilic property. Meanwhile the TGA indicate that no thermal degradation on the encapsulated oil. The encapsulation efficiency ranges from was 84 -92 % for the fabricated nanofibers. In conclusion, CP extraction method is the best way to extract oil in its native form compared to other extraction methods that involve the use of chemical, enzyme and heat. Likewise, the coaxial method of electrospinning has produce nanofiber that can combat rancidity of oil and makes the product viable for further usage in other industries.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Moringa oleifera (Moringaceae) is a fast-growing softwood tree indigenous to sub-Himalayan tracts of Northern Indian (Leone et al., 2016, Tsaknis and Lalas, 2002). The tree has been used in a many ways to support nutrition in both plant and animal (Ruttarattanamongkol and Petrasch, 2015a, Rashid et al., 2008, Pereira et al., 2016). All part of the tree is useful for both traditional and modern medication. The seeds are rich in bioactive compound which have antimicrobial, anti-genotoxic, anti-inflammatory and anti-tumour properties (Leone et al., 2016). The oil with a brilliant yellow colour, pleasant taste and aroma has been used for cooking, medicine and pharmaceutical purposes because of its nutritional contents. The oil is rich in unsaturated fatty acid with 70% of it being oleic acid, (Abdulkarim et al., 2005b, Ruttarattanamongkol and Petrasch, 2015a, Ruttarattanamongkol et al., 2014, Ogunsina et al., 2014b). Oil rich in oleic acid have been reported to be stable at food processing application requiring high temperature like frying, it has the ability to prevent ulcerative colitis, prevent cell from free radicals damage, reduce cholesterol and blood pressure (Adegbe et al., 2016). Likewise the seed flake which can be use in purification of waste water, and soil conditioning (Ruttarattanamongkol and Petrasch, 2015a, Ndabigengesere and Narasiah, 1998, Tsaknis and Lalas, 2002).

Edible seed oils constitute an important source of bio-component in food such as saturated and unsaturated fatty acids, phospholipids, carotenoids, tocochromanols, phytosterols, squalene, lignans, and phenolic compounds Górnaś et al. (2014) which promote proper functioning of the organism and good health.

Natural qualities of these edible oil are easily affected during their processing (Kiralan et al., 2014). The refining process of oil has an adverse effects that causes reduction in their antioxidant activities by an estimate of 80%, reduction of carotenoid concentration by 70% and that of polyphenols by 26 – 55% (Teh and Birch, 2013, Górnaś et al., 2014). This highlights the need for alternative green methods using cold pressing - a cheap, simple, eco-friendly and less labour intensive method of extraction and enzyme as a means of pre-treatment to improve yield quantity of cold pressing method (Abdulkarim et al., 2006, Yusoff et al., 2016, Ezeh et al., 2016).

Oxidation is a chemical reaction that gives free radicals, which leads to chain reactions that can cause cell destruction (Tsao and Deng, 2004, Esmaeilzadeh Kenari et al., 2014). This free radicals react with lipids and cause lipid oxidation that occur during raw material storage, processing and heat treatment (Tomaino et al., 2005). Oxidation leads to change in colour and odour of oil thereby limiting the oil quality and nutritional values (Suja et al., 2004). Natural antioxidants such as phenolic compounds, flavonoids, and other phytochemicals can act as free radical scavengers which can also delay the lipid oxidation process and contribute longer shelf life of food products (Tohidi et al., 2017, Meullemiestre et al., 2014).

Thorough research on different extraction methods used to produce oil from different plants seed have been reported in literatures (Augustin et al., 2016, Luque de Castro and Priego-Capote, 2010, Armenta et al., 2015). Many more research on oil produce from *Moringa oleifera* through solvent extraction (Masime et al., 2017, Abdulkarim et al., 2005b, Bhutada et al., 2016, Yusoff et al., 2016) and supercritical fluid extraction (Zhao and Zhang, 2014b, Rai et al., 2017), have also been reported. However, not many research findings have been reported on the use of cold pressing to produce oil from *Moringa oleifera* seed perhaps because of the low oil yield and oil recovery associated with cold pressing (Uquiche et al., 2008, Al-Obaidi et al., 2013) and *Moringa oleifera* seed being classified as non-oily seed. Equally, Soxhlet extraction with higher oil yield is non-environmentally friendly and have impure product because of the solvent used during the process, thus becomes a source of health concerns on its oil and by-product. There are unanswered research questions on the influence of cold pressing on the native oil quality of *Moringa oleifera* kernel, stability of bio-active compounds contained in the extracted oil, quality of the metabolites composition of the extracted oil and isolation of the essential compounds of the oil are rarely mentioned in literatures.

Pretreatment have shown improvement in the oil yield and oil recovery of *Moringa oleifera* kernel (Delfan-Hosseini et al., 2017, Abdulkarim et al., 2006, Yusoff et al., 2016). Different pretreatment methods have been employed for cold pressing *Moringa oleifera* seed, Da Porto et al. (2016) used microwave assisted treatment; enzymatic (Yusoff et al., 2016); aqueous (Delfan-Hosseini et al., 2017); CO_2 (Ruttarattanamongkol et al., 2014). However, most of these treatment techniques leaves an after effect on the products (Song and Milner, 2001, Kidmose and Kaack, 1999). Thus the use of natural antioxidant such as tocopherol content can improve the oil quality without interfering on the natural composition of the oil.

Generally, edible oils are naturally not very stable and are susceptible to rancidity. Research efforts have shown that use of antioxidant, proper packaging and microencapsulation of oil can cause delay in rancidity, does prolonging the shelf-life and enhance availability of the valuable products to other industries. There are different types methods used in microencapsulating oil from plant seed, among them is electrospinning method which is now commonly used because of its simplicity and efficiency. However, the effectiveness depends on the solutions, ambient and processing parameters of the materials involved in producing fiber encapsulates. Coaxial electrospinning technique is a more efficient method that has a greater control over encapsulant layer, loading capacity and retention of core material (Yao et al., 2016, Yang et al., 2017). However, this techniques has never been applied to encapsulate oil extracted from *Moringa oleifera* kernel with zein polymer as wall material.

In view of the aforementioned literature gaps, the present study examines the influence of different extraction methods on the natural quality of *Moringa oleifera* kernel oil. Also, thermal behavior of the oil when expose to heat before and after enrichment is evaluated. Furthermore, the oil produce from *Moringa oleifera* kernel was microencapsulated and the oil in its nanofiber form was examined and the efficiency determined.

1.2 Problem Statement

There are different extraction methods: rendering, supercritical-fluid, solvent, enzymes, Soxhlet etc., that are used in producing oil from plants seeds and kernel (Azwanida, 2015, Abdulkarim et al., 2005a, Samaram et al., 2015). But this methods of extraction impact the oil and its by product in a manner that affects the potency and nutritional value of the oil because of the processes involved during extraction which interacts with the oil natural quality (Tongnuanchan and Benjakul, 2014, Al Juhaimi and Özcan, 2018, Delfan-Hosseini et al., 2017).

Cold pressing is an age long technique use in extracting oil from plant parts. The method is very simple, inexpensive, environmentally friendly with preserved natural colour and flavor (Augustin et al., 2016, Górnaś et al., 2014) and exclude the use of chemical and heat interaction during processing. However, the major set-back in this method is the low oil yield and oil recovery after processing. Although, several researchers have used different pretreatments techniques such as: enzyme treatment, size reduction, microwave treatment and dehulling to improve the oil yield and oil recovery in cold press method (Ezeh et al., 2016, Yusoff et al., 2016). The low oil yield and oil recovery can be compensated for by the natural oil quality preservation.

Heating oil at high temperature during food processing alters its molecular structure in an unfavorable manner and affect the oil nutritional value making it carcinogenic (Guillen and Goicoechea, 2009, Santos et al., 2005, Abdulkarim et al., 2007). Literature reviews shows enrichment of oil with natural antioxidant (i.e. tocopherol) can reduce the effect of degradation during frying processing. However, quality attributes of *Moringa oleifera* oil characteristic under thermal analysis has not been reported in literature. The thermal analysis knowledge is important in studying the thermal stability of *Moringa oleifer*a oil.

Oils are chemically unstable and susceptible to oxidative deterioration and loss of volatile compounds, especially when exposed to oxygen, light and moisture. The oil quality may deteriorate due to oxidative degradation, forming unpleasant tastes and off-flavors and the generation of free radicals. These changes have a negative effect on the shelf-stability, sensory properties, and overall acceptability of the developed products (Zhang et al., 2017b, Abdulkarim et al., 2007). Nowadays, researchers have focused attention on microencapsulation techniques to mitigate against these set-backs. Thus far, microencapsulation technique can improve stability in oil, maintain biological assay compounds and functional characteristics of oil. Although, there are different techniques of microencapsulation of oil in existence but not all the methods of microencapsulation are very effective towards mitigating against aforementioned problems. Microencapsulation of oil through electrospinning methods have gained prominence due to its effectiveness and simplicity, but, the effectiveness of the methods lays on accurate selection material and optimization of the experimental and operation parameters.

1.3 Objectives of the study

1. To examine the influence of different extraction methods (cold press, enzyme assisted cold press, enzyme assisted aqueous extraction and Soxhlet extraction) on oil yield and microstructure of *Moringa oleifera* kernel and flake.
2. To evaluate the quality of extracted *Moringa oleifera kernel* oils using physicochemical properties, fatty acid profile, antioxidant activities and oxidative stability properties.
3. To determine the thermal behavior, perform thermal degradation analysis and stability on the *Moringa oleifera* kernel oil before and after enrichment.
4. To microencapsulate *Moringa oleifera kernel* oil and examine the microstructural morphology and physical characteristics.

1.4 Scope of Study

In this research work, the *Moringa oleifera* plant used belongs to the family of (*Moringaceae).* Matured plant - pods were harvested from Southwest region of Nigeria. The pods were cracked, sorted, winnowed to remove unwanted particles and sun dried for a period of three days during a sunny weather condition. Later on the seed (endocarp layer) were cracked to expose and remove the kernels. Proximate composition and micromorphology was performed on the *Mo* kernel and kernel-flake. The extraction methods employed to produce oil from the *Moringa oleifera* kernel are limited to four types (cold press extraction, enzymes assisted cold press, enzymes assisted aqueous extraction and solvent extraction). The oil yield and oil recovery produced were compared among the four extraction methods utilized. In addition, physicochemical properties, characterization, oxidative properties and antioxidant activity were conducted on the oil produced from each of the four types of extraction methods. The extracted oil samples were compared amongst the different extraction methods to evaluate their influence on the extracted oils. The oil produced from the optimal extraction methods in terms of native oil quality retention was now quantified to determine the tocopherol contents and presence of bio-assay compounds (phytochemicals) followed by enrichment with natural antioxidant (tocopherol) after which, the stability studies was conducted on the pure *Moringa oleifera* kernel oil and enriched *Moringa oleifera* kernel oil on monthly basis for a duration of ninety days. Furthermore, the oil sample was subjected to thermal analysis test to determine the oil behaviour under varying temperature and heating cycle. The thermal analysis test was conducted for the oil samples before and after enrichment with tocopherol. Thereafter, the oil sample were microencapsulated using coaxial electrospinning method and the oil in its nanofiber form was evaluated appropriately using different analytical tools and physical evaluation test.

The summary of the research work is illustrated in flowchart as shown in Figure 1.1.

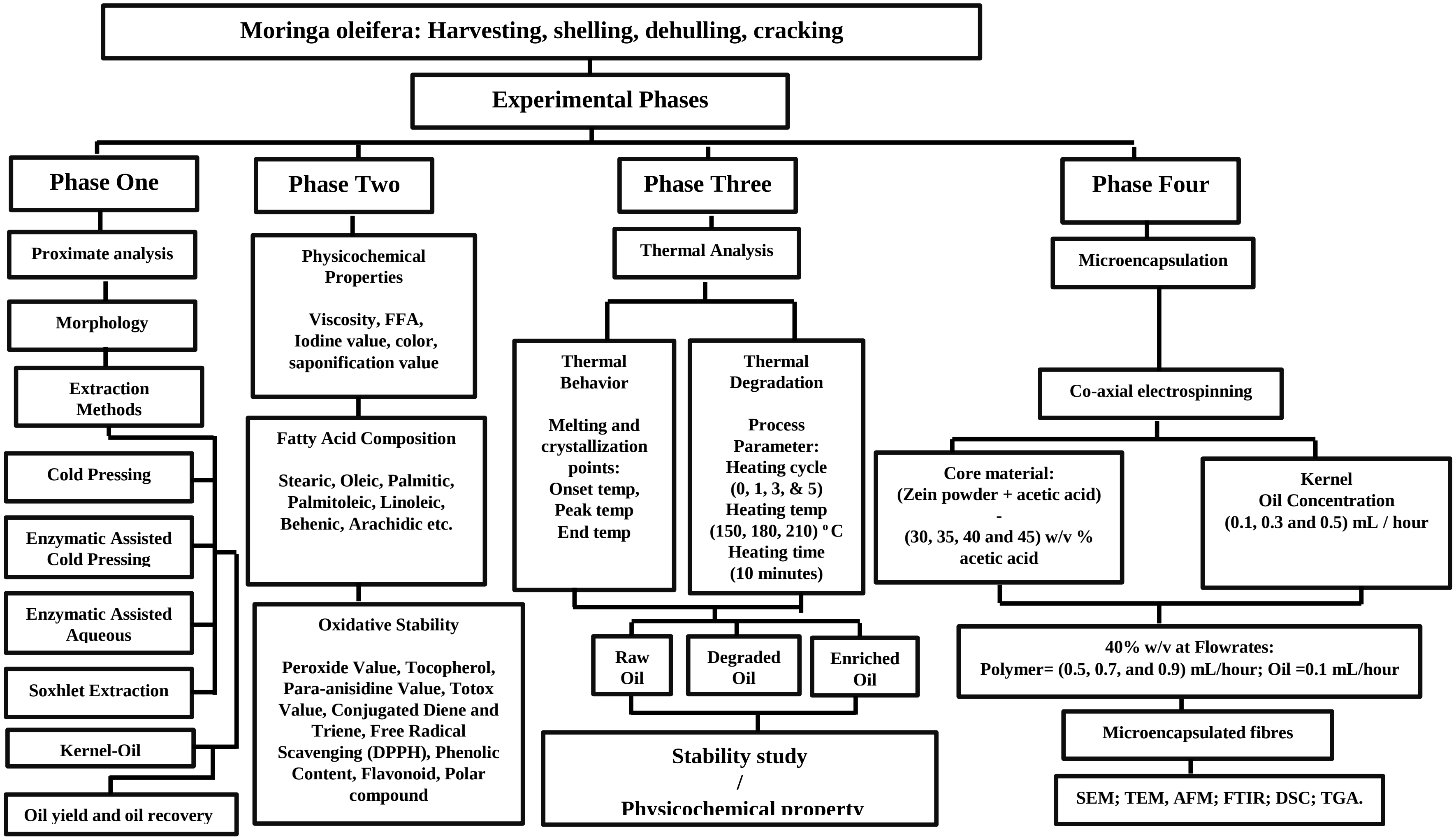

Figure 1.1: Flow Chart of Experimental Implementation.

CHAPTER 2

LITERATURE REVIEW

2.1 Historical Information on *Moringa oleifera*

Moringa oleifera is one of the mono generic families of *Moringacea,* which has fourteen (14) known species of the shrubs and trees that have its native origin from the Himalaya part of Northwest India, from where they have been introduced in many warm countries Pakistan, Afghanistan, Nigeria, Senegal, Tanzania, Brazil, Mexico, Philippines Cambodia, Central America, North and South America, and the Caribbean Islands (Abdulkarim et al., 2005a, Salem and Makkar, 2009, da Silva et al., 2010, Crosby, 2007, Animashaun and Toye, 2013, Anwar and Bhanger, 2003). The plant are also known as Horseradish tree, Drum stick tree or Ben oil tree. *Moringa oleifera* (*Mo*) seeds are expected to germinate within 5–12 days after seeding and can be implanted at a depth of 2 cm in the soil.The tree can grow up to 5-12m in height. They are mostly cultivated in arid, semiarid and warm areas, with temperatures varying between 25 and 35 $^{\circ}$C, in some area they able to tolerate up till 48 °C and in weak frost conditions in subtropical areas (Gopalakrishnan et al., 2016, Gupta et al., 2017).

It is tolerable to drought and grows with annual precipitation, it can adapt to drought between 250 and 3000 mm in altitudes under 600 m. Furthermore, it evolves in soil with pH varying from 5.0 to 9.0, but it prefers neutral and well-drained soils. It also adapts better in sandy- clay, well-drained soil and it can grow in clay soil without standing water. The tree (Figure 2.1a) does not require soil fertility as it can developing itself in poor soil. The flowering appears twice in a year, about 8 months after being planted, it can be cultivated by seed, leaves, tree-bark and stem-cutting (Crosby, 2007, Pereira et al., 2016, Salaheldeen et al., 2014, Anwar and Bhanger,

2003) The tree production rates is considered fast because it reach maturity within 3 years of being planted, the pod (Figure 2.1c-e) are approximately 50cm long, when matured to give a round or triangular light wooden seed shell with three papery wings (Figure 2.1f), (Abdulkarim et al., 2005a). A single tree can produce between 1000–1600 seed pods per year with an average of 24000 seeds (Ayerza, 2011, Pereira et al., 2016, Leone et al., 2016) which when shelled produces the kernel (Figure 2.1g).

Mo plants are cultivated for their nutritional compositions, medicinal and herbal connotation among other reasons. Every part of *Mo* is a storehouse of important nutrients**.** *Mo* plant parts such as leaves, flower, roots and seeds are useful for food consumption (Gupta et al., 2017, Brilhante et al., 2017), medicinal purposes (Sujatha and Patel, 2017, Karagiorgou et al., 2016) and forage (Kasolo et al., 2010, Anwar et al., 2007); the plants has exceptional compositions of vitamins, minerals, amino acids and other useful nutrients for human and animal diet (Salem and Makkar, 2009, Gupta et al., 2017, Gopalakrishnan et al., 2016); the kernel oil constitute food components that serves as antimicrobial, anti-inflammatory, anti-tumour and antipyretic promoting activities (Lalas et al., 2012, Chuang et al., 2007b, Ogbunugafor et al., 2011). *MO* kernel can be use in treating hyperthyroidism, Chrohn's disease, antiherpes-simplex virus arthritis, rheumatism, gout, cramp, epilepsy and sexually transmitted diseases (Gopalakrishnan et al., 2016). For non-food purposes such as cosmetic, water treatment, biofuel has been reported (Sutherland et al., 1994, Richter et al., 2003, Fahey, 2005, Abdulkarim et al., 2005a, Abdulkarim et al., 2006, Anwar et al., 2007, Leone et al., 2016).

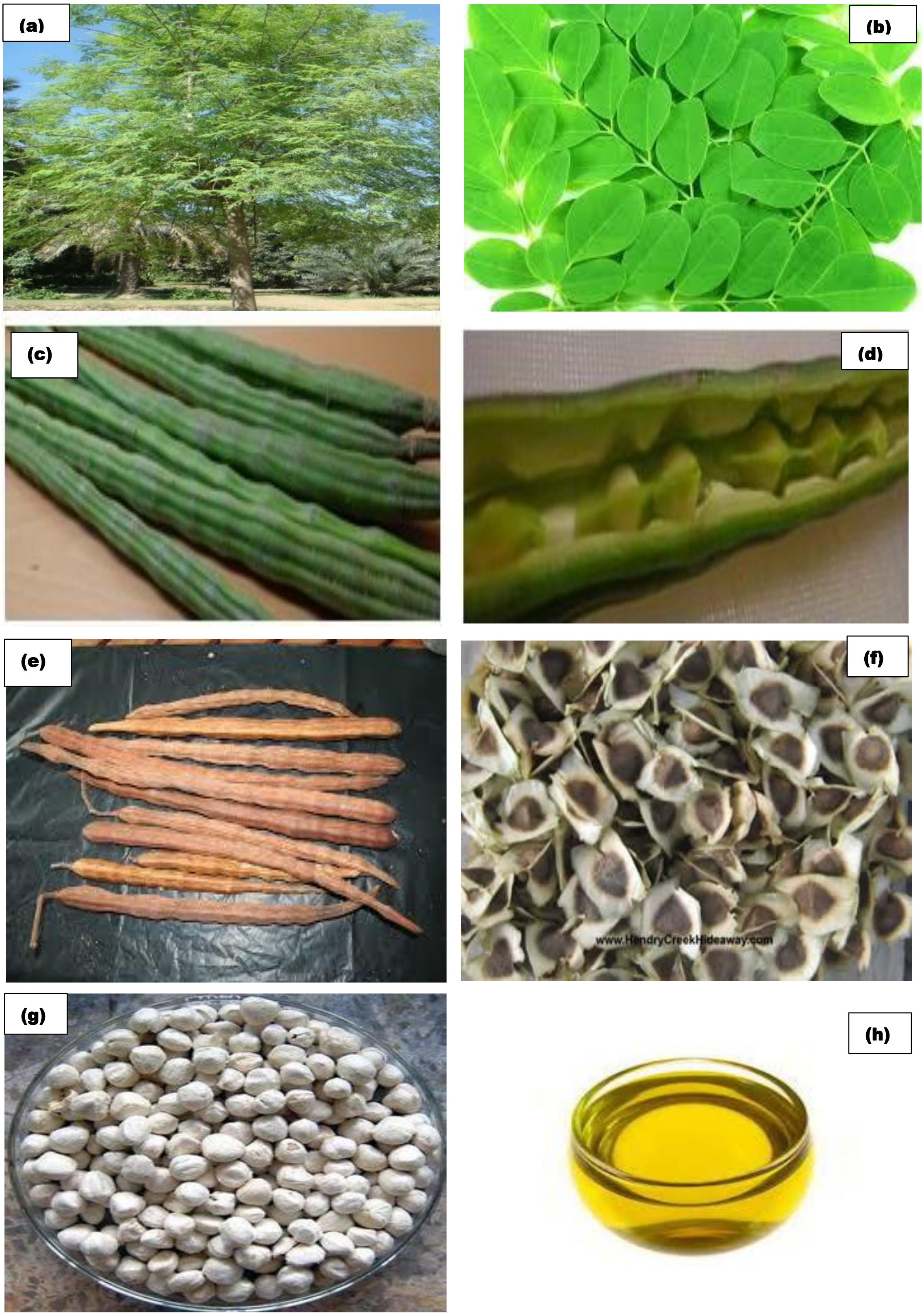

Figure 2.1: Moringa Oleifera (a) Tree; (b) Leaves; (c) Immature Pod; (d) Immature Seed; (e) Matured Pod; (f) Matured Seed; (g) Kernel and (h) Oil.

2.2 Factors Influencing Nutritional Composition of *Moringa oleifera*

The inherent nutritional composition of *Mo* plants varies with species and geo-ecological conditions. The mineral element concentrations in different edible parts of *Moringa* spp. are affected by the environment in which they grow (Kumssa et al., 2017). For example the tree grown in India has slightly different nutritional components than a tree grown in Nigeria according to the report of Asante et al. (2014), a study on the nutritional differences in the leaves from two ecological locations i.e. semi-deciduous and Savannah regions was performed. The result showed that the latter was less nutritious than the former and attributed the differences to high temperatures at the Savannah regions. Proteins and enzymes get denatured at higher temperature and this could be the cause for the difference in nutrient content. Similarly, in the study of Kumssa et al. (2017), reported the impact of environment on mineral elements concentration on *Mo* parts in Ethiopia. Their findings shows concentrations of Ca, Fe, and Zn in *Moringa* leaves grown in mid-altitude areas during the rainy season were 24800, 578 and 24.3 mg kg/1 dw for *Moringa Oleifera* and 14900, 700 and 24.7 mg kg/1 dw for Moringa stenopetala respectively. A study conducted by (Olson et al., 2016) indicated variation in leaf elemental concentration between 12 Moringa species grown in a common garden experiment. Likewise in Nigeria, it was reported that Ca, Mg, Fe and Cu concentration in the leaves, pods and seeds were higher in tissues collected from Sheda region than Kuje, Abuja (Anjorin et al., 2010).

2.2.1 Harvesting *Moringa oleifera*

The two most useful part of a *Mo* plant are the leaves and the seed. The tree has a compound leaf which means one leaf is made up of multiple leaflets. The manual harvesting of shoots and leaves are done with a pair of shears, a sickle or a sharp knife.

Mechanical harvesters are also been used in large-scale, intensive leaves production. Harvesting can also be done by removing the leaves, picking them directly off the tree. They are easily removed at the base of the petiole. In most cases, the produce are harvested at the coolest time of the day: early morning or late in the evening. It is important to make sure there is no dew on the produce before harvesting, especially in the morning, to avoid rot during transport, (Adu-Dapaah et al., 2015, Gunstone, 2011). Likewise in the seed farms, pods should be harvested as early as possible when they reach maturity, i.e. when they turn brown and dry. The harvested pods are crushed and the seeds are removed. The seeds are sundried, crushed to remove the kernel. The removed kernels are bagged, and stored in a dry place.

2.3 Nutritional Composition –*Moringa oleifera* kernel

Moringa oleifera kernel are eaten either in the immature pod or when fully maturated. The matured seeds can be pressed to produce Moringa oil, which is used in cooking, cosmetics and pharmaceuticals. *Mo* kernel contains approximately 25-40% oil depending on plant variety and climate condition (Ruttarattanamongkol et al., 2014). *Mo* oil is rich in monounsaturated fatty acid which is mainly oleic acid (Leone et al., 2016, Rahman et al., 2014), followed by behenic, stearic, palmitic and arachidic acid with traces of linoleic and palmitoleic acid, (Muyibi and Evison, 1995, Ogunsina et al., 2014a). *Mo* flake contains positively charged molecules that serves as coagulant and source of animal feed (Sutherland et al., 1994, Salem and Makkar, 2009). Table 2.1 describes the proximate and mineral composition of *Mo* kernel.

Table 2.1: Proximate Composition of *Moringa oleifera* Kernel (mg/100g) Dry Mass

Nutrients / Minerals	**Authors / Years**			
	Olagbemide and Alikwe (2014)	Stadtlander and Becker (2017)	Taher et al. (2017)	Amabye et al. (2015)
Crude fat	38.67±0.03	37.42±0.42	35.10±0.02	10.31±0.21
Crude protein	35.97±0.19	37.52±0.23	29.40±0.03	10.71±0.83
Carbohydrate (By Difference)	8.67±0.12	11.51±0.31	8.52±0.02	7.61±2.19
Crude fibre	2.87±0.03	5.28±0.07	11.23±1.03	6.82±0.03
Ash content	3.87±0.09	4.43±0.02	4.56 ± 0.06	7.29±1.84
Moisture content	9.97± 0.09	6.43±0.52	10.74±1.05	3.34±1.36
Phosphorous	635.00±8.66	452.00±3.41	455.00±0.11	505.01±0.03
Potassium	75.00±0.00	67.00±0.02	61.01 ± 0.04	97.00±0.54
Calcium	251.63±0.03	246.14± 0.01	210.12±0.01	263.02±0.01
Iron	5.20±0.15	4.04±0.12	1.23 ± 0.01	7.03±0.11
Zinc	0.05±0.00	0.12±0.00	0.45 ± 0.01	1.01±0.03
Manganese	45.00±0.00	38.00±0.01	45.01± 0.22	38.01±0.13
Vitamin B1 (mg)	0.05	0.04	0.05	0.05
Vitamin B2 (mg)	0.06	0.04	0.05	0.06
Vitamin B3 (mg)	0.2	0.3	0.3	0.2
Vitamin C (mg)	4.20±0.13	2.8±0.13	4.5± 0.17	4.4±0.13
Vitamin E (mg)	751.11± 0.04	701.33±0.00	722.02±0.01	718.02±0.04

2.3.1 Moringa oleifera Kernel Oil

Vegetable oils are commonly extracted from seeds of plant, although some other part of plant can yield oil (Cao et al., 2017). There are two categories of seed-plant for extraction purpose; the oily-seed and non-oily seeds plants. The oily-seed plants are referred to as seed-plant with oil yield of 50% and above while non-oily seed have less than 50 %. *Mo* seed is classified as a non-oily seed, with low oil yield during extraction (Augustin et al., 2016); the oil from the kernel contains strong antifungal activities against Zoophilic dermatophyte causing inflammatory reaction in human (Chuang et al., 2007a). The effectiveness of the oil has been proven to optimize neurological transmission and blood function (Sumich et al., 2013); the presence Polyphenols: quercertine, kaemferol, isoquercertine, kampterin and rhaminetin proves the ability to strengthen cell membrane, repair worn out tissue. (Saito et al., 2006, Ogbunugafor et al., 2011, Siddhuraju and Becker, 2003); reduces low density lipoprotein (bad cholesterol); the glycemic index, total cholesterol, due to the presence of oleic acid (Martinez-Herrera et al., 2006). Table 2.2 shows some examples of vegetable oil seed plants and the statistics for world market and trade with production in million metric tons

Table 2.2: Oil content of some oil bearing vegetable materials and global oil seed production

Author / Year	**Types of Plant - Seed**	**%Composition range of Oil**
Jiao et al. (2014), Siano et al. (2016),Türkmen et al. (2017)	Pumpkin	65 – 75
Ghosh et al. (2014), (Deli et al., 2011)	Copra seed	60 -70
Mohammed et al. (2018), Shakeri et al. (2016),Gharby et al. (2017)	Sesame seed	55 - 60
Omoriyekomwan et al. (2016),Ma et al. (2017), Oh et al. (2016)	Palm kernel	50 - 55
Alenyorege et al. (2015), Alhassan et al. (2017), Khalid (2017)	Groundnut	45 - 50
Hansen et al. (2017), Gao et al. (2017), Angın and Şensöz (2014)	Rapeseed	40 - 45
(Chemat et al., 2015), (Moralı et al., 2018)	Sunflower seed	35 - 45
Ganesapillai et al. (2016), Al-Farraji et al. (2017),Ruttarattanamongkol and Petrasch (2015b)	Olive kernel	25 - 30
Seal et al. (2015), Önal et al. (2017)	Cotton seed	18 - 25
Sawada et al. (2014), Mamauag et al. (2017)	Soybean	15 - 25
Yusoff et al. (2015),Abdulkarim et al. (2006)	*Moringa Oleifera*	25- 40

Types of Seeds	**Worldwide oil seed production in million metric tons**				
	2018-2019	**2017-2018**	**2016-2017**	**2015-2016**	**2014-2015**
Copra	5.829	5.734	5.512	5.319	5.424
Cotton seed	43.825	45.154	39.083	35.759	4.357
Palm kernel	19.112	18.399	17.362	15.958	16.567
Peanut	41.951	44.905	44.153	41.232	41.547
Rapeseed	70.971	74.028	69.436	68.740	70.429
Soybeans	367.497	338.567	348.946	316.242	320.565
Sunflower	50.443	47.406	48.008	40.540	39.189
Total	599.574	574.193	572.500	523.790	538.018

2.4 Extraction Methods

Extraction of oil for domestic and industrial application is an important step towards isolating the essential nutrients and biological active compounds present in the seeds (Samaram et al., 2015). The extraction of oil is a long held practise and there are different types of extraction methods applied to plant materials. The different types of extraction methods can be broadly classified into three distinct groups; the rendering method, mechanical method and solvent method.

2.4.1 Rendering Method

Rendering processing involves primarily thermal means of initiating separation of components. The basic procedures employed in rendering typically include size reduction, cooking, grinding and pressing Rosenthal et al. (1996), to separate fat and protein. The method is less efficient and it has been practiced for centuries but now obsolete. The two subgroup of rendering process is either dry rendering (the oil bearing material is subjected to direct heating to extract the fat) and the wet rendering (the oil bearing material will be boiled in water which leads to partial separation of oil from the material Ofori-Boateng et al. (2012) and afterwards the oil are skimmed off from the boiling vessel and stored.

2.4.2 Mechanical Method

The process of extracting oil from plant material using expression means to release the liquid content is referred to as mechanical pressing. It is usually use for oily plants parts with more than 20% oil content (Koubaa et al., 2016). This method is relatively efficient as it recovers 70-80% of the available oil content in the material (Tongnuanchan and Benjakul, 2014). However, for plant with seed-oil content below

20%, the efficiency is relatively low and depends on other process parameter employed during extraction (Rosenthal et al., 1996). There are two broad categories of mechanical expression method of oil from plant-seed, namely batch hydraulic pressing (small and laboratory scale) and continuous mechanical pressing (screw pressing).

2.4.2(a) Hydraulic Pressing Machine

During hydraulic pressing, the energy is delivered through the fluid pressure in the chamber which causing the chamber to expand and compress the samples by the use of pumps and valves. The merit of the machine is about the duration / distance operation, large capacity and under constant speed. However, the process is relatively expensive compared to other pressing machines types. Figure 2.2 describes the schematic diagram of a hydraulic pressing machines.

Figure 2.1: Image of Hydraulic Press Machine (Adesina et al, 2018)

2.4.2(b) Screw Pressing Machine

The operating energy is derived from the rotatory motion to turn a large screw. In this type of machine, a force is applied to the shaft through to the screw's head by a friction disk and pushes a ram and gain mechanical advantage (Muangrat et al., 2018). Screw presses expression is relatively slow and takes longer duration to operate compared with hydraulic press machine. The schematic diagram of a screw pressing machines is described in Figure 2.3.

Figure 2.2: Image of Screw Press Machine (Adetola et al, 2014)

Conventionally, both the hydraulic and the screw pressing methods can be operated either by pressing hot (involves heat treatment) or by cold pressing (without heat treatment) (Koubaa et al., 2016, Sultana et al., 2009). However, cold pressing is cheaper, simpler to operate with less processing steps, the extracted oil has better preserved native organoleptic properties and generally acceptable to consumers than hot pressed oil. Although cold pressing has low oil yield because the method of extraction cannot remove the total available oil which will still remain in the pressed cake (Ofori-Boateng et al., 2012, Koleva et al., 2002).

2.4.3 Solvent Method

The partitioning that involves separation of compounds based on solubility and boiling point of using two immiscible liquids is referred as solvent or liquid – liquid extraction methods. The solvent penetrate into the cell wall of the seeds and break open the cell structure to free the available oil. This method can be employed to oily and non-oily seeds due to its high oil yield and oil recovery. However, the effect on the produce and by-product natural quality remains a source of health concerns(Manubolu et al., 2018).

2.4.4 Some Recent Development in Methods of Oil Extraction

The conventional method of oil extraction are either producing low yield (quantity), or low quality with long processing time. These deficiency can be overcome with alternative methods that are quicker, less expensive and do not require the use of solvent. The alternative methods can be employed alone or in combination with other extraction methods. The pretreatment process are easily combined herewith to improve yield production, shorten extraction time, better heat regulation and enhanced oil quality.

2.4.4(a) Supercritical Fluid Extraction (SFE)

SFE is a viable alternative method of extraction compared to the conventional mechanical pressing and solvent extraction for plant materials (Ruttarattanamongkol et al., 2014). SFE has ability to recover valuable natural nutrients, enhanced yield production and better quality of plant-seed extracts. The carbon dioxide (CO_2) is mostly used as fluid in food industry because of its low critical temperature and pressure (T_c =31.1°C, Pc = 7.28MPa), non -toxic, non-inflammable, high purity and inert as it does not react with food constituent (Pourmortazavi and Hajimirsadeghi, 2007, McHugh and Krukonis, 2013). This supercritical carbon dioxide (SC-CO_2) has been successfully used for extraction in grape seed (Rombaut et al., 2014), flaxseed (Özkal and Yener, 2016), muskmelon (Maran and Priya, 2015), moringa oleifera seed (Ruttarattanamongkol et al., 2014), camelina sativa seed (Belayneh et al., 2017), and quinoa seed (Przygoda and Wejnerowska, 2015)

2.4.4(b) Ultra Sound Assisted Extraction (USAE)

USAE can be used as a pretreatment which has multiple applications such as freezing, drying, cutting, bleaching, sterilization, emulsification, homogenization, crystallization, foam removal and importantly extraction of oil because of its high replicability, accuracy and due to its cavitation phenomena and mass transfer enhancement (Boukroufa et al., 2015). USAE can function alongside with Solvent extraction and Supercritical fluid as pretreatment to extract oil from plant-seeds such as pumpkin seed (Jiao et al., 2014); papaya seed (Samaram et al., 2015); flax and canola seed (Teh and Birch, 2014); oleaginous seed (Sicaire et al., 2016), and still shows a reduced extraction time, increase extraction yield and non-toxic effect to the produce (Boukroufa et al., 2015).

2.4.4(c) Enzymatic Extraction

During enzymatic extraction, enzymes are used to degrade the cell walls of plant seed with water acting as the solvent causing high pressure on the cell wall which generate swelling of the plant cell. This generated pressure pushes the cell-wall from inside, stretching and immediately rupture it to facilitate leaching out its constituents. Enzymes extraction makes fractionation of the oil from the seed much easier and has low cost of maintenance and safe to operate (Koubaa et al., 2016, Kaufmann and Christen, 2002, Mandal et al., 2007). However, the efficiency of enzymes extraction is a function of the soaking time and temperature (Koubaa et al., 2016, Li, 2002, Mandal et al., 2007). It is estimated that the costs of enzymes extraction process are much greater than hexane extraction Koubaa et al. (2017).

2.5 Classification of Extraction Method

There are four broad categories in which extraction methods can be classified, however, the classification based on mechanical expression and solvent is adopted in the present study. This study subdivide this classification of extraction method into four types, mainly, the cold pressing (CP), enzymes assisted cold pressing (EACP), enzymes assisted aqueous extraction (EAAE) and Soxhlet extraction (SE) methods

2.5.1 Cold Press Extraction Method.

The process of expressing oil from plant-seed with a screw press or hydraulic press without heat is referred to as cold-pressing (Rosenthal et al., 1996). Cold-pressing is a viable alternative extraction method of oil from plant-seed as it does not require the use of organic solvent or heat (Teh and Birch, 2013). Cold pressed method is an age-long technique, yet effective method to extract oil from oil seeds by subjecting the seeds to pressing load till oil recovery. Cold pressed method can potentially extract the largest number of bioactive compounds such as essential fatty acids, phenolic, flavonoids and tocopherol since it's a nondestructive procedure which do not modify bioactive compounds through chemical or physical means (Azmir et al., 2013). However, the disadvantage of cold-pressing is on the low yield during extraction (Teh and Birch, 2013).

In order to preserve the natural properties of plant extracts such as oil, the qualitative and quantitative measures mostly rely on the selection of proper extraction method to maintain the bioactive compounds from plant materials (Azmir et al., 2013). During Soxhlet extraction, the solvent is usually recovered by evaporation. The extraction and evaporation temperatures have a significant effect on the quality of final products (Mamidipally and Liu, 2004) . Many nutraceuticals such as phenolic, alkaloids and glycosidic compounds are poorly soluble in carbon dioxide and hence not extractable using supercritical fluid extraction (Pourmortazavi and Hajimirsadeghi, 2007, Wang and Weller, 2006). Corrales et al. (2008), extracted bioactive compound such as anthocyanins from grape by-product using various techniques and found better extraction of anthocyanin mono-glucosides by pulsed-electric field (PEF) extraction, though number of bioactive compounds were fewer to other methods because of the positively charged electron effects on the chemistry of the plant extracts. Furthermore, the use of methanol as a modifier requires a slightly higher temperature to reach the supercritical state and this could be disadvantageous for thermolabile compounds (Özkal and Yener, 2016). It is obvious from several literatures that extraction method plays a key role in the quality of the plant extracts but unfortunately, there are no universal accepted standard extraction method for classified different plant extracts.

Thus far, the ability of cold pressed method to maintain the bioactive compounds of the oil and by-products can compensate for the low oil yield and oil recovery associated with cold pressed method. In addition, the use of inorganic solvent such as n-hexane during extraction can impact the natural quality of the oil and by-product in a way that raises safety and health concerns among consumers and manufacturers. Furthermore, the expensive, complicated and time consuming process of extracting oil and by-product from plants using other extraction methods are among the key factors why cold press method should be regarded as the most viable green method to produce oil plant seeds.

2.5.1(a) Factors Affecting the Process Efficiency of Cold Pressing.

In cold-pressing, the two main factors that influence the yield performance are the plant-species of the seed used for extraction of the oil and the process parameters procedures during extraction of the oil (Gavahian et al., 2015). The plant-species which exhibits direct influence on the oil yield are the nature of plant-seed i.e. oily or non-oily plant-seed (Silvia et al., 2012); the moisture level of plant-seed (Pradhan et al., 2011, Ionescu et al.), and particle size of the plant-seed (Kim et al., 2007, Deli et al., 2011). Meanwhile, the process parameters which have indirect impact on the oil yield are the pressing speed of the hydraulic /screw press (Kartika et al., 2010); the size of the mould, nozzle and screw (Pradhan et al., 2011), the pressure / weight exerted on the plant-seed (Karaj and Müller, 2011, Silvia et al., 2012, Koubaa et al., 2015); the quantity of feed material (Ionescu et al.); the duration (time) of the exerted pressure / weight on the plant-; ambient condition and temperature of pressing (Ionescu et al., Raynie, 2006, Silvia et al., 2012); pretreatment method applied on the plant-seed before extraction procedure (Pradhan et al., 2011) and rate of seedbed compaction