Extraction, Characterization and Determination of Physicochemical Properties of Elketan *(Linum usitatissimum L.)* Seed Oil

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Date of Examination 28/7/2020

Declaration

I hereby declare that this dissertation is my own original word, and wherever contributions of others are involved, every effort is made to indicate this clearly with references and acknowledgement. The result embodied in this dissertation has not been submitted to my other University or Academic Institution for the award of my scientific degree.

Name: Twasul Abd Elmohssin Merghani Mohammed

Signature :

Place: University of Gezira **Date:** 28/7/2020

Dedication

I dedicate this work to;

My father, My mother, brothers, sisters and friends

With love and eternal appreciation

TWASUL

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First of all, I would like to thank our almighty Allah for shedding on me good health, and keeping my brain work to extent completing this research.

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Abstract

Vegetable oils have been used for centuries as source of energy and industrial applications. Flax is an annual, self-pollinate species, it provides raw materials for food, medicine and textiles. Flax seed is a rich source of different types of phenolics. This study aimed to determine its physicochemical properties such as fatty acid composition, antioxidant activity and to study flax seed oil refining. All the physicochemical properties were determined according to the American Oil Chemists Society (AOCS) official methods. Flax seed samples were collected from the Baraka shop -Wad Medani. Physicochemical properties of flax seed include moisture content, oil content, crude protein, and crude fiber. For flax seed these were 1.92%, 37.9%, 24%, 22% respectively. The proximate analysis of flax seed Oil (FSO) includes moisture content, free fatty acids, peroxide value, saponification value, unsaponifiable matter, refractive index, and color for FSO. These were 0.005%, 0.52%, 6.4 meq/kg, 146 mgKOH/g,0.031%,1.4818, Red: 2.7 Yellow: 70.0 respectively .Fatty acid composition was determined by GC-MS. The most abundant unsaturated fatty acids in the FSO were linolenic acid 50.01% in FSO followed by Linoleic acid 26.80% while the most abundant saturated fatty acids in the FSO was palmitic acid 11.9% followed by Stearic acid 9.35%. The antioxidant activity was determined by DPPH assay. The antioxidant activity of FSO extracted by cold pressing it was 20% while for the oil extracted by n-hexane in detectable. The oil has good physicochemical properties and is rich in unsaturated fatty acids. Cold pressing showed higher antioxidant activity than solvent extraction. Free fatty acids and color were determined for refined FSO as 0.051%, (Red 0.3 - yellow 4.1). It is recommended that further studies should be made to determine the minor components of the oil. Also the stability of the oil need to be studied because of its high contents of unsaturated free fatty acids.

استخلاص وتوصيف وتحديد الخواص الفيزيائية والكيميائية لزيت بذور الكتان

توسل عبد المحسن ميرغني محمد

مخلص الدراسة

استخدمت الزيوت النباتية لعدة قرون كمصدر للطاقة وتطبيقات الصناعية. الكتان نبات سنوى ذاتى التلقيح ، يستخدم الكتان في انتاج المواد الخام للأغذية والدواء والمنسوجات . بذور الكتان هي مصدر غني لأنواع مختلفة من الفينول . اجرئ هذا البحث لاستخلاص زيت بذور الكتان ثم تحديد الخواص الفيزيائية والكيميائية للزيت، ومعرفة تركيبة الاحماض الدهنية وتكريره وقياس نشاط مضادات الأكسدة فيه. وفقاً للطرق المعتمدة للجمعية الأمريكية الكيميائية للزيوت. جمعت عينة بذور الكتان من سوق المحلي ود مدني. وقد شملت الخصائص الفيزيائية والكيميائية لبذور الكتان محتوى الرطوبة ومحتوى الزيت والبروتين الخام والألياف الخام حيث كانت لبذور الكتان 1.92% ،37.9 %، 24% ، 22 % على التوالي، وقد شملت الخصائص الفيزيائية والكيميائية لزيت بذور الكتان محتوى الرطوبة، الأحماض الدهنية الحرة، رقم البيروكسيد، ، رقم التصبين، المواد غير القابلة للتصبن، معامل الانكسار، درجة اللون والكثافة حيث كانت في الكتان 0.005% و0.52 % و6.4 ملى مكافئ/كجم و 146ملجم هيدروكسيد بوتاسيوم/جم و 0.031% و1.4818 والون الأحمر: 2.7 والأصفر: 70.0 جم/مل على التوالي قدرت الأحماض الدهنية بواسطة كروماتوغرفيا الغاز، ووجد أن نسبة حمض اللينولينيك 50.01 ٪ يليه حمض اللينوليك الذى نسبته 26.80 % أما الأحماض الدهنية المشبعة فتشمل حمض البالمتيك بنسبة 11.90% يليه حمض الاستياريك بنسبة 9.35%. حددت مضادات الأكسدة بطريقة كسح الجذور (DPPH)، وأظهرت النتائج أن مضادات الأكسدة في زيت الكتان المستخرج بالعصر البارد كان نسبة 20% أعلي من تلك الموجودة للزيت المستخلص بواسطة مذيب الهيكسان . الزيت المستخلص بواسطة العصر البارد أظهر نشاطاً مضاداً للأكسدة اكثر من الزيت المستخلص بالمذيب . تم تحديد نسبة الأحماض الدهنية الحرة ودرجة اللون لزيت بذور الكتان المكرر وكانت 0.051 % و (الأحمر 0.3 – الأصفر 4.1) يوصى بإجراء مزيد من الدراسات لتحديد المكون الثانوي للزيت . كما يجب دراسة ثبات الزيت اذ انه محتواه العالي من الأحماض الدهنية الحرة غير المشبعة.

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List of Abbreviations

AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists Society
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DMSO	Dimethyl Sulfoxide
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acids
GC-MS	Gas chromatography- Mass spectrometry
ME	Methyl Ester
MUFA	Mono Unsaturated Fatty Acids
NIST	National Institute of Standard and Technology
SFA	Saturated Fatty Acids
SV	Saponification Value
PV	Peroxide Value
PUFA	Poly Unsaturated Fatty Acids
PAHs	Polycyclic aromatic hydrocarbons

CHAPTER ONE

INTRODUCTION

1.1 Vegetable Oil

The oils and fats of commerce are mixtures of organic molecules. They are mainly triacylglycerols (commonly referred to as triglycerides), accompanied by lower levels of diacylglycerols (diglycerides), monoacylglycerols (monoglycerides) and free fatty acids, and by other minor components, some of which are important materials in their own right(Hamm *et al.*,2013). The human body uses oils and fats in the diet for three purposes: as an energy source, as a structural component and to make powerful biological regulators. They also play an important role in metabolic reactions in the human organism. In particular, vegetable oils are beneficial and popular due to their cholesterol-lowering effect. In contrast to animal fats, which are predominantly saturated and do not react readily with other chemicals, especially oxygen, unsaturated vegetable oils are more reactive (Llorent-Martínez *et al.*, 2011). Vegetable oils are obtained from plants, such as seeds, nuts ,fruits, and vegetables, and are the major source of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in the diet. Three oils are rich sources of saturated fatty acids (SFA): coconut oil, cocoa oil, and palm oil. Cocoa oil is the first ingredient in ice cream produced by the industry, and palm oil is the usual oil in fast-food preparation. They are also a dietary source of fat-soluble vitamins and antioxidants. Oils made from walnuts, almonds, olives, sesame seeds, and flax seeds have been used for 5000–6000 years for several purposes (Savva and Kafatos, 2016).The importance of these oils and fats will increase considerably in the future because they represent a vast potential of naturally renewable raw materials in which the chemical and pharmaceutical industries have a special interest (Bockish, 1998). Vegetable oils are used in various industrial applications such as emulsifiers, lubricants, plasticizers, surfactants, plastics, solvents and resins (Erhan, 2005).

Almost all plants contain fats or oils, mainly in their seeds; the amount varies from very little to as much as 40 - 70% (Bockish, 1998). The chemical and physical properties of fats and oils are largely determined by the nature of their molecule

(O'Brien, 2009). The fatty acid composition of vegetable oils is the main factor influencing their nutritional value and properties (Holt, 2016). Analyses of fats and oils are required for a number of applications, beginning with commodity trading. In every fat and oil processing plant, there are analytical requirements for process quality control. In refining, for example, evaluating the free fatty acids (FFAS) content of the oil is necessary to determine the caustic treat, and to serve as a quality indicator in other areas. Melting points, fat solids content, and other physical evaluations indicate that the product will function as developed. For final edible-oil products, organoleptic evaluations, peroxide value, free fatty acids, and other analyses are utilized for assurance that the product has the required bland flavor, with predictive analysis, such as active oxygen method (AOM) stability being utilized to ensure proper shelf life (O'Brien, 2009). The world's supply of vegetable oil is currently in excess of 100 million metric tons. The demand is increasing at a rapid pace due to increasing need for non–food uses of vegetable oil, for example in biodiesel, oleochemicals, lubricants, pharmaceuticals and cosmetics. However, only about 12 of the ~500,000 known plant species are currently commercially exploited, by big companies with vast resources for promotion and marketing, to produce vegetable oils (Mabaleha *et al.*, 2007).

Flax (*Linum usitatissimum* L.) is an annual, self-pollinated species. It provides raw materials for food, medicine and textiles and hence it has been of great importance to human culture and development (Nag *et al.*, 2015). Flax is a food and fiber crop harvested in the cold regions of the world. The flax plant first originated in the Upper Paleolithic age and flax fibers were found in the Dzudzuana Cave. The corroboration of flax utilized by humans was in Republic of Georgia, where spun and knotted flax fibers for textiles were found. Flax was then domesticated and naturalized in Egypt where the linen made from flax were used to entomb the mummies as it was considered a symbol of saintliness and rectitude. It was later peddled in the whole of the Mediterranean by the Phoenicians who used it for sails and linen textiles. Finally the linen industry came into light in the European Middle ages where about 90% of the world's output was concentrated in Russia (Banerjee and Thiagarajan, 2015). Flaxseed is a rich source of different types of phenolics such as lignans, phenolic acids, flavonoids, phenylpropanoids and tannins (Kasote, 2013). Flax seed, is a remarkable dietary substitute and a rich source of omega-3 fatty acids (α-linolenic acid), omega-6 fatty acids, phytochemicals, phytoestrogenic compounds, rich in

protein and both soluble and insoluble fibers. The natural non-volatile oil approximately contains 30-35% of oleic and linoleic acid as well as about 69% of linolenic acid (Banerjee and Thiagarajan, 2015). It was found that consumption of ground seeds add nutritional benefits because flax seeds are also a rich source of lignans, having anticancer properties (Nag *et al.*, 2015).

1.2 Research Objectives

1.2.1 General Objectives

The main objective of this research is to study the characteristics of flax seed oil.

1.2.2 Specific objectives

a) Study the physicochemical properties of flax seed oil.
b) Determine chemical constituents of flax seed oil.
c) Ascertain the antioxidant activity of flax seed oil.
d) Refining of flax seed oil.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutrient Composition of Vegetable Oils

Vegetable oils are mainly composed of triacylglycerols, that is, esters of fatty acids with glycerol, but they also contain free fatty acids, monoacylglycerols, diacylglycerols, and nonglyceridic nutrients. The fatty acid composition of vegetable oils varies, and most edible oils are high in mono unsaturated fatty acids (MUFA) or poly unsaturated fatty acids (PUFA), whereas three of them are high in Saturated Fatty Acids (SFA). Nevertheless, the rapid increase in vegetable oils consumption represents the most important factor that contributed to the elevation of dietary omega-6-to-omega-3 ratio to its current value of 20–30 compared with a ratio of 2 in the hunter-gatherer diets (Savva and Kafatos , 2016).

2.1.1 Triglycerides

An oil or fat will usually contain at least 95% triacylglycerols before refining. After refining, this number will generally be in the range 97–99%, depending on the level of unsaponifiable material the oil or fat still contains. Triacylglycerols are fatty acid esters of the trihydric alcohol glycerol (1, 2, 3-trihydroxypropane) and contain three acyl chains in each molecule, usually from two or three different fatty acids. Accompanying the triacylglycerols are low levels of diacylglycerols, monoacylglycerols and free acids. These can result from incomplete biosynthesis in immature seeds or from post-harvest lipolysis. Almost all of the free acids and most of the monoacylglycerols will be removed by refining, but diacylglycerols tend to remain in the product. These are usually in the range 0–2% (Hamm *et al.*, 2013).

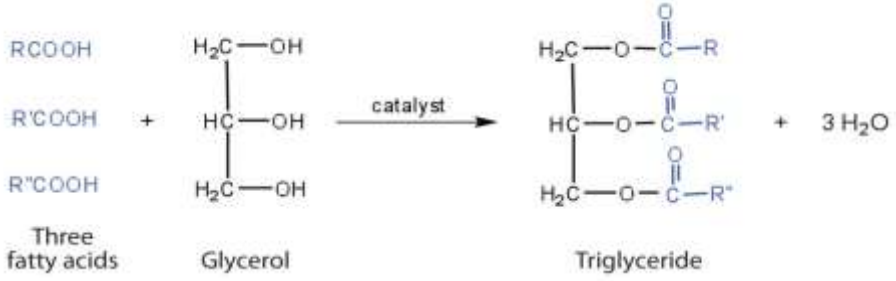


Figure (2-1) formation of triglyceride

2.1.2 Tocopherols

Tocopherols are lipid compounds and natural source of vitamin found in vegetable oil. It is a mixture of alpha, beta, gamma and delta tocopherol with fat-soluble antioxidant properties (Holt, 2016). The four tocopherols differ in the number of methyl groups attached to the heterocyclic moiety. The tocols have two valuable properties: they show vitamin E activity and they are powerful antioxidants. The tocols are themselves very sensitive to oxidation and are more stable in an esterified form when the all-important hydroxyl (phenolic) group is not free (Hamm *et al.*, 2013).

2.1.3 Phospholipids

Crude oils generally contain phospholipids, which are removed during refining at the degumming stage (Gunstsone, 2002). They have adverse effects on product quality and refined oil yield (O'Brien, 2009). They are used in the food industry as stabilizers, emulsifiers and antioxidants (Holt, 2016).The phosphatides are divided into two categories: hydratable and nonhydratable, depending on the effect that water has on them. Pretreatment of good-quality crude oils with phosphoric or citric acid before refining is successful in removing both nonhydratable and hydratable phosphatides to a phosphorus level of approximately 20 to 30 ppm. (O'Brien, 2009).

2.1.4 Color Compounds

Main pigments extracted along with the oil are carotenoids, chlorophyll, gossypol, and related compounds. Chlorophyll can promote oxidation in the presence of light, also act as catalyst poisons. Pigments are removed by adsorbents such as activated earth, activated carbon, amorphous silicas, and other adsorbents. (Reddy and Kawakatsu, 2001). Most of the carotenes are removed from the oil by heat bleaching in deodorization. Most of the chlorophylls are removed from the oil during the bleaching process using bleaching clay (Gupta, 2017).

2.1.5 Trace Metals

Vegetable oil contains trace amounts of metals, which at higher concentrations increase the oxidation rate of oil. Metals present in vegetable oils can be from mineral uptake from the soil as well as from other sources such as the application of

agrochemicals like fertilizers and pesticides. Storage and transportation could contribute to the metal contamination. Many metals are beneficial to humans but their levels in foods are of significant interest because, above certain levels, they may become harmful. Consequently, it is important to ascertain the levels of both beneficial and toxic metals in foods. (Nnorom and Ewuzie, .2015). Trace quantities of copper, iron, manganese, and nickel substantially reduce the oxidative stability of fats and oils, whereas calcium, sodium, and magnesium reduce the efficiency of the refining, degumming, bleaching, and hydrogenation systems (O'Brien, 2009).

. 2.2 Fatty Acids in Common Vegetable Oils

Fatty acids are carbon chains with a methyl group at one end of the molecule and a carboxyl group at the other end .The carbon atom next to the carboxyl group is called the (α) carbon, and the subsequent one the (β) carbon. Fatty acids play a number of key roles in metabolism – major metabolic fuel (storage and transport of energy), as essential components of all membranes, and as gene regulators. In addition, dietary lipids provide polyunsaturated fatty acids (PUFAs) that are precursors of powerful locally acting metabolites. As part of complex lipids, fatty acids are also important for thermal and electrical insulation, and for mechanical protection. Moreover, free fatty acids and their salts may function as detergents and soaps owing to their amphipathic properties and the formation of micelles (Rustan and Drevon, 2001).

2.2.1 Saturated Fatty Acids

Saturated fatty acids are filled with hydrogen. Most saturated fatty acids are straight hydrocarbon chains with an even number of carbon atoms. The most common fatty acids contain 12–22 carbon atoms (Rustan and Drevon, 2001).The most common saturated fatty acids in seed oil are lauric (C12:0), myristic (C-14:0), palmitic (C-16:0), stearic (C-18:0), arachidic (C-20:0) and behenic (C-22:0) (O'Brien, 2009).

2.2.2 Unsaturated Fatty Acids

Unsaturated fatty acids contain fewer hydrogen atoms than required to fully satisfy the valence of each carbon atom in the molecule Gupta, 2017). Monounsaturated fatty acids have one carbon–carbon double bond, which can occur in different positions. The most common monoenes have a chain length of 16–22 and a double bond with the cis configuration. Tran's isomers may be produced during industrial processing. The presence of a double bond causes restriction in the mobility of the acyl chain at that point. The cis configuration gives a kink in the molecular shape and

cis fatty acids are thermodynamically less stable than the Trans forms. The cis fatty acids have lower melting points than the trans fatty acids or their saturated counterparts (Rustan and Drevon, 2001). Hydrogenation of unsaturated fatty acids improves the oxidative stability of the oils (O'Brien, 2009). In polyunsaturated fatty acids (PUFAs) the first double bond may be found between the third and the fourth carbon atom from the (ω) carbon (Rustan and Drevon ,2005).The most important unsaturated fatty acids are oleic (C-18:1), linoleic (C-18:2) and linolenic (C-18:3) (O'Brien, 2009).

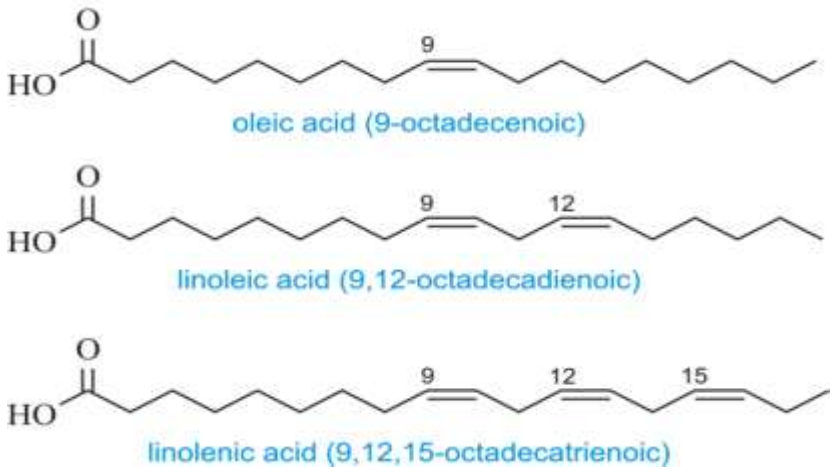


Figure (2.2): Structure of some unsaturated fatty acids

2.3 Flax

Flax or linseed (*linum usitatissimum* L.) is among the oldest crop plants cultivated for the purpose of oil and fiber. It belongs to the genus linum and family linaceae .The Mediterranean and southwest Asia have both been proposed as the center of origin (Jhala and Hall,2010). It is mainly grown in Canada, Argentina, America, China and India (Pradhan *et al.*, 2010). The fiber has been converted to yarn, which served as a major source to manufacture textiles for table or bed coverings and clothing (Zuk *et al* ,2015).Generally flax seed oil is used for the manufacture of paints, varnishes, inks, soap, etc. In recent time, flax seed oil has become more popular as functional food in the health food market because of its reported health benefits and disease preventive properties on coronary heart disease, some kinds of cancer, neurological and hormonal disorders. Most of the observed health benefits

and disease preventive properties of linseed oil have been attributed to their omega-3 fatty acid, -linolenic acid (ALA, 18:3) content (Kasote *et al.*, 2013).

2.4 Morphological characterization of flax (*linum usitatissimum* L.)

2.4.1.1 Root

Shallow root system of flax consists of a main root and many of lateral roots. The taproot is short, thin, with fibrous branches, which may in light soil extend to 0.9-1.2 m. the main root is straight. Lateral roots are mostly in upper part of root system where secondary and further branching occurs. (Nozkova *et al.,* 2011).

2.4.1.2 Stem

The cross-section of flax stem is usually round to oval. The stem width ranges from less than 1.2 mm to more than 2.0 mm. The plant develops lateral branches on the lowest part of the stem above the ground. Their development is influenced by density of planting and soil fertility. The branching in the upper part of stem is determined by the genotype, but it is also influenced by the environment and the density of planting. The oil type can branch from middle part of stem. The length of flax plant ranges approximately from 200mm to 1500mm (Nozkova *et al.*, 2011).

2.4.1.3 Leaf

The leaves are alternate smooth , have three veind and their size range from 10 to 30 mm .The shape of leaves varies – linear, lance late-egg shape. In initial growing stage they are covered by a thick wax coat that makes them more resistant to herbicides. The leaf density depends on the use type the fiber type has less (80-100) and thinner leaves than the oil type that has more leaves (more than 120) and they are large. (Nozkova *et al.,* 2011).

2.4.1.4 Flower

Flower of flax has five sepals , but sometimes there can occur 4, 6 or 7 . the sepals are smooth-edged, membranous at the edge , and usually do not drop. The surface is without or dotting , with smaller or larger intensity of dots. This character is important for varieties distinguishing . The petals are inversely wedge-shaped. The top part of petals is round and some genotypes have petals with a longitudinal folded out or inward. The color of petals varies from white, light blue, blue, dark blue, pink, violet, to red-violet. The veins are colorless or colorful (pink, biue or violet) (Nozkova *et al., 2011*).

2.4.1.5 Fruit

The flax fruit is capsule. The fruit varies in shape from oblate to globular and cylindrical or conical. The capsule has five carpels. Each carpel has two septa separated by incomplete septum. The septum inside of capsule can be hairy or smooth. The maximum number of seeds per capsule is 10. Acapsule with 6 to 7 carpel occurs sometime. Commonly , the fiber flax produces 7 to 8 seeds per capsule but large-seeded can from up to 10 seed (Nozkova *et al.,* 2011).

2.4.1.6 Seed

Flax seeds are usually flattened , slightly convex in the middle, ovoid or oblong elliptical, rounded at the base, and acute at the apex .Flax seed shape depends on seed width ; it can be round or elongated. The size of the seeds varies among genotypes the seed length varies from less than 4.0 to more than 5.25mm. The ripe seed is fully colored. The color of seeds varies from yellow, different hues of brown , to black, can be olive and even multi-colored seeds can be found (Nozkova *et al*., 2011).

2.4.2 Adaptation

Flax is widely adapted to a broad range of soil and environmental conditions. Cool temperatures after flowering tend to increase oil (particularly linolenic acid) content. As a result, flax oil yield and quality are generally better in higher latitudes. Fiber flax also will grow in many environments; however, cool weather conditions during crop development are essential to produce high-quality fiber. The best quality flax fiber generally comes from temperate regions (near 45° latitude) with a strong coastal climatic influence (Ehrensing,2008).Flax generally does best on well-drained soils with good water holding capacity, such as silt-loams and clay loams. Flax does not tolerate poorly drained soils well. Surface crusting on heavy soils can retard germination and interfere with good crop establishment (Ehrensing,2008).

2.4.3 Flax Seed Oil (FSO)

Flaxseed oil differs from whole and ground flaxseed by being devoid of both fiber and lignans (Roy *et al* ,. 2007) . The direct use of unprocessed conventional flax oil in the human diet is limited by product stability (Jhala and Hall,2010). The use of flaxseed oil in food preparation by the ancients is seldom dealt with in detail apart from acknowledging its long culinary use in Egypt, India and China. Flaxseed oil (FSO) is seldom used for frying or preparation of food when heat is involved, but in

major flax-growing regions in China , flaxseed oil has been used as cooking oil and is favored by the local people over rapeseed or mustard oil (Choo *et al. , 2007)* . Flaxseed oil is sold locally after extraction, and foods are cooked at home and consumed immediately, which probably shortens the time between oil extraction and its consumption. (Choo *et al. , 2007)*.In the 1950s, the limiting factor for the use of flaxseed oil in food was its susceptibility to "flavor reversion," an off-flavor that developed in industrial prototypes of both salad oil and shortenings. This defect appeared to be associated with flaxseed oil's high ALA content (Muir and Westcott,2003).Experimental high temperature processing of flaxseed oil to polymerize ALA (275°C for 8–12 hr.) succeeded in averting flavor reversion. As a result, the commercial processing of flaxseed oil did not proceed at that time. Today, the high-ALA flaxseed oil sold in health food stores is cold pressed at < 35°C, processed in a low-oxygen environment and packaged in light-proof containers to maintain stability. The increasing consumer demand for cold pressed flaxseed oil appears promising (Muir and Westcott,2003). Flaxseed is most commonly used as a laxative and also used during menopause for hot flashes and breast pain. Flax seed oil is used for various conditions including arthritis. Both flaxseed and (FSO) have been used for high cholesterol levels and in the prevention of cancer (Roy *et al.* 2007).Flaxseed is rich in fat, protein and dietary fiber. The compositions of flaxseed averaged 30–40% fat, 20–25% protein, 20–28% total dietary fiber, 4–8% moisture and 3–4% ash, and the oil contains vitamins A, B, D and E, minerals, and amino acids (Pradhan *et al.*, 2010). Flaxseed oil is mainly found astriacylglycerols (98%) with lower contents of phospholipids (0.9 %) and free fatty acids (0.1%) (Rubilar *et al* .,2010).

2.4.3.1 Uses and Applications of Flax Seed Oil (FSO)

Flax (*Linum usitatissimum*) is cultivated for the production of textile fibre, seed and flaxseed (linseed) oil. In recent years flaxseed has become known as a functional food due to its nutritional composition, which has positive effects on disease prevention providing health-beneficial components such as alpha-linolenic acid (Jhala and Hall,2010).Lignans and polysaccharides (other than starch), these components may prevent or reduce the risk of various important diseases such as diabetes, lupus nephritis, arteriosclerosis and hormone dependent types of cancer.. Flaxseed oil, the principal component of this seed, is rich in alpha-linolenic, linoleic

and oleic acids, and for years has been the focus of interest in this seed (Gutiérrez *et al* ., 2010). When this flax oil is exposed to air, the double bonds of ALA react with oxygen and result in relatively soft, durable film. This property is known as "drying" quality of linseed oil, is responsible for extensive use in manufacturing varnishes, oilcloth, printer's ink, imitation leather and also as an anti-spalling and curing agent for concrete surfaces on highways (Jhala and Hall,2010).The drying quality of oil can be improved by addition of metal catalyst to promote oxidation and also by partially pre-oxidizing oil through exposure to the air. Along with the use of flax oil as an oil paint carrier, it is also being used as a painting medium, making oil paints more fluid, transparent and glossy. Linseed oil can also be used as "finishing oil" for wooden furniture to prevent it from denting. It does not cover the surface of wood but soaks into the pores, leaving a shiny but not glossy surface (Jhala and Hall,2010).

2.5 Antioxidant Activity

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules (Aluyor and Jesu, 2008). They are used for the stabilization of polymeric products, petrochemicals, foodstuffs, cosmetics and pharmaceuticals (Pisoschi and Negulescu, 2011). Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive specie(Kumar, 2014). Numerous methods have been developed to control the rate and extent of lipid oxidation in foods, but the addition of antioxidants is most effective, because of their unique properties of extending the shelf-life of food products without any adverse effect on their sensory or nutritional qualities(Sardarodiyan and Sani ,2016). Antioxidants for use in food system is inexpensive, nontoxic and effective at low concentrations; highly stable and capable of surviving processing; have no odour, taste or colour of their own; easy to incorporate; and have good solubility in the product (Sardarodiyan and Sani ,2016). The methods of antioxidant capacity evaluation, include spectrometry, chromatography and electrochemical techniques (Pisoschi and Negulescu, 2011). Tocopherols are the main antioxidant in vegetable oils. Flaxseed contains the lowest amount of tocopherols when compared with other oilseeds with similar amounts of polyunsaturated fatty acids (PUFA) (Barthet *et al* ., 2014).

2.6 Refining Methods

Purpose of refining is to convert crude oil or fat into a product more suitable for its end purpose. This will involve the removal of undesirable components and will usually result in a product with minimal colour and flavour. The processes have been devised to minimise changes in the triacylglycerols and in the levels of those minor components which confer nutritional benefit. If the oil is to be processed with the aid of a catalyst (hydrogenation, interesterification, etc.) (Gunstone *et al*, 2002).

The compounds removed by refining include phospholipids, free acids, mono- and di-acylglycerols, colour, trace metals, oxidation products and environmental contaminants. Oils may be refined by a series of processes that can be grouped together as 'chemical refining' or 'physical refining'. The former involves degumming, chemical neutralization, bleaching and deodorization, while the latter requires only bleaching and steam distillation (deodorization). Physical refining is preferred in that it is a more economical process requiring less chemicals, producing less waste and giving higher oil yields (Gunstone *et al*, 2002).

2.6.1Blending

The mixing of oils and fats is produce blends with improved nutritional or physical properties. Oils are also blended to obtain the desired mix at minimum cost and computer programs hSave been developed to give the best blend (Gunstone *et al*, 2002).

2.6.2 Degumming

degumming techniques are employed to reduce the phospholipid levelsto meet quality standards.(Gunstone *et al*, 2002).The phosphorus content of the oil has a profound influence in the flavor, color, and hydrolytic and oxidative stability of the refined, bleached, and deodorized oil, as well as it causes reduced refining yield. Various degumming processes are used in the vegetable oil industry: water degumming, acid conditioning, acid degumming and enzymatic degumming (Gupta, 2017).

2.6.3 Bleaching

The purpose of bleaching is to provide lighter colored oil, and to purify it from traces of soap and other impurities that may be present in the oil. It follows the water washing and vacuum drying step in physical process or the alkali refining in chemical refining. (O'Brien, 2009). Bleaching is usually carried out with neutral clays, activated earths, synthetic silicates, silica gel, and activated carbon by

adsorption. Bleaching earth is the most common adsorbent used for refining many edible oils due to its low cost (Chemat, 2017).

2.6.4 Neutralization

This is effected by treatment with alkali . The resulting soap is separated . A similar result can be achieved by steam distillation (physical refining), but this can only be applied to oils of low phospholipid level (Gunstone *et al*, 2002).

2.6.5 Deodorization

Deodorization is the last step in the refining of oils. The primary objective of deodorization is to remove compounds responsible for undesirable odors and flavors, such as residual, aldehydes, ketones, alcohols, Polycyclic aromatic hydrocarbons PAHs , dioxins, and pesticide residues. Aldehydes, ketones, and alcohols are generated due to hydrolytic and oxidative instability of oil during production and storage. Deodorization is essentially steam distillation performed at high temperatures (170–270°C) and under high vacuum (3–8 mmHg absolute pressure (Chemat, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

Flax seeds samples were collected from the local shop (Wad Medani). It is classified by Medicinal and Aromatic Plants Institute Research – Khartoum

Figure (3-1) flax seeds

3.2 Chemicals and Reagents

The chemicals and reagents used were analytical grade (AR):

Sodium hydroxide(0.1N, 10N, 0.02,N, 0.5%), Sodium thiosulphate(0.1N), Potassium hydroxide (1.33%,50%), Potassium iodide, Hydrochloric acid (0.5N), n-hexane (99 %), Petroleum ether(99 %), Ethanol(10%,95 %), Acetic acid – chloroform solution (3:2 v/v), Phenolphthalein indicator, Distilled water, Starch indicator. Sulphuric acid(1.25%), Phosphoric acid(85 %), Bleaching earth (1.2%), Boric acid(3%), Diethyl ether, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) (300µM), Dimethyl Sulfoxide (DMSO).

3.3 Instruments

Gas chromatography- Mass spectrometry GC-MS 2010 PLUS- Shimadzu- Japan, Lovibond model PFX- i 995, Refractometer model RX-7000 α- Japan, Electrical oven, Rotary evaporator. Screw pressing, Digital balance, Multiplate reader spectrometer, Soxhelt apparatus, kijeldahl apparatus.

3.4 Extraction of Flax Seed Oil

The extraction of flax seed oil was performed at the National Oil Seed Processing Research Institute (NOPRI), University of Gezira.

3.4.1 Solvent Extraction

The flax seeds were cleaned from any foreign matter and ground into fine powder using mortar and pestle to be ready for extraction and analysis. Forty grams of ground seeds were weighed into thimble and transferred to soxhlet extractor, 250 ml of n-hexane were added into flask, the temperature was set at 60^{o}C for 6 hours. n-hexane was evaporated using rotary evaporator (Heidolph Instruments GmbH and Co.KG91126 Schwabach), the extracted oil was kept for analysis.

3.4.2 Mechanical Pressing

The flax seeds were cleaned from any foreign matter and three thousand gram were taken and they were crushed by a table top screw pressing machine (OEKO TEC-IBG MONFORTS, type CA 59G, 2006, Machine No.20201550 Germany).

Figure (3-2) Lab scale screw mill used in this study.

3.5 Proximate Analysis of Flax Seeds

3.5.1 Moisture Content

The moisture content was carried out according to AOCS official method (2003). The flax seeds were cleaned manually to remove all foreign matter such as dirt, dust, stones and chaff as well as immature and broken seeds. Fifty grams of sample were weighed into dish, then the dish was placed in the oven and dried at 103°C for 3 hr. After that the dish was removed from the oven, immediately was cooled in

desiccators to room temperature and was weighed. The moisture content was calculated as follows:

$$\text{Moisture Content \%} = \frac{\text{original weight of sample - weight of dried sample}}{\text{original weight of sample}} \times 100$$

3.5.2 Oil Content

The oil content was determined according to AOCS official method (2003). The oil was obtained by using soxhlet. Amount of 39 grams of flax seeds powder were taken into a thimble after grinding and drying in oven at 103 ^{0}C for three hours. A round bottom flask containing known volume (250ml) of hexane was fixed to the end of the apparatus and a condenser was tightly fixed at the bottom end of the extractor in a heating mantle was used for six hours using n-hexane as a solvent, followed by solvent removal with a rotary evaporator. The oil content was determined as the ratio of the weight of the extracted seed oil to the weight of the oilseed powder sample as described below.

$$\text{Oil content (\%)} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

3.5.3 Crude Protein

Crude protein was determined according to (AOAC, 1995) through the following stages: -

3.5.3.1 Digestion Stage

One gram of flax seeds powder was placed in kijeldahl tube and a 4g mixture (catalyst; sodium sulphate and copper sulphate) was added. The mixture was digested with concentrated sulphuric acid (25ml) for 2 hours in fume hood until the solution became clear to light green.

Seeds (H_2SO_4 conc/catalyst) $\longrightarrow$ $(NH_4)_2SO_4$

3.5.3.2 Distillation Stage

A total of 120ml distilled water were added to the solution and allowed to cool. Sodium hydroxide (10N) was also added 75ml without agitation. The flask was then connected to the distillation bulb with the tip of the condenser immersed in a standard acid solution (boric acid 3%) containing 5 drops of the indicator. The flask was then heated to release ammonia into the indicator solution.

$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + H_2O + NH_3$

$NH_3 + H_3BO_3 \longrightarrow NH_4^+H_2BO_3^-$

3.5.3.3 Titration Stage

The excess standard acid in the distillate was titrated with (0.1N) standard HCL. The conversion factor of 6.25 was used and % of Nitrogen calculated as below:

$$NH_4^+H_2BO_3^- + HCL \longrightarrow NH_4CL + H_3BO_3$$

$$\text{Crude Protein (\%)} = \frac{(B - T)\ N \times 14 \times 6.25 \times 100}{W_S \times 1000}$$

Where:

T= Titration reading.

B= Blank titration reading.

N= HCl normality.

Ws= sample weight , gram

3.5.4 Crude Fiber Content

Crude fiber contents were determined for samples according to (AOAC, 1995). Two grams of flax seed powder were transferred into a 200 ml labeled beaker after which 50 ml sulphuric acid (1.25%) and 150 ml distilled water were added. The sample mixture was then boiled for 30 minutes under reflux flask and later treated with 50 ml potassium hydroxide (1.33%) and 150ml water the solution was re-boiled again for 30 minutes and registered using vacuum crucible filtrate on system. The sample in the crucible was rinsed with water followed by acetone. The samples were put into a pre-weighed crucible and transferred to the oven to dry for 4 hours, cooled in desiccators and weighed. The weighed sample was used in the furnace set at 660°C for 5 hours until it became grey ash which was cooled in the desiccator and weighed. The weight of ash was then calculated as follows:

$$\text{Crude fiber \%} = \frac{W_1 - W_2 \times 100}{W_S}$$

Where:

W_1= Weight of crucible with sample before ashing.

W_2= Weight of crucible with sample after ashing.

Ws= Weight of sample , gram .

3.6 Physical parameters Characteristics of Flax Seed Oil extracted by pressing machine

3.6.1 Specific gravity

Specific gravity was determined according to AOCS to (1a-64, 2003). Specific gravity is the ratio between weights of specified volume of oil at a certain temperature to the weight of the same volume of water . Specific gravity of Flax seed oil was determined by bottles method. The experiment was conducted at 25°C and specific gravity of the sample was calculated by using the following formula

$$\text{Specific gravity} = \frac{(W_3-W_1)}{(W_2-W_1)}$$

Where:

W_1= weight of empty specific gravity bottle

W_2= weight of water + specific gravity bottle

W_3= weight of test sample + specific gravity bottle

3.6.2 Color

The color was measured using the Lovibond tintometer (model PFX 880), equipped with standard color slides as described in AOCS official method (Cc 13e-95, 2003). Pure crude flax seeds oil was placed into 1inch (25.4mm) cell to Lovibond apparatus. The Lovibond was read estimating the number of red, yellow and neutral or blue unit's needed to obtain the match with the standard colour slides.

3.6.3 Refractive Index

This was carried according to AOCS Official method (Tp,1a- 64,2003).The refractive index is the ratio of the speed of light in one medium to that in another medium. The refractive index of oils was measured at 20°C using refractometer (Rx-7000, Japan).

3.7 Chemical Parameters

3.7.1 Free Fatty Acids (F.F.As)

The free fatty acids are the number of milligrams of sodium hydroxide required to neutralize the free fatty acids in 1 g of oil. The determination of the free fatty acids for oil was carried out according to AOCS official method (3d. 63, 2003). Five game of the oil samples were taken into 500 ml conical flask then 50 ml of neutralized ethanol and (2-3) drops of phenolphthalein indicator were added to sample. The solution was titrated with (0.1N) sodium hydroxide (with vigorous shaking) to the appearance of the pink colour at the end point.

$$NaOH + R(COOH) \longrightarrow RCOO^- Na^+ + H_2O$$

The percentage of free fatty acids was calculated as follows:

$$\text{F.F.A \% (as oleic acid)} = \frac{(28.2) \times N \times V}{Wt}$$

Where:

N=Normality of sodium hydroxide.

V= volume of NaOH solution used.

Wt=weight of sample , gram.

3.7.2 Peroxide Value (P.V)

Peroxide value was determined according to AOCS official method (Cd 8-53, 2003).Five gram of the oil sample were taken into 500 ml conical flask and 30 ml of acetic acid - chloroform solution (3:2/volume) were added, then 0.5 ml of saturated potassium iodide was added to it. After that the solution was shaken for one minute and 30 ml of distilled water were added to it. The solution was then titrated with 0.1N sodium thiosulfate until the yellow color of the solution appeared and then a starch indicator solution (2ml) was added to the solution and the titration was continued with vigorous shaking until the blue color of the solution has just disappeared. A blank determination was similarly conducted simultaneously with the samples.

Oil/fat + KI acetic acid/ chloroform 3:2 $\longrightarrow$ I_2

$2Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$

The peroxide value was calculated as follows:

$$\text{P.V (meq/kg)} = \frac{(B - S)(N)(1000)}{Wt}$$

Where:

B=ml of titration of blank.

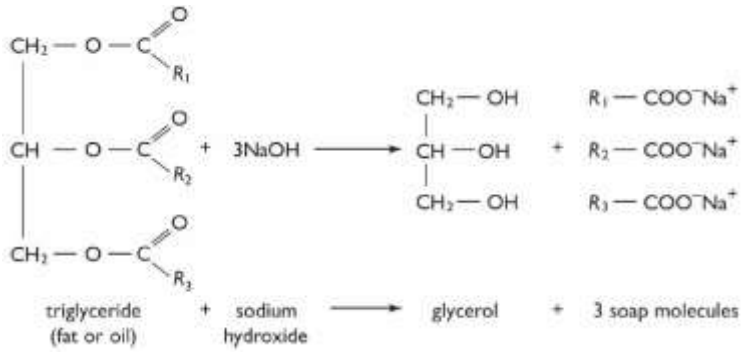
S= ml of titration of sample.

N= Normality of sodium thiosulfate.

Wt = Weight of sample, gram .

3.7.3 Saponification Value (S.V)

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the fatty acids resulting from the hydrolysis of one gram of oil or fat. The saponification value was determined according to AOCS official method (Cd 3.25, 2003). Five grams of the oil sample were taken into 500 ml round bottom flask then, 50 ml of ethanolic potassium hydroxide were added to the round bottom flask, then a reflux condenser was connected and the solution was boiled gently in a water bath but steadily until the sample was completely saponified (one hour). The flask was allowed to cool but not sufficiently to form a gel. The insides of the condenser were washed with small amount of distilled water and after that the condenser was disconnected. One ml of phenolphthalein indicator solution was added to the flask, and then the solution was titrated with 0.5N hydrochloric acid until the pink color just disappeared. A blank determination was similarly conducted.



The saponification value was calculated as follows:

$$\text{S.V (mg KOH/g)} = \frac{(B - S)(N)(56.1)}{Wt}$$

Where:

B= ml of titration of blank.

S= ml of titration of sample.

N=Normality of hydrochloric acid.

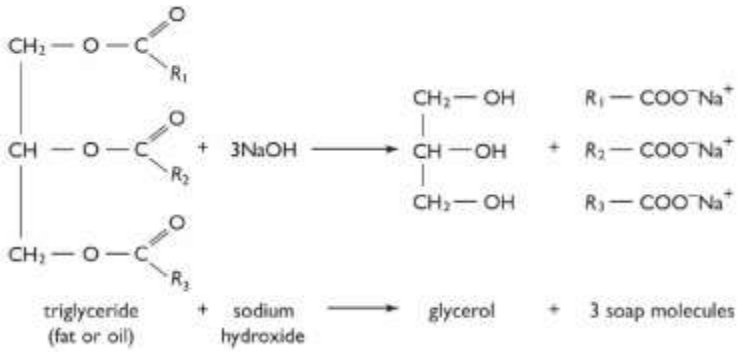
Wt = Weight of sample, gram

3.7.4 Unsaponifiable Matter

This test was carried out according to AOCS official method (Cd 3.25, 2003). Five grams of the oil samples were weighed into round bottomed flask. Amount of 30 ml of ethanol and 5 ml of aqueous KOH 50% were added. The round bottomed flask was attached to reflux condenser and boiled gently for 1 hour. Reaction mixture was

transferred into separating funnel. The flask was washed by 10 ml ethanol followed by 20 ml warm and then 20 ml cold distilled water, all the washings were transferred to the separating funnel. After that 10 ml petroleum ether were added. Then the solution of separating funnel was cooled at room temperature before adding 50 ml of petroleum ether. The separating funnel was stoppered and shaken vigorously for 1 minute; allowed to stand until there was completely separation of the two phases. The soap layer was drawn off to second separating funnel and 50 ml of petroleum ether were added, and it was repeated five times. The combined extracts were washed three times with 25 ml portions of 10% (v/v) ethanol and shaken vigorously. The ethanol layer was drawn off after each wash. The petroleum ether extract was transferred into 250 ml round bottomed flask was previously dried and weighed. The petroleum ether was evaporated from solution by using rotary evaporator; the drying was completed in a vacuum oven at (70 – 80) $^{\circ}$C. The residue was dissolved in 50 ml of warm 95% ethanol previously neutralized to faint pink color using phenolphthalein indictor, Titrated with 0.02 N sodium hydroxide and the weight of free fatty acid.

was calculated



The unsaponifiable matter percentage was calculated as follows:

$$\text{Unsaponifiable matter \%} = \frac{100(W_1 - W_t)}{W}$$

Where:

W: the weight in grams of the oil sample

W_1: the weight in grams of the residue

W_t: the weight in grams of the fatty acids

3.8 Fatty acid Composition

The fatty acid composition analysis was carried out at the University of Medical Sciences and Technology, Khartoum, Sudan. To identify the constituents of the fixed

oils extracted from one accession, GC-MS Instrument (QP 2010- Ultra), equipped with a capillary column (0.25 diameter; film thickness 0.25 µm) was used. The carrier gas used was helium (99.99%), before injection of the oil sample in the GC the fatty acid methyl ester (FAME) was prepared by taking 2 ml of seed oil sample into test tube, 7 ml of alcoholic NaOH,was prepared by dissolving 2 g of NaOH in 100 ml methanol, and 7 ml of alcoholic H_2SO_4 1% (prepared by mixing 1 ml conc. H_2SO_4 added to 99 ml methanol) and shaken by vortex for 5 minutes, all the contents were left to overnight.1 ml of saturated NaCl and 2 ml of n-hexane were added, shaken for 3 minutes, n- hexane layer was collected. Five µl of collected hexane was taken and diluted with 5 ml diethyl ether; 1g of sodium sulphate was added as drying agent, and filtered through syringe filter 1 µm, filtrate was transfered directly to the GC-MS vial. Fatty acid composition of the flax seed oil was determined by identifying comparison of their retention time (RT) with the mass spectral library of the GC-MS data software system national institute of standard and technology (NIST library). The total running time for a sample was 27 min.

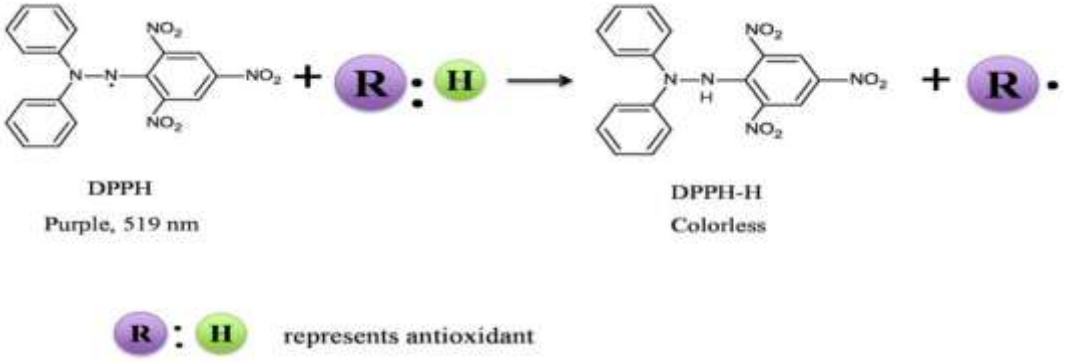
3.9 Oil Refining

The refining process was carried out according to (Chemat, 2017). Crude flax seed oil was acid-degummed by pretreating the oil with 0.3% w/w of phosphoric acid (85% concentration) at 70^{o}C for 10 min. Then, distilled water (3% w/w) was added into the oil and stirred in a heated water bath at 70^{o}C for 30 min. After cooling the mixture, the gums were removed from the degummed oil by centrifugation. A stoichiometric quantity of sodium hydroxide solution (16^{o}Baume) with an excess level of 0.5% was added into the degummed oil to neutralize the free fatty acids. The soap stock was removed by centrifugation. The neutralized oil was washed three times with distilled water to remove the residual soap in the oil. The neutralized oil was bleached with 1.2% w/w of bleaching earth at 95^{o}C for 30min. The bleaching of oil was followed by the final deodorization step performed using a lab-scale glass deodorizer at 200^{o}C for 1 h.

3.10 DPPH Radical Scavenging Activity

The experiment was conducted at the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging was determined according to the method described by (Shimada *et al.*, 1992). In 96-well plate the test samples were allowed

to react with DPPH stable free radical for half an hour at 37°C. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO (Dimethyl sulfoxide) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm, multiplate reader spectrometer was used. Percentage radical scavenging activity by samples was determined in comparison a DMSO treated control group.



Radical scavenging activity =

$$\frac{\text{(Absorbance of control – Absorbance of sample) X 100}}{\text{Absorbance of control}}$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physicochemical Properties of Flax Seed

The oil content, moisture content, crude protein, crude fiber and flax seeds are shown in table (4-1).

4.1.1 Oil content

The oil content of the flax seed by mechanical pressing was found to be 20% these are lower than that reported by(Kasote *et al.*, 2012).Flax seed oil by solvent extraction was found to be 37.9% which is within the range reported by(El-Beltagi *et al.*, 2007) Who stated that Linseed contains about 36–40% of oil.

It is clear that the oil content by solvent extraction is greater than that got by pressing machine.

4.1.2 Moisture content

The moisture content of flax seed was 1.92 % these are lower than that reported by (Rubilar *et al.*, 2010) at 3–4%

4.1.3 Crude protein

The crude protein content of the flax seed was 24%. Which is in range that reported by (Rubilar *et al.*, 2010) at 20 – 25 %,

4.1.4 Crude fiber

The fiber content of the flax seed was 22% ,which is in the range that reported by (Rubilar *et al.*, 2010) at 20 – 25 %

Test	Oil Content %	Moisture content %	Crude Protein %	Crude Fiber %
Flaxseed	**37.9 %**	**1.92**	**24%**	**22.52%**

Table (4-1) Physiochemical Properties of flax seed

4.2 Physiochemical Properties of flax seed Oil by Mechical Pressing

The moisture content, free fatty acids, peroxide value, saponification value, unsaponifiable matter, refractive index, colour and density for flax seed oil are shown in table (4-2).

4.2.1 Moisture content

The moisture content of the flax seed oil was (0.005) these are similar to that reported by Kasote *et al* (2012). at (0.0%)

4.2.2 Free Fatty Acids (FFA)

The free fatty acids of the flax seed oil was (0.52%) these are lower than that reported by(Kasote *et al.*, 2012) with a value of 0.9 %.

4.2.3 Peroxide Value

The peroxide value of the flax seed oil was (6.4meq/kg) these are higher than that reported by (Kasote *et al.*, 2012) at (2, 1, 1.8) meq/kg.

4.2.4 Saponification Value

The saponification value of the flax seed oil was (146 mgKOH/g) these are within the range reported by (Kasote *et al.*, 2012) at (146,183, 196) mgKOH/g.

4.2.5 Unsaponifiable Matter

The unsaponifiable matter of the flaxseed oil was (0.031%) these are less than that reported by (Choo *et al*,. 2007) at 0.0.39%.

4.2.6 Refractive index

The refractive index of the flax seed oil was (1.4818) it is higher than that reported by (Kasote *et al.*, 2012) .

4.2.7 Colour

The colour of the flax f seed oil is brownish yellow similar to that reported by (Kasote *et al.*, 2012).

Test	Flaxseed oil
Moisture content	**0.005 %**

Free Fatty Acid as oleic	**0.52%**
Peroxide Values	**6.4** meq/kg
Saponification Value	**146** mgKOH/g
Unsaponifiable Matter	**0.031%**
Refractive Index	**1.4818**
Colour	Red: 2.7 Yellow:70.0

Table (4-2) Physiochemical Properties of flax seed oil

4.3 Fatty acid composition

The fatty acid composition of the flaxseed oils is shown in table (4-3) . oil sample is high in total unsaturated fatty acids consisted mainly of linolenic and linoleic acids , and total saturated fatty acids, consisting mainly of palmitic and stearic acids. The most abundant unsaturated fatty acid in flax seed oil was linolenic acid $C_{18:3}$ 50.01% followed by linoleic acid $C_{18:2}$ 26.80%while the least was 11,14,17-Eicosatrienoic acid $C_{21:3}$ 0.16%. On the other hand, the most abundant saturated fatty acid was palmitic acid with 11.90% followed by stearic acid $C_{18:0}$ 9.35% while the behenic acid $C_{23:0}$ was also detected 0.31% The total unsaturated fatty acid 77.06% less than that reported pradhan etal (2010) as (80%). The total saturated fatty acids 21.93 % higher than that reported pradhan *et al* (2010). as (11.93%)

No	Fatty Acid	(FSO)%

Table (4-3): Fatty acid composition of flaxseed oil

1	Palmitic acid $C_{16:0}$	11.90
2	Stearic acid $C_{18:0}$	9.35
3	behenic acid $C_{23:0}$	0.31
4	Linoleic acid $C_{18:2}$	26.80
5	linolenic $C_{18:3}$	50.01
6	11,14,17-Eicosatrienoic acid $C_{21:3}$	0.16

4.4 Antioxidant activity by DPPH assay

The antioxidant activity of flax seed oil (FSO) extracted by cold pressing was inactive FSO table (4-4); for the n-hexane extracted oil it was 20% for FSO Oil extracted by cold pressing showed less antioxidant activity than that extracted by solvent .Because the oil extracted by solvent is more pure than that extracted by cold pressing.

Table (4-4) Antioxidant activity of flax seed oil

No	Sample	%RSA ± SD (DPPH)*
1	FSO by solvent	20±0.1
2	FSO by cold pressing	In active

4.5 Oil Refining

Results in table (4-5) show the FFA% and colour for FSO .The FFA are within the range reported by Gupta (2017) as 0.03 – 0.05 %.

Table (4-5) FSO refining results

Test	FSO Crude	FSO Refined

FFA %	0.56	0.05
Colour	Red:2.7 Yellow: 70.0	Red: 0.3 Yellow : 4.1

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- The oil extracted from flax seed was fully refined and characterized.
- Flax seed oil content was (37.9) % and its protein content was (24)%, which are suitable comparable to commercial oilseeds.
- The fatty acid composition of flax seed oil consists of unsaturated fatty acid mainly linolenic (50.1) % and linoleic (26.80) % acids and saturated fatty acids which consist mainly of palmitic (11)% and stearic (9.35)% acids.
- Flax seed oil showed antioxidant activity by DPPH assay.
- Refined flax seed oil showed a reduction in free fatty acids and colour.

Recommendations

- It is recommended to plant flax in the Sudan in industrial scale.
- It is recommended to study the stability of flax seed oil since it is high in unsaturated fatty acids.
- To explore the possibility of application of flax oil in soap industry.

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Appendices

Appendix **(1)**

Lovibond tintometer (model PFX 880)

Appendix(2):

Refract meter (Rx-7000, Japan)

Appendix(3):

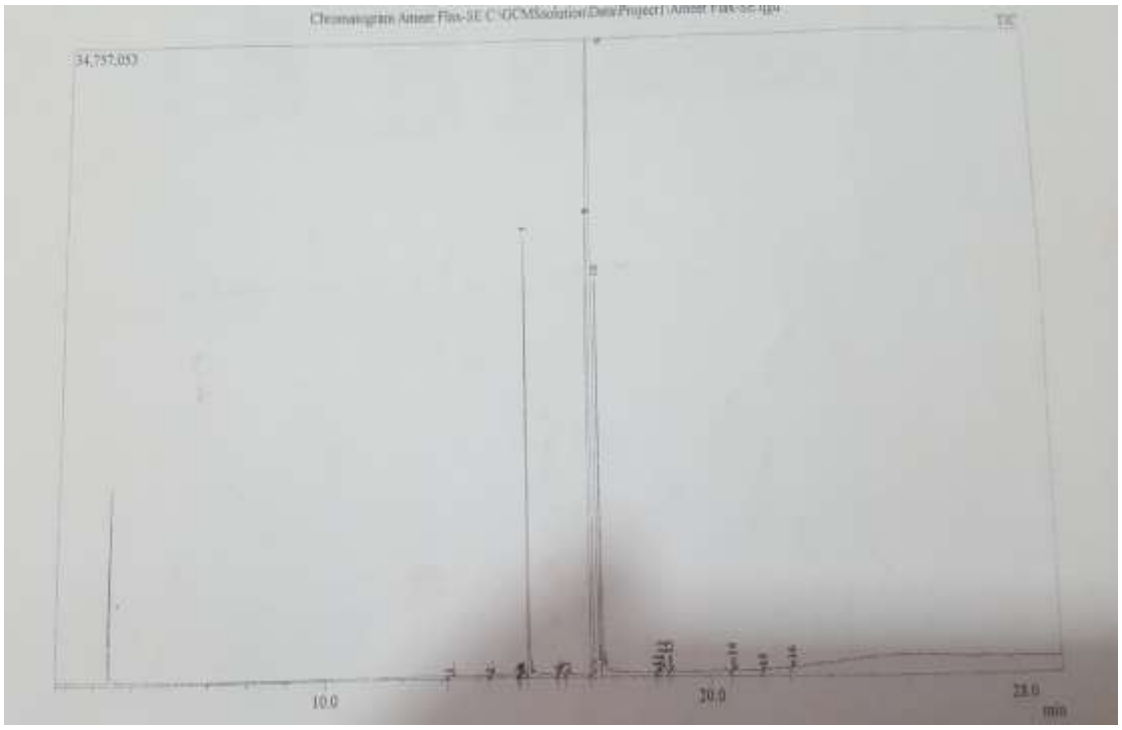

Figure (1) GC-MS Chromatogram with identifications of Fatty acid methyl esters of flax seed oil(FSO)

Appendix(4):

Fig.3a Degumming of flax seed oil

Fig.3b Neutralization of flax seed oil

Fig.3c Bleaching of flax seed oil

Fig.3d Deodorization of flax seed oil

Fig.3f : Curde and refined flax seed oil