Sudan University of Science and Technology
College of Graduate Studies

A Comparative Study of Physicochemical Properties of Moringa, Sesame and Peanut Oils

دراسة مقارنة للخواص الفيزيوكيميائية لزيوت المورنقا، السمسم والفول السودانى

A Thesis Submitted in Partial Fulfillment for the Requirements of M.Sc. in Chemistry

By

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Dedication

To

My parents

Brothers

and Sister

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ABSTRACT

The purpose of this study was to determine the oil yield and physiochemical properties of *Moringa oleifera*, sesame and peanut seeds samples extracted by n-hexane. The properties were compared to show the possibility of using moringa as a source of edible oil.

The oil yields obtained by the cold method extraction were found to be 43% for *Moringa oleifera*, 41% for sesame and 44% for peanut but that of hot method extraction were 42% for *Moringa oleifera*, 40% for sesame and 42% for peanut.

The measured physicochemical properties include; density, P^H value, viscosity, refractive index, saponification value, peroxide value, acid value, boiling point, as well as, sodium, potassium and calcium content using flame photometer. Their concentrations were found to be 29.71ppm, 116.20ppm and 2.78ppm respectively.

Fatty acids constituents were determined by GC-MS. The analysis showed that *Moringa* oil contains greater a mounts of unsaturated fatty acids especially oleic acid (11.00%) and linoleic acid (18.12%). The main saturated acids were found to be palmitic and stearic acids in the four oil samples.

Peroxide value (1.8 meqO_2\kg oil) of *Moringa* oil may indicate high stability to oxidative rancidity.

The GC-MS analysis showed some significant differences between the n- hexane extracted oil and the commercial oil of *Moringa oleifera*.

المستخلص

الغرض من هذه الدراسة هو تحديـد كميـة الزيـت الناتـج وتقـدير الخـواص الفيزيوكيمائيـة للزيـت المسـتخلص بالهكسـان العـادى لعينـات مـن بـذور المورنجا،السمسم والفول السودانى. تمت مقارنة الخواص الفيزيوكيمائية للزيوت الثلاث لتحديد إمكانية إستخدام المورنقا كمصدر لزيت الطعام.

نسـبة كميـات الزيـت الناتـج بطريقـة الإسـتخلاص البـارد، كـانت 43% للمورنجا أوليفيرا و41% للسمسم 44% للفول السودانى . عند إستخدام طريقة الإستخلاص السـاخن كـانت النسـبة 42% للمورنجـا أوليفـرا،40% من للسمسم و42% للفول السودانى.

الخـــواص الفيزيوكيميائيـــة المقاســـة شـــملت، الكثافـــة، قيمـــة الأس الهيدروجينى، اللزوجة، معامل الإنكسـار،رقـم التصـبن، رقـم البيروكسـيد، رقم الحموضة، درجة الغليان بالإضافة إلى تركيـز الصـوديوم، البوتاسـيوم، والكالسيوم بإستخدام مطيافية اللهب. وجد أن تراكيز العناصر الثلاث هــى 29.71 ppm ، 116.20ppm 2.78 و ppm على الترتيب.

يمكـن أن يعتـبر رقـم البيروكسـيد لزيـت المورنقـا (1.8 meqO_2\Kg) مؤشرا لإستقرارية الزيت ضد تأثيرات الأكسدة.

أن GC-MS أعطى التحليل للأحماض الدهنية الأساسية مقارنة مع نتائج زيت المورنجا الناتج يحتوى علي نسبة عالية من زيت الأوليك واللينولينيـك الغير مشبعين. أما الأحماض الدهنية المشبعة والأكـثر وفـرة هـى كـل مـن البالمتيك والإستيريك.

أظهرت (GC-MS) قياسات جهاز كروماتوغرافيا الغاز ومطيافية الكتلة بعض الإختلافات فروقا واضحة بين زيـت المورنقـا المسـتخلص بالهكسـان وعينة الزيت التجارى.

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Introduction

1. Introduction

1.1 Fats and oils

Fats and oils are rationally important because they form one of the three major classes of food. Oils are used in variety of ways. Oils are used for food texturing, baking, and frying. They are also used inclusively in the manufacture of soap, detergents, cosmetics and oil's paints. In plants, oil is deposited mostly in seeds in the endosperm along with carbohydrates where they jointly nourish the embryo (Oyeyiola, 1993,). Oil is also found in some plants mascara e.g. in plant fruits. In animals, oil is found in various parts of the body e.g. liver (Oyeyiola, 1993). Nutritional and industrial processes have increased the demands for oils and this in turn has led to the search for oils from different types of seeds.

1.2 Characterization of *Moringa oleifera*

The *Morningaceae* is a single-genus family with 14 known species. One of these is *Moringa oleifera* which is most widely known and utilized species. It is small or medium-sized tree about 10m high is a wild plant indigenous to Sudan. *Moringa oleifera* is also indigenous to many countries in Asia, Saudi-Arabia, Pacific and Caribbean islands and South Africa. It is also indigenous to other countries in Africa. In some parts of the world *Moringa oleifera* is referred to as the "drumsticle tree" or the horseradish ", whereas in other parts is known as "kelor tree".

In the Nile valley, the name of the tree is *Moringa oleifera* or "shagara al rauwag", which is indigenous to the subcontinent. It is a small or medium–sized tree, 10 m high, found as wild plant and cultivated throughout the plains. It is often cultivated in hedges and it thrives best in house yards under the tropical insular climate, and it is plentiful near countries like Pakistan in Asia, *Moringa* is represented by only two species, *Moringa concanesis* and *Moringa oleifera.* The former is not common and perhaps confined to only a remote locality. The later, *Moringa oleifera* is known locally as "sohanjna". This species is grown and cultivated in Kordofan where as *Moringa concanesis* is grown and cultivated in northern Sudan plains, particularly in temperate and tropical regions of the country.

The flowers and fruits of this tree appear twice a year. It can be propagated either by seeds or cuttings and the latter is more performed flowers are white and fragrant, and the fruits are usually 20-45 cm long.

Moringa oleifera has recently been characterized with regard to its seed oil potential. It's oil is high in oleic acid and resembles in context of fatty acid composition with seeds of other *Moringa* species, which includes the *Moringa stenopetala*, *Moringa pregame* and *Moringa concanesis*. (Abdukonm SM, Lano, Muhammed S.K.S, long K. Ghazali H. 2007).

High-oleic oils are gaining importance especially for replacing polyunsaturated vegetable oils and are reported to exhibit good oxidative stability during frying.

(Abdukonm SM, Lano, Muhammed S.K.S, long K. Ghazali H. 2007).

1.3 Scientific classification

Kingdom: Plantae

(unranked):Angiosperms

(unranked):Eudicots

(unranked):Rosids

Order: Brassicales

Family: *Moringaceae*

Genus**:** Moringa

Species: *Moringa Oleifera*

1.4 Nutritional importance

Moringa Oleifera is a traditionally important food commodity. That leaves, flowers, seeds and roots of this tree are locally used as vegetables.(Nasir, E., and Ali, S. 1973).The tender pods are cooked or pickled and used in culinary preparations. The fresh beans are roasted to be a meal. Seeds are also consumed after frying and being tasted like roasted groundnut's seeds.

1.5 Medicinal uses

A number of medicinal and therapeutic properties have been ascribed to various parts of this tree, which include the treatment of ascites, rheumatism and venomous bites and used as cardiac and circulatory stimulants. Parts of this plant have been reported to show antitumor , antipyretic, antiepileptic, anti-inflammatory and anti-ulcer

effects and are used in native medicines and folk remedies. The roots of the young tree and root bark are rubefacient and vesicant. Leaves are rich in vitamins A and C (Dahot. M. 1988) and are considered to be useful in catarrhal afflictions, said to have purgative properties and to promote digestion and are used as an external application for wounds. The flowers of this plant are considered to possess medicinal value as a stimulant, aphrodisiac, diuretic and cholagogue. Flowers have been also reported to contain flavonoid pigments (Dahot.M 1988).

Interest in the composition of *Moringa Oleifera* seeds and the extracted oil, which is known commercially as "Ben" or "Bhen" oil, has existed many years. Olivera and Silveria (1999) described the compositional and nutritional attributes of seeds. The seeds are considered to be anti-pyretic, acrid, and bitter. They are reported to show microbial activity and are also utilized for waste water treatment and purification of muddy river water.

Ben oil has been used for illumination and is considered to be particularly suitable as lubricant for machines (Qaiser, Nasir. E and Ali.S.I,et al 1973). The oil was erroneously reported to resist rancidity and used extensively in the "effleurage" process whereby delicate fragrances are extracted from flowers (New Delhi, India, 1962). Some studies have been reported in the literatures that represent the composition and the characteristic of seed's fat of *Moringaceae* family, however, very little is

known about its production as edible oil. Ibrahim 1974 reported that the oil content and its properties are varied over a wide range, mainly depending on the species and environmental conditions.

1.6 Commercial Importance

The uncontrolled world population growth coupled with industry has widened the gap between demand and production of vegetable oils. This has resulted in ever–increasing imports, requiring the expenditure of valuable foreign exchange and a deficiency in people's fats intake in many developing countries (Dietz.M, Metzeler.R and Zarate, et al 1994). Now, when sustainable socio-agro forestry is gaining recognition as an appropriate means to improve national economies. The research for alternative sources of additional fats and vegetable oils has played a crucial role (Delpanque. B, et al 2000).

In views of growing demand and scientific awareness about the nutritional properties of oils, the quality assessment and composition of oils from some non-conventional oilseeds are of much concern till now. This has prompted us to design an analytical protocol and further investigations on this oil (Anwar.F,Dahot.M.U and Bhanger.M.I,et al 2001)

1.7 Characterization of sesame

The sesame is one of the most ancient oil seed known to mankind. Sesame family is a small family of 60 genera and 60 species. Among the 60 species of this family

(Pedaliaceae family), 37 species belong to seasamum genus. However, only sesame is cultivated between these 37 species (Facciola, et al 1990).

Sesame, an ancient cultivated plant which is thought to be originated from Africa and Turkey, is known to be second genetic resource Sesame is one of the most important oil seed worldwide (kocu et al 2007). These numerous varieties and ecotypes of sesame adapted various ecological conditions. However, the cultivation of modern varieties is limited due to insufficient genetic information. Many farmers continue to grow local sesame (souzaet et al., 1991), bean (phaseolusvelgris L.) (singhetet al. 1991), cotton (gossypiumhirstum L.) (Brown, 1991), Tritieales (Royo et al., 1995), and soya bean (ghycine max L.) (Perry et al, 1997).

Two studies that used morphological characters to group genotypes into clusters found a wide genetic diversity in Indian sesame genotypes (Ganesh et al. 1995, Patil et al. 1994). Multivariate analysis based on morphological characters provides genetic information that will allow the breeder to improve populations by selecting from specific geographic regions (Souza et al. 1991).

1.8 Fatty acid composition and uses of sesame

Genotypic factors are not only affecting the fatty–acid composition, but also play an important role in the process resulting in the fact that each genotype shows a different

fatty- acid composition. The determinate growth habit, which is a very useful character enabling the possibility of mechanized harvesting by providing synchronous flowering in Sesame. It should also be influenced by genotypic factors as well as the environment with respect to fatty-acid composition. However, there is no detailed study on the fatty acid composition of the determinate character even though it was first induced as early as the 1980s (Uzun et al., 2002).

Sesame is grown primarily for its oil-rich seed, which comes in a variety of colors from cream-white charcoal black. In general, the paler varieties of sesame seem to be more valued in the west Middle East, while the black varieties are highly prized in the east of India.

Sesame oil has a mild odor and a pleasant taste as such is a natural salad oil. Sesame oil is very popular as cooking oil in many countries and more expensive than other vegetable oils. Despite sesame oil's high proportion (41%) is of high smoke points which is defined as the temperature at which the oil is decomposed (Fazel M, Sahari MA and Barzegar M.2008).Light sesame oil has slightly lower smoke point and unsuitable for deep-frying instead it can be used for stir-frying of meats and vegetables and in making of omelet.

1.9 Medicinal uses of sesame oil

Sesame oil can be used or applied to darken the human being hair. It is used also as a soap fat in pharmaceuticals and as a synergist for insecticides. Because of the belief that sesame oil reduces the heat of the body and thus helps in preventing hair loss, sesame oil is a source of vitamin E which is an anti-oxidant. The uses of sesame oils as natural anti-oxidant have been reported. The oil has a potential lowering of cholesterol levels in the human body (Mohammed and Hamza, et al. 2008).

Sesame oil contains magnesium, copper calcium, iron, zinc and vitamin B6. (Nissitis M. and Tasioula. 2002 , Alpaslan M, Boydak E and Demircin M 2001).

Sesame oil is one of the most stable natural oils, but it can still benefit from refrigeration and limited exposure to light and high temperature during extraction, processing and storage in order to minimize nutrient loss through oxidation and rancidity. Storage in amber-colored bottles can help minimize light exposure (Uzun *et al*.,2002).

Sesame oil is polyunsaturated (PUFA) semi-drying oil. Commercial sesame oil varies in color from light to deep reddish yellow depending on the color of the seed processed and the method of milling (Alasalvar et al. 2002, Pigott and Tucker 1987, Citil et al.2011). Although the oil is milled from well-cleaned seed, it can be refined and bleached easily to yield light- colored limpid oil (Alasalvar et al. 2002, Pigott and Tucker 1987, Citil et al.2011). .

Sesame oil is rich in oleic and linoleic acids, which together account for 85% of the total fatty acids (Nazikouet et al.2009 and Egbekun and Ehieze et. al1997).

1.10 Peanut family (Arachishypogaea)

Peanut is a legume which is widely grown as a food crop. It is an herbaceous plant of which there are different varieties such as Boro light, Boro red, Mokura, Campala, Gut and Ela (Anayser et. al 2009).

Peanut is an important source of edible oil for millions of people. Edible oils from plant sources are of interest in various food and industrial applications. They provide characteristics flavors and textures to food as integral diet components (Odoemlam et al. 2005), and can also serve as a source of chemicals (Morrison et. al, 1995).

Oil content of peanut differs in quantity depending on the relative proportion of fatty acids, geographic location, seasons and growing conditions (Aclyeyeye and Ajewde et. al, 1992).

Peanut seed contains 44 to 56% oil and 22 to 30% protein on dry basis and is a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamin K and B group (Savage and Keenon , 1994).

1.11 Nutritional and Medicinal uses

Peanut protein is increasingly becoming important as food and feed sources, especially in developing countries where proteins from animal sources are not within the

nutrients of the majority of the populace (Ayoola and Adeyeye, 2010). Peanut seeds are reported to contain 9.5 to 19.5% total carbohydrates as both soluble and insoluble (Croker and Barton, 1957; Oke, 1967, and Wood roof, 1983).

The chemical composition of peanut seeds has been evaluated in relation to protein level (Yomng et al., 1973) and fatty acid composition (Grosso and Quznam, 1995) in several countries.

Vegetable oils are in high demand due to diseases associated with fat from animal origin. The peanut cake has several uses in feed and infant food formulations (Asibuo et al., 2008).

The literature has reported many health benefits associated with consumption of peanuts including cancer inhibition. This benefit is mainly attributed to micronutrients such as tocopherol, folate, minerals and health promoting phytochemicals, particularly resveratrol, ferulic acid and other phenol compounds (yu et al., 2004).

Barku et al, 2012 have reported some changes in the chemical composition as a result of processing. However, little information on the effect of traditional processing on peanuts quality was reported. Peanuts are very good sources of saturated fats, the type of fat that is emphasized in the heart-health of Mediterranean diet.

Peanuts are good sources of vitamin E, niacin, folate, protein and manganese (Savage and Keenan,1994).

Oils from nuts are edible and non-edible depending on their type of the nuts. These oils are often available as raw materials for chemical and industrial applications (Abalaka, et al.1978).

Nuts provide an interesting national supply due to their high nutritive and energetic value (Mitchel 1962 and Musa 1992). Peanut oil is also used to decrease appetite as an aid to weight loss. Some people used it to help preventing cancer.

Peanut oil is used in ointment and medicinal oils for treating constipation. Pharmacists compare the use of peanut oil in various products which they prepare for internal and external use.

1.12 Health benefits of peanuts

Peanuts are rich in energy (567 calories per 100g) and contain health benefit nutrients, minerals, antioxidants and vitamins that are essential for optimum health. (Asibuo et al., 2008) and (Savage and Keenan,1994).

They compose sufficient levels of mono–unsaturated fatty acids especially oleic acid. It helps lowering LDL or "bad cholesterol" and increasing HDL or "good schoolyard" level in the blood. Research studies suggest that Mediterranean diet, which is rich in

monounsaturated fatty acids help to prevent coronary artery disease and strokes by favoring healthy blood lipid profile.

The kernels are good source of fibrous protein and compose fair quality of amino acids that are essential for growth and development (Young et al., 1973 and Ayoola and Adeyeye,2010).

Research studies have shown that peanuts contain high concentrations of poly phenol-antioxidants like primarily P-Comerica acid. This compound has been thought to reduce the risk of stomach cancer by limiting formation of carcinogenic nitrosamines in the stomach.

Peanuts are an excellent source of resveratorol, another poly phenol antioxidant. Resveratorol has been found to have protective function against cancers, heart disease, degenerative nerve disease, Alzheimer's disease and viral fungal infections (Crocker and Barton, 1957; Oke, 1967; Woodroof 1983).

Furthermore, studies suggest that resveratorol reduce stroke risk by altering molecular mechanisms in the blood vessels (reducing susceptibility to vascular damage through decreased activity of angiotensin; a systemic hormone responsible for blood vessel constriction that would elevate blood pressure), and by increasing production of vasodilator hormone, nitric oxide (Savage and Keenan,1994).

Recent research studies suggest that roasting/ boiling enhances antioxidants bioavailability in the peanuts. It has been found that boiled peanuts have two and four food increase in isoflavone antioxidants biochanin-A and genistein content, respectively. (Journal of agricultural and food chemistry.

The kernels are an excellent source of vitamins E (α - tocopherol), containing about 8g per 100g. Vitamin E is a powerful lipid soluble antioxidant which helps maintain the integrity of cell membrane of mucus membranes and skin by protecting from harmful free radicals.

The nuts are packed with many important 13-complex groups of vitamins such as riboflavin, niacin, thiamin, pantothenic acid, vitamin B-6, and the recommended daily intake (RDI) of niacin, which contribute to brain health and blood flow. (Asibuo et al 2008 and Yu et al.2004)

The nuts are rich source of minerals like copper manganese, potassium, calcium, iron, magnesium, zinc and selenium (Savage and Keenan, 1994).

1.13 Minerals content in edible oils

Sesame and peanuts seeds are an excellent source of magnesium, copper and a very good source of magnesium, calcium, phosphorus, iron, zinc, molybdenum and selenium (Savage and Keenan,1994).This rich assortment of minerals translates into the following health benefits.

a) Copper

Copper is known for its use in reducing some of the pain and swelling of rheumatoid arthritis. Copper's effectiveness is due to the fact that this trace mineral is important in a number of anti inflammatory and antioxidant enzyme systems. In addition, copper plays an important role in the activity of lysyl oxidase, an enzyme needs for the cross-linking of collagen and elastin, the ground substances that provide structure, strength and elasticity in blood vessels, bones and joints.]

b) Magnesium

Magnesium’s usefulness is clearly shown in preventing the airway spasm in asthma, lowering high blood pressure, a contributing factor in heart attack, stroke and diabetic heart disease. It is shown also in preventing the trigeminal blood vessel spasm that triggers migraine attack and restoring normal sleep patterns in women who are experiencing unpleasant symptoms associated with menopause.

C) Calcium

Calcium has been shown in recent studies, to help protect color cells from cancer- causing chemical prevent the bone loss that can occur as a result of menopause or certain conditions such as rheumatoid arthritis, prevent

migraine head ages in those who suffer from them, and to reduce following symptoms during the luteal phase (the second half) of the menstrual cycle. Calcium assists in teeth development (Brody, 1994).

d) Potassium

Potassium is an essential nutrient and has an important role in the synthesis of Amino acid and proteins (Malik, 1982).These facts indicate the beneficial food importance of the investigated oils under this study.

1.14 Fatty acids content

a- Oleic acid

Oleic acid is a fatty acid that occurs naturally in various animals and vegetable fats and oils. It is odorless, colorless oil, although commercial samples may be yellowish. In chemical terms, oleic acid is classified as a monounsaturated omega-9fatty acid, abbreviated with a lipid number of 18:1 cis-9. It has the formula CH_3-(CH_2)7-CH=CH-(CH_2)$_7$COOH. The term "oleic" means related to, or derived from, oil of olive, the oil that is predominantly composed of oleic acid (Thomas Alfred 2000 and Young,Jay A. 2002)

b- Linoleic acid

Linoleic is a polyunsaturatedomega-6 fatty acid. It is a colorless liquid at room temperature. In physiological literature, it has a lipid number of 18:2 *cis*, *cis*-9,12. From the chemistry perspective, Linoleic acid is a carboxylic acid with an 18-carbon chain and two *cis* double bonds;

with the first double bond located at the sixth carbon from the methylend. Linoleic acid belongs to one of the two families of essential fatty acids, which means that the human body cannot synthesize it from other food components (Burr, G. O ,Burr ,M, M.and Miller, E. (1930).

The word "linoleic" derived from the Greek word *linon* (flax). *Oleic* means "of, relating to, or derived from oil of olive" or "of or relating to oleic acid" because saturating the omega-6 double bond produces oleic acid.

c-Palmitic acid

Palmitic acid is the most common fatty acid (saturated) found in animals, plants and microorganisms .Its chemical formula is $CH_3(CH_2)_{14}COOH$. As its name indicates, it is a major component of the oil from palm trees (palm oil), but can also be found in meats, cheeses, butter, and dairy products. Palmitate is a term for the salts and esters of palmitic acid. The palmitate anion is the observed form of palmitic acid at physiologic p^H (7.4). Aluminum salts of palmitic acid and naphthenic acid were combined during World War II to produce napalm. The word "napalm" is derived from the words naphthenic acid and palmitic acid (Kingsbury, K. J.; Paul, S.; Crossley, A.; Morgan, D. M. 1961)

d- **Stearic acid**

Stearic acid is a saturated fatty acid with an 18-carbon chain and has the IUPAC name octadecanoic acid. It is a waxy solid and its chemical formula is $C_{17}H_{35}CO_2H$. Its name comes from the Greek word στέαρ "*stéar*", which means tallow.

The salts and esters of stearic acid are called stearates. As its ester, stearic acid is one of the most common saturated fatty acids found in nature following palmitic acid. The triglyceride derived from three molecules of stearic acid is called stearin.

e- Behenic acid

Behenic acid is a carboxylic acid, the saturated fatty acid with formula $C_{21}H_{43}COOH$. In appearance, it consists of white to cream color crystals or powder with a melting point of 80 °C and boiling point of 306°C.

f- Arachidic acid

Arachidic acid is the saturated fatty acid with a 20-carbon chain. It is as a minor constituent of peanut oil (1.1%–1.7%), corn oil (3%). and cocoa butter (1%).Typical Fatty acid composition (%): Its name derives from the Latin *a rachis* — peanut. It can be formed by the hydrogenation of arachidonic acid. Arachidic acid is used for the production of detergents, photographic materials and lubricants. Reduction of arachidic acid yields arachidonic alcohol..

1.15 Aim of the study

1. The study was aimed to determine the oil yield obtained by n-hexane extraction of *Moringa oleifera* seeds and the characterization of it's physicochemical properties compared with sesame and peanut oil samples obtained by the same method.

2. The main objective of this study was characterization and exploitation of moringa oil for the edible and commercial purposes.

Chapter two

Materials and methods

1. Materials and methods

2.1 Materials

2.1.1 Samples collection and treatment

Moringa oleifera, Sesame and peanut seeds were collected from Khartoum North market under storage for six month.

The seeds were cleaned, dried under direct sunlight and powdered by a mechanical grinder and then by mortar to reduce the seeds to smaller sizes to make them more accessible to the solvent. The sample was sun-dried in order to reduce the moisture content, then about 500g of the sieved *Moringa oleifera* seeds was weighed using an electronic weighing balance.

Table 2.1 Chemicals and Reagents

Chemical	Source
n- hexane	CDH, India
Petroleum ether	CDH, India
Diethyl ether	CDH, India
Glacial acetic acid	CDH, India
Chloroform	CDH, India
Potassium iodide	Mumbai 400005 India
Potassium thiosulphate	Mumbai 400005 India
Potassium hydroxide	Mumbai 400005 India
Sulphuric acid	CDH, India
Iodine mono chloride	CDH, India
Hydrochloric acid	Mumbai 400005 India
Ethanol	Mumbai 400005 India

2.1.2 Instruments

a- P^H-meter

P^H meter – company: JENNAY- UK

Model: 3505 P^H meter.

Range: 2.00 to1.99

Resolution: 0.001 to 0.1 PH

Accuracy: ± 0.003PH

b- Viscometer

Abbe 60 refract meter, viscometer, Ostemald. U Tube.

c- Flame photometer:

Instrument: Flame photometer

Company: JENWAY UK

Model: PFP7 flame photometer

d-Gas chromatography Mass Spectrometer \GC-MS:

Instrument: GC-MS

Model: QP 2010 Plus

Company: SHIMADZU – Japan

2.2 Methods

2.2.1 Oil extraction

a-Hot method extraction

500g of each of the three samples were fed to a soxhlet extractor fitted with a 1-liter round–bottom flask and a condenser. The 500g of the ground seed's samples were placed into a thimble and extracted with n- hexane. The extraction was executed on a water bath for 4-5hours with 0.5 L of the n- hexane. The solvent was then distilled off under vacuum in a rotary evaporator.

b- Cold method extraction

The 500g of each ground sample were macerated in one liter-hexane in500 ml necked rounded flask. The three flasks containing the different samples were allowed to stay overnight for 72 hours. The mixture was then filtered. In a necked flask with around bottom. The solvent was distilled off under vacuum to yield the oil.

2.2.2 Refractive Index

The refractive index (RI) was determined by Abbe 60 refract meter as described by AOAC method (1984). Double prism was opened by means of screw head and a few drops of oil were placed on the prism. The prism was closed firmly by tightening the screw head. The instrument was then left to stand for few minutes before reading, in order to equilibrate the sample temperature with that of the instrument (32±2°C).The prisms were cleaned between the readings by whipping off the oil with soft cloth, then with cotton moistened with petroleum ether and let to dry. The refractive index was recorded.

2.2.3 Determination of Viscosity

The viscometer was suspended in the constant temperature bath (32±2ºC). The capillary was vertical. The instrument was filled to the mark at the top of the lower reservoir with the oil by means of pipette inserted into the side arm, so that the tube wall above the mark is not wetted. The instrument was then let to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument (32±2ºC).

By means of the pressure on the respective arm of the tube, the oil moved into the other arm so that the meniscus is1cm above the mark at the top of the upper reservoir. The liquid was then allowed to flow freely through the tube. Then the time required for the meniscus to pass from the mark above the upper reservoir was recorded.

2.2.4 Density Determination

Clean and dry 25 ml density bottle was weighed and then filled with oil to the mark, stoppered and weighed again. The method was repeated for each oil sample. The density of each oil type was calculated.

2.2.5 Determination of P^H value

The P^H-meter was standardized using Buffer solutions 4, 7 and 9. The PH value for each sample was measured.

2.2.6 Boiling Point Determination

U-Thiele boiling point determination apparatus was used for measuring the B.P for each oil sample.

2.2.7 Determination of Iodine value

About 0.2 grams of oil were weighed and placed in a dry clean 250ml iodine flask specially offered for the test. 10 ml of chloroform was used for dissolving the oil. 25 ml of iodine mono chloride solution was added and finally (20ml) of KI (0.1N) were added to the contents of the flask. The flask was then Stoppard and the mixture allowed standing for 10 minutes in a dark place. The stopper and the side of the flask were rinsed with enough amount of distilled water.

The contents of the flask were then shaken and titrated against 0.1N sodium thiosulphate using an indicator. A blank determination was carried out simultaneously.

2.2.8 Determination of Peroxide value

About 5.00g of each oil sample was accurately weighed in a conical flask, then 30ml of a mixture of acetic acid and chloroform (2:1) was added to it. The mixture was boiled for one minute. The solution was poured into a flask containing 10ml of potassium iodide solution, well shaken and titrated against (0.1M) potassium thiosulphate until the yellow color almost disappeared. 0.5ml of starch indicator was added and the titration continued until the end point (where the blue black color just disappeared). A blank titration was also performed.

2.2.9 Determination of Saponification value

5.00g of the oil sample were accurately weighed in a conical flask. 25ml of 0.1 N of alcoholic potassium hydroxide was added to the conical flask, and the content was continuously stirred for 1 hour followed by reflux. Phenolphthalein indicator was then added to the conical flask and titrated with 0.5M HCl till the solution changes to colorless. A blank titration was carried for comparison.

2.2.10 Determination of acid value

25 ml of each ethanol and diethyl ether were mixed in a 250ml beaker, then 5.00g of oil sample was added to the resulting mixture in250ml conical flask. The contents were heated to dissolve the oil, few drops of phenolphthalein indicator were added to the mixture. The mixture was then

titrated with 0.1M KOH and noted that the pink color was appeared. A blank titration was carried.

2.2.11 Determination of Minerals

One gram of each oil sample was ignited to white ash, in a muffle furnace at 550ºCfor 8hours.The ignition was repeated three times for each sample.The was dissolved in 20.00 ml of 2.5% HCl. Then the volume was decreased to 7.0 ml by heating in steam bath and transferred quantitatively to a 50ml volumetric flask. The new volume was diluted to 50ml with distilled water and kept in clean polyethylene samples bottles. The minerals contents were determined using flame photometer.

2.2.12 GC-MS Analysis

The four oil samples were analyzed using GC-MS for determination of the chemical components of each sample.

Chapter Three

Results & Discussion

3. Results and Discussion

3.1 Oil content

Results presented in table 3.1 showed the amount of oil extracted from 500g of *Moringa oleifera*, peanut and sesame seeds.

The cold method extraction gave higher yield compared with hot method extraction. This may be due to the increased ability of the polar solvent to overcome forces that bind lipids within the sample matrix (Lumely and Colwell, 1991).

Variations in oil contents reflect difference in the method used. The study showed that *Moringa Oleifera* seeds were a good source of oil.

Table 3.1 Oil content in samples

Parameter / Type of sample	**Weight of sample**	**Time of extraction**	**oil content in ml**	
			Hot method	Cold method
Moringa oleifera	500g	6	233.84	238.12
Sesame	500g	6	219.95	224.75
Peanut	500g	6	232.97	240.95

3.2 Physical properties

The physical properties of *moringa oleifera*, sesame and peanut oils are shown in table 3.2. These physical parameters do not show any significant variations. The

boiling point values of samples extracted by hot method for *Moringa oleifera*, sesame and peanut obtained were as follows, 228ºC, 227ºC and 226ºCrespectively. These values are close to samples oils extracted by cold method which are 227ºC, 227ºC and 226ºC respectively. All these results are in a good agreement with those in the literature of Standard Codex (2001).The P^H measuring of the three samples oils especially that extracted with the hot method gave an average values of 2.92, 2.29 and 2.94 respectively.

There was no significant difference in the refractive index of *Moringa* oil among the two methods of extraction. The refractive index1.4655 for *Moringa* oil extracted by hot method was found to be within the reference range (1.4549-1.4665) and was not varied from the other two sesame and peanut oils extracted by the same method which were 1.4645 and 1.4665respectively.The variations of refractive index values for oils extracted by cold method may be attributed to the place of planting and moreover to the additional chemical constituents associated with the extracted samples.

Table 3.2 Physical proprieties of oil samples

Measured value / Type of oil sample		Refractive index	Viscosity	P^H values	Density	Boiling points
Mori	Hot method sample	1.4655	28.94	2.92	0.90208	228°C
	Cold method	1.3565	27.99	2.96	0.91157	227°C

sample					
Commercial sample	1.4549	28.15	3.25	0.91441	227°C
Sesam Hot method sample	1.4645	29.10	2.29	0.910772	227°C
Cold method sample	1.4624	28.99	3.05	0.90555	226°C
Commercial sample	1.3663	29.35	2.95	0.91455	226°C
Pean Hot method sample	1.4665	28.75	2.94	0.90425	226°C
Cold method sample	1.422	27.96	2.96	0.91332	226°C
Commercial sample	1.2972	29.73	2.88	0.90255	228°C

3.3 Chemical properties

The iodine values were high suggesting the presence of unsaturated fatty acids especially in *Moringa oleifera* oil which gave an average result of 114 Wij's compared with 117.5 and 113.5 for peanut and sesame oils respectively. It indicates the degree of instauration in the fatty acids of tri-acyl glycerol. These values could be used to quantify the amount of double bonds present in the oils, which signifies the susceptibility of oil to oxidation.

The peroxide value of *moringa* oil was 1.85 ($meqO_2$ /kg oil) which fell in the range from 1.5-2.4, as reported by codex standard (2001). The other peroxide values of sesame and peanut oils were 1.9 and 2.1 ($meqO_2$/kg oil) respectively were also found to be in a good agreement with those of *moringa* oil. Fluctuations of these values may be attributed to immaturity and storage effect of seeds.

The *moringa* oil showed high oxidative rancidity as the results of the measured peroxide values indicate.

The measured saponification values in (*meq* KOH/g oil) of *moringa* oils (hot and cold method samples) have an average of 190.87.These values have an inverse relationship with molecular weight of lipids. The values of sesame and peanut oils were 190.38 and 192.63 respectively. All of these values were within the range 186-195, as reported by codex standard (2001).

The acid value is an indication of the amount of fatty acid present in the oil sample. It is a reflection of P^H value of oil. If the acid value increases, the P^H of oil will decrease. The acid values of the *moringa*, sesame and peanut were found to be5.77%, 5.86% and 5.91% respectively.

Table 3.3 Chemical properties of oil samples

Oil Sample \ Measured value		Iodine value	Peroxide value	Sap.value	Acid value
Moringa	Hot method sample	116	1.8	189.88	5.77%
	Cold method sample	113	1.9	191.85	5.75%
	Commercial sample	112	1.89	195.85	5.25%
Sesa	Hot method sample	113	2.0	190.85	5.86%
	Cold method sample	114	1.8	190.76	5.80%
	Commercial sample	110	1.9	189.25	5.11%
Pean	Hot method sample	118	2.0	192.45	5.91%
	Cold method sample	117	2.2	192.82	5.92%
	Commercial sample	98	2.0	190.55	4.59%

3.4 Sodium, potassium and calcium content

Moringa oil contained significant amount of important minerals (Table 3.4).Potassium content was 110.547ppmin oil extracted by hot method and 110.547ppm in oil extracted by cold method. The calcium content of *moringa* oil by hot method was 2.52ppmwhile sesame and peanut oil extracted by the same method gave 3.02ppm and 3.018pmm respectively. The mean values of sodium content for *moringa*, sesame and peanut oils were: 3.00ppm, 28,833ppm and 30.2775ppmas tabulated below**.**

Table 3.4 Sodium, potassium and calcium content

Measured value / Oil Sample		Na (ppm)	K (ppm)	Ca (ppm)
Mor	Hot method sample	30.667	110.547	3.018
	Cold method sample	29.333	110.547	2.015
	Commercial sample	30.333	106.592	3.018
Sesa	Hot method sample	29.333	110.547	3.520
	Cold method sample	28.333	114.501	2.015
	Commercial sample	28.889	114.501	3.520

Peanut	Hot method sample	28.333	110.547	3.018
	old method sample	32.222	110.547	3.018
	Commercial sample	30.556	110.547	3.018

3.5 GC-MS analysis

The extracted oils of *moringa oleifera*, sesame and peanut were analyzed by GC-MS (table 3.5). The chemical constituents of these samples were determined using GC-MS for qualitative and quantitative analysis. The retention time and % area of each fatty acid and other constituents were compared with those reported in national institute of standards and technology (NIST), with help of HPCHEM software and published mass spectra**.** The details were summarized in the table 3.5.

The oil content of *moringa* was significantly higher compared to commercial oil sample.

Tables 3.5 and 3.6 show the chemical constituents of oil samples. Palmitic acid content was (0.98%) in *moringa*, (13.62%) in sesame and in peanut (11.36%) compared with (7.91%) in the commercial oil sample; oleic acid (11.00%) in *moringa*, (66.83%) in sesame and (57.93) in peanut where in commercial sample was found to be (68.49%). Stearic acid was found to be (2.95%) in *moringa*,(0.17%) in sesame and(3.76%) in peanut where in commercial sample was (3.76%)**.** Arachidic acid was found to be (0.34%) in sesame, (0.2%) in peanut, and not detected *moringa* but it was not detected in commercial

sample. Lauric acid (4.40%) in *moringa*,(1.25%) in sesame and (0.35%) in peanut and not detected in the commercial sample. Linoleic acid was (18.12%) in moringa, (4.75%) in sesame and not detected in peanut. 9,12-Octadecadienoic acid was found in all samples as follows: (4.40%) in *moringa* , (1.89%) in sesame, (11.40%) in peanut but it is (3.7%) in the commercial sample. Some chemical constituents were found in least amounts such as stearic acid anhydride (2.95%) in *moringa*, (2.58) in sesame and not determined in peanut. Nonenal and nonanal were found in very low percentages in the oil samples but nonenal was not detected in both sesame and commercial samples. Beta-tocopherol (3.10%) and (0.49%) were found only in extracted and commercial moringa samples respectively and not detected in other oil samples. Sesamin (25.87%) was detected only in the extracted oil whereas amounts of tri-methyl phenyl silane (13.69%) in moringa and (1.92%) in sesame were measured.

The main unsaturated fatty acids in oil samples were oleic (C18:1) and linoleic acid (C18:2), which were found in high percentages as discussed before. The main saturated acids detected in the oil samples were palamitic acid, lauric acid, and stearic acid.

Table 3.5 Chemical constituents of oil samples using GC-Ms

Oil type / Chemical constituents	***Moringa Oleifera***		**Sesame**		**Peanut**	
	Retention Time (min)	% Area (min)	Retention Time (min)	% Area (min)	Retention. Time (min)	% Area (min)
Nonenal	16.090	0.1	16.350	0.07	16.180	0.2
Nonanal	16.389	0.05	-	-	16.394	0.17
Palmitic acid	41.072	0.98	37.747	13.62	37.672	11.36
Lauric acid	28.870	4.40	46.652	1.25	21.049	0.35
Stearic	45.494	2.95	39.375	0.17	41.138	3.67
Oleic acid	40.732	11.00	41.443	66.83	41.288	57.93
Arachidic acid	-	-	44.541	0.34	44.508	0.2
Oleic acid anhydride.	45.422	3.14	45.422	3.14	-	-
Stearic acid anhydride	45.494	2.95	45.613	2.58	-	-
Sesamin	59.640	25.87	-	-	-	-
9,12 Octadecadiconic	37.347	4.40	45.492	1.89	46.351	11.40
Linoleic acid chloride	45.913	0.78	-	-	-	-
Bête-Tocopherol silane	46.283	3.10	-	-	-	-
Tri methyl phenyl silane	61.066	13.69	46.435	1.92	-	-
Linoleic acid	40.649	18.12	40.382	4.75	-	-
Diethyl methyl borane	-	-	42.533	1.13	55.268	0.58
Oleic acid chloride	-	-	-	-	45.5084	13.8
Stearic acid anhydride	45.494	2.95	45.613	2.58	-	-
Diethyl methyl	-	-	42.533	1.13	55.268	0.58

borane						

The results obtained in table (3.6) were in a good agreement with those reported by Nzilou et.al.(2009). Palmitic acid is mainly the predominant of the saturated fatty acids which was found in all oil samples. Higher amounts of 66.83O% and 57.93% of oleic acid (C_{18}:1) were measured in the comparative samples of sesame and peanut oils respectively, whereas commercial and moringa oil samples showed a measuring readings of 68.49% and 11.00% respectively. The greatest proportion of oleic acid was found in commercial moringa oil sample with its lowest linoleic acid content (0.59%).

Sesame oil is rich in oleic and linoleic acids, which together account for 85% of the total fatty acids (Nazikouet et al.2009 and Egbekun and Ehieze et al. 1997).

Table 3.6 Chemical constituents of commercial sample using GC-Ms

Oil type / Chemical constituents	**Retention Time (min)**	**% Area under the peak**	**Height % of the peak**
Octadecanoic acid	37.930	3.76	-
9- octadecenoic acid	46.353	5.42	-
Heptanoic acid	49.521	0.49	-
Oleic acid	40.873	68.49	38.21
Stearic acid	41.092	3.76	9.47
Lauric acid	-	-	-
Palmitic acid	42.51	7.91	2.51

Oleic acid chloride	45.508	5.34	13.8
Arachidic acid	-	-	-
Stearic acid anhydride	45.921	0.63	-
9-Eicosadione	55.207	0.54	-
Hexadeconic acid	43.291	0.92	-
Linoleic acid	46.521	0.59	20.09
9,12 Octadecanoic acid	37.930	3.76	-
Palmitic acid chloride	42.514	1.38	-
Beta- Tocopherol	49.52	0.49	1.09
Diethyl methyl borane	56.700	3.27	-

Recommendations

Further research may be needed on *moringa oleifera* oil such as purification and more GC-MS analysis may be required to know exactly the main amount of essential fatty acids.

Microbial activity detections may be required to enhance further investigations on fatty acids contents and the viability of *moringa* oil.

The results showed that, the properties of *moringa oleifera* oil in Sudan could be employed for edible and cosmetics applications.

The oil exhibit good physicochemical properties and could be useful for industrial applications.

Moringa oleifera could be cultivated in Sudan large tropical areas because, the tree is rapidly growing even in poor soils, and it is not affected by drought especially in poor and developing countries (Sengupta and Gupta, 1970, Morton,1991).

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