

بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**  
**College of Graduate Studies**

**Effect of Roasting and Germination on Physicochemical  
Characteristics of Kernels and Oil from seeds of *Moringa*  
*peregrine***

أثر التحميص والإنبات فى الخواص الفيزيوكيميائية لأنوية وزيت بذور المورينجا برقرينا

A Thesis Submitted in Partial Fulfillment of the Requirements of the Degree of  
Master in Chemistry

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## **Dedication**

This thesis is dedicated to my parents; Nafisah Mahjoob and Ahmed Suliman.

## **Acknowledgement**

First of all I would like to thank Almighty Allah for giving me strength and patience to complete this research.

I would like to thank my supervisor Dr. Essa Esmail Mohammad for his guidance and advice throughout this research.

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## Abstract

The purpose of this study was to examine the effects of roasting and germination processes on the proximate compositions of the kernels and the physicochemical characteristics of the extracted seeds oil from *Moringa peregrina*. Roasting was carried out at 180°C for 25 minutes whereas germination was carried out at ambient conditions in a wet jute bag for five days. The results have shown that the roasting and germination processes have significantly affected the proximate composition of kernels. Roasting has decreased both protein content from 30.16% to 29.72% and fat content from 40.8% to 36.81%. Furthermore, roasting has observably increased moisture content from 2.90% to 3.32%, ash content from 2.6% to 2.97%, and total carbohydrate content from 23.54% to 27.18%. On the other hand, germinating has increased moisture content from 2.90% to 3.90%, ash content from 2.60% to 3.91%, and total carbohydrate content from 23.54% to 24.74% whereas the protein content was decreased from 30.10% to 27.25% and fat content did not show any appreciable changes (40.80 to 40.20%). Moreover, the physical and physicochemical properties of the extracted oil from raw and processed samples were determined. Both roasting and germinating have altered the measured parameters to varying degrees. The density almost remained unchanged as a result of roasting and germination whereas marginal variations in refractive index were seen between raw and processed samples. Furthermore, both roasting and germination have significantly altered the color of the extracted oil from (12.2Y, 0.1R) to (23.2Y, 0.3R) and from (12.2Y, 0.1R) to (32.3Y, 1.3R) respectively. On the other hand, roasting has noticeably decreased the viscosity of the roasted oil while germination has significantly increased it. Peroxide and acid values were both changed observably by processing. In the case of the roasted sample the peroxide value has changed from 0.57 to 0.95 meqO<sub>2</sub>/Kg oil and the acid value from 0.68 to 0.25 mgKOH/g while for the germinated sample the acid value changed from 0.68 to 20.03 mgKOH/g and there was no detectable peroxide value (PV).

## ملخص البحث

هدف هذا البحث إلى دراسة تأثير التخميص والإنبات على التركيب الكيميائي للنواة والخواص الفيزيوكيميائية للزيت المستخلص من بذور المورينجا برقرينا. أجريت عملية التخميص عند 180 درجة مئوية لمدة 25 دقيقة بينما تمت عملية التنبيت في جوال خيش رطب في الظروف الطبيعية لمدة خمسة أيام. أوضحت النتائج أن عمليتي التخميص والإنبات لهما تأثير مهم على التركيب الكيميائي للأنوية. حيث أدت عملية التخميص إلى انخفاض محتوى كل من البروتين من 30.16% إلى 29.72% و الزيت من 40.80% إلى 36.81%. بالإضافة لما سبق فقد بينت النتائج زيادة ملحوظة في محتوى الرطوبة من 2.9% إلى 3.32% ومحتوى الرماد من 2.6% إلى 2.97% وكذلك محتوى الكربوهيدرات الكلية من 23.54% إلى 27.18%. على الجانب الآخر فإن عملية الإنبات قد زادت محتوى الرطوبة من 2.90% إلى 3.90% و محتوى الرماد من 2.60% إلى 3.91% وكذلك محتوى الكربوهيدرات الكلية من 23.54 إلى 24.18% بينما إنخفض محتوى البروتين من 30.1% إلى 27.25% و محتوى الزيت لم يظهر أي تغير ملموس (40.80% إلى 40.2%). بالإضافة إلى ما سبق فقد تم تحديد الخصائص الفيزيائية والفيزيوكيميائية للزيوت المستخلصة من العينات الخام والمعالجة. بينت النتائج أن كل من التخميص والإنبات أدبا إلى تغيير المعاملات المقاسة بدرجات متباينة. أوضحت النتائج أن الكثافة بقيت تقريبا دون تغير يذكر بينما تغير معامل الانكسار بصورة هامشية للعينتين المعالجتين. بالإضافة إلى ذلك فإن عمليتي التخميص والإنبات للزيوت أحدثتا تغييرا في اللون من - أصفر 12.2، أحمر 0.1 - إلى -أصفر 23.2، أحمر 0.3 - ومن - أصفر 12.2، أحمر 0.1 - إلى - أصفر 32.3، أحمر 1.3 - على التوالي. من الناحية الأخرى فقد أدى التخميص إلى نقصان ملحوظ في اللزوجة للزيت بينما قاد الإنبات إلى إحداث زيادة معتبرة في لزوجة الزيت. أدت معالجة العينات إلى تغييرا ملحوظا في رقمي البيروكسيد و الحمض. في حالة العينة المحمصة تغير رقم البيروكسيد من 0.57 إلى 0.95 ملي مكافئ أوكسجين/كيلوجرام زيت ورقم الحمض من 0.68 إلى 0.25 ملغرام هيدروكسيد البوتاسيوم/جم زيت بينما العينة المنبتة فإن رقم الحمض قد تغير من 0.68 إلى 20.03 ملغرام هيدروكسيد البوتاسيوم/جم وأما بالنسبة لرقم البيروكسيد لم يتم رصد قيمة له.

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## Chapter One

### Introduction

*Moringa Peregrina* is a genus of shrubs and trees up to 5 m high, branches whip-like with multi-purpose uses. It occurs naturally in arid or semi-arid countries bordering the Red Sea, from Somalia and Yemen to Israel and Syria. In Africa, it is reported in Sudan, Ethiopia, Eritrea, Djibouti and Somalia. Occurrence of the tree in Iran and Pakistan was also reported in the literature. In Sudan, it is distributed in several parts which include Red sea hills, Blue Nile, Kordfan, Darfure and Equatoria [1-7].

The *Moringa* tree has a wide range of uses in the areas of agriculture, health, and industry so it is called a multipurpose tree. *Moringa* serves as a medicinal plant, animal fodder, and a food source for humans. The tuber of the young plant is eaten in Yemen and Oman. The seeds are used as coagulant to purify water. The oil is used to treat abdominal pain as well as it used in many applications which include medicine, cosmetics, human food, lubricant oil for small machinery, and bio-fuels. The *Moringa peregrina* oil is reported to be active against microorganisms and have antioxidant activity [7-11].

Study by Gharibzahedi *et al.* have shown that *M. peregrina* seeds oil contains a low amount of polyunsaturated fatty acid methyl esters (C18:2 and C18:3), which is a significant difference compared to other oils such as canola, soybean and sunflower. Therefore, this oil could be useful for edible purposes and for some industrial applications like hydrogenation, shortening production and others [6].

Only limited studies [5,6,9,11] were found in the literature on the characteristics of the oil of *Moringa peregrina*. To the best of our knowledge, no study was found in the literature which investigates the effects of thermal processing of seeds such as roasting as well as the germination of seeds on the proximate composition of the kernels and the physicochemical properties of the extracted oil.

#### 1.1 Objective of study

The objective of this study is to investigate the effects of roasting and germination of seeds on the proximate composition of the kernels and the physicochemical characteristics of the extracted oil of *Moringa peregrina* grown in Sudan.

## Chapter Two

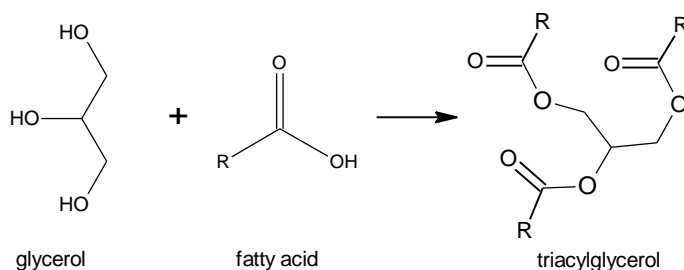
### Literature review

#### 2.1 Lipids: general introduction

Fats and oils (Lipids) are a mixture of esters of fatty acid usually triglycerides. Lipids consist of a wide group of compounds that are soluble in organic solvents and insoluble in water. They have lower densities than water and nonvolatile. Based on their structure and composition, oils and fats could be liquids or solids at normal room temperature [12,13].

#### 2.2 Components of fats and oils: major components

The major components present in vegetable oils are triacylglycerols. They are non-polar and water insoluble substances which composed of three fatty acid residues esterified to a glycerol backbone.



**Scheme 2.1:** The structure and constituents of a triacylglycerol

If all three fatty acid are identical it is a simple triglyceride. The more common forms however are the “mixed” triglycerides in which two or three kinds of fatty acids are present in the molecule. A crude oil or fat will usually contain at least 95 per cent of triacylglycerols. After refining, this will rise to 97- 99 per cent, depending mainly on the level of (unsaponifiable) material insoluble in aqueous alkali after hydrolysis [14-16].

#### 2.3 Components of fats and oils: minor components

The primary constituents in crude fats and oils are the triglycerides, but they also contain varying amounts of minor components, many of which significantly affect their chemical and physical properties. Several minor components such as wax esters, tocopherols, tocotrienols, phenolic compounds, pigments and phospholipids are present in vegetable oils. Many of these components are associated with color and distinctive taste of different types of oils as well as their anti-oxidant properties [16-18].

### **2.3.1 Mono- and diacylglycerols**

Acylglycerols are the predominant constituent in oils and fats of commercial importance. Glycerol can be esterified with one fatty acid they terms monoacylglycerol. When glycerol is combined with a single fatty acid it may form two isomeric monoacylglycerols. And the glycerol is combined with two fatty acids; it may form two isomeric also called diacylglycerols. Monoacylglycerols and diacylglycerols may be present as minor components, either as intermediates in the biosynthetic pathway or as products of partial lipolysis [13,15].

### **2.3.2 Free fatty acids**

Free fatty acids are the unattached fatty acids present in a fat. The levels of free fatty acids are reduced in the refining. Fully refined fats and oils usually have a free fatty acid content of less than 0.1% [14].

### **2.3.3 Phosphatides**

Phospholipids or phosphatides are natural surfactants and emulsifiers consisting of an alcohol such as glycerol, one or two molecules of fatty acids and a phosphoric acid compound. They are found in all plants and animals and include such substances like lecithin, cephalin, and sphingomyelin. Crude oils generally contain phospholipids, which are removed during refining at the degumming stage [16,19].

### **2.3.4 Sterols**

Sterols are minor components of all natural fat and oils. They have high melting points, colorless, heat stable and relatively inert. Sterols free are esterified with fatty acids, and are found in small concentrations (0.1 – 5.6%) in edible fats. Cholesterol is primary animal fat sterol and at relatively low amounts in certain plant lipids. Vegetable oil sterols collectively are termed phytosterols [16,17].

### **2.3.5 Tocopherols and tocotrienols**

Tocopherols and Tocotrienols are natural antioxidants found in plant-based oils, include four tocopherols and four tocotrienols isomers, each designated as alpha ( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ) and delta ( $\delta$ ). The  $\alpha$ -isomer is the most active biologically, whereas the  $\gamma$ -tocopherol is perceived as the best antioxidant. The tocopherols have a saturated, branched, polyisoprenoid C<sub>16</sub> side chain and tocotrienols have three double bonds in the side chain. These inhibit lipid oxidation in foods by stabilizing hydroperoxides and other free radicals, and their presence has a major effect on oil flavor quality. Antioxidants are sometimes intentionally added to oil to improve oxidative stability [16,17,20,23].

### **2.3.6 Carotenoids and chlorophyll (Pigments)**

Chlorophylls and carotenoids are the main pigments in vegetable oils. Carotenoids are a group of tetraterpenoides consisting of isoprenoid units. Double bonds in carotenoids are conjugated forms and usually are all trans forms.  $\beta$ -Carotene is one of the most studied carotenoids. Edible oils, contain  $\beta$ -Carotene. Chlorophylls are common pigments present in edible vegetable oil. It is the green coloring matter of plants which plays an essential part in the photosynthetic process. The levels of most of these color materials are reduced during the normal processing and refining of oils [12,16,23].

### **2.3.7 Vitamins**

Most fats and oils are not good sources of vitamins other than vitamin E. Vitamin E compounds include the tocopherols and tocotrienols. The fat-soluble vitamins A and D are sometimes added to foods which contain fat because they serve as good carriers and are widely consumed [12,13].

### **2.3.8 Glycerol**

Glycerol (Propane-1,2,3-triol) is the one and only alcohol to which fatty acids are esterified into triglycerides. Glycerol is asymmetrical triple alcohol and is important as the basic component of all triglycerides [16,22].

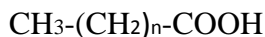
### **2.3.9 Wax esters**

The wax esters formed by the reaction of alcohols and free fatty acid are present in seed and fruit. During the oil extraction process a fraction of these esters is transferred into the oil depending on the oil extraction system. So solvent extracted oils contain higher concentration of wax esters compared with cold pressed and centrifuged ones. The determination of waxes is important to evaluate the quality and genuineness of some vegetable oils [16,19].

### **2.3.10 Fatty acids**

Fatty acids differ from one another in the number of carbon atoms in the hydrocarbon chain, the degree of unsaturation and the relative positions of these double bonds in the various chains. The degree of saturation of fatty acids plays an important part in their definition. Fatty acids without any (C=C) double bonds are referred to as saturated. Unsaturated fatty acids can be grouped further into monounsaturated (only one double bond is present) and polyunsaturated (with two or more double bond). The *cis*, *trans*, and positional configurations of unsaturated fatty acids have different physical and physiological properties. *cis*-Isomers are the natural configuration, whereas the *trans*-isomers and positional isomers develop with processing or heating but predominately with selective hydrogenation. Trans fatty acids can possibly cause diseases such as coronary heart disease, allergies, cancer and diabetes.

Saturated fatty acids are chemically the least reactive and have a higher melting point than do corresponding fatty acids of the same chain length with one or more double bonds. Among saturated fatty acid found in vegetable oils are myristic acid, palmitic and stearic acid.



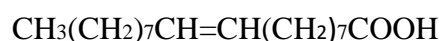
n = 12, Myristic acid.

n = 14, palmitic acid.

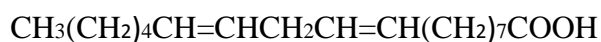
n = 16, stearic acid.

**Scheme 2.2:** the structure of saturated fatty acids

Unsaturated fatty acids include oleic acid, linoleic acid, linolenic acid and palmitoleic acid.



Oleic acid



Linoleic acid



Linolenic acid

**Scheme 2.3:** the structure of unsaturated fatty acids

These common fatty acids are easily recognized and separated by gas chromatography of their methyl esters, and this technique is a standard analytical procedure in quality-control laboratories [17,18,20].

## 2.4 Vegetable oils: properties and characteristics

The physical and chemical characteristics of oils are dependent upon degree of unsaturation and the length of the carbon chains. The fats which include large amount of saturated fatty acids are solid at room temperature whereas the ones which contain high amount of unsaturated fatty acids are liquid at room temperature. As the chain length of a saturated fatty acid increases the melting point also increases. Thus a short chain saturated fatty acid such as butyric acid has a lower melting point [13,14].

## 2.5 The chemical properties of oils

### 2.5.1 Iodine value (IV)

The iodine value is a measure of degree of unsaturation, which is the number of (C=C) double bonds in relation to the amount of fat or oil. IV is defined as the grams of iodine absorbed per 100 g of sample. The higher the amount of unsaturation the more iodine is absorbed and the higher the IV [21,22].

### 2.5.2 Saponification value (SV)

The saponification value is defined as the amount of alkali necessary to saponify a given quantity of fat or oil. It is expressed as the milligrams of KOH required to saponify 1g of the sample. The SV is an index of the mean molecular weight of the weight of the triacylglycerols in the sample [21].

### 2.5.3 Free fatty acids (FFA) and acid value

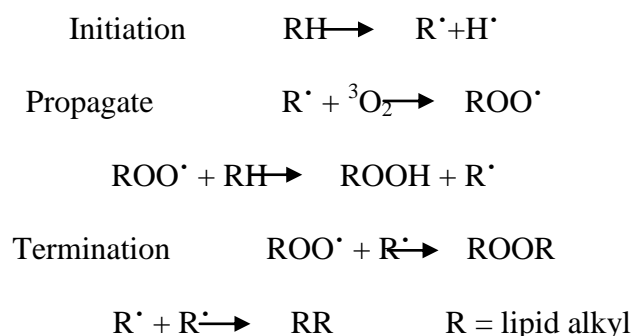
Free fatty acid is the percentage by weight of a specified fatty acid (e.g. percent oleic acid). Acid Value is defined as the mg of KOH necessary to neutralize the free acids present in 1g of fat or oil [21].

### 2.5.4 Peroxide value (PV)

The peroxide value is defined as the milli-equivalents of peroxide per kilogram of sample. It is a redox titrimetric determination. The assumption is made that the compounds reacting under the conditions of the test are peroxides or similar products of lipid oxidation [21].

## 2.6 Fats oxidations

The term rancidity refers to the off odors and flavors resulting from lipolysis (hydrolytic rancidity) or lipid oxidation (oxidative rancidity). Lipolysis is the hydrolysis of fatty acids from the glyceride molecule because of their volatility, hydrolysis of short chain fatty acids can result in off odors. Fatty acids shorter than C12 (lauric acid) can produce off-odors in foods. Lipid oxidation (also called autoxidation) as it occurs in bulk fats and oils proceeds via self-sustaining free radical mechanism [21,23-25].



**Scheme 2.5:** Mechanisms of Autoxidation in edible oils [23].

The oxidation process is affected by oxygen concentration present, heat, metals and presence of antioxidants. Oxidative deterioration is a chemical process that can be inhibited by removal of reactants (oxygen present in air), by removal of compounds and conditions that promote the reaction (light, metal, temperature) and by addition of compounds that slow down (inhibit) the oxidation process called antioxidant [15,23].

Edible oils naturally contain antioxidants such as tocopherols, tocotrienols, carotenoids, phenolic compounds and sterols. Antioxidants are sometimes intentionally added to oil to improve oxidative stability. Antioxidants can donate hydrogen atom to free radicals and convert them to more stable non-radical products [23].

## **2.7 Methods of extraction and processing**

Most oil seeds require some degree of cleaning and preparation before the oil is separated from the solid portion of the seed. Seeds are pre-treated in a series of processes involving cleaning, dehulling or decorticating, size-reduction if necessary, cooking at 90-115°C and flaking to about 0.3-0.4 mm thickness to rupture the cell. The most common methods for oil extraction include expeller or screw press extraction and solvent extraction [15,17].

### **2.7.1 Solvent extraction**

Organic solvents are used for the extraction of oils. The type of solvent and the actual method of lipid extraction depend on both the chemical nature of the sample and type of lipid extract desired. The most important characteristic of the ideal for lipid extraction is the high solubility of lipids coupled with low or no solubility of proteins, amino acids, and carbohydrates [26].

Glycolipid are soluble in alcohols and have a low solubility in hexane, in contrast triacylglycerols are soluble in hexane and petroleum ether, which are non-polar solvent [21]. The oil is generally extracted from crushed seeds by the soxhlet procedure using hexane or suitable hydrocarbon fraction such as that boiling between 40 and 60°C [15].

## **2.8 Seeds processing prior to oil extraction**

Seeds are treated by cooking (commonly roasting and boiling processes) to improve the quality and the amount of oils as well as the nutritive value of cake [27,28].

### **2.8.1 Roasting**

Roasting of seeds was reported by many authors to have positive effects on the quality of the extracted oils. However, in some cases no effects were noticed or even negative influences on the properties of the extracted oils were observed [23,28,29]. Roasting is achieved by heating seeds using various heating sources such as electric roasters (equipped with stirrers and temperature controllers), ovens, and microwaves for varying times and temperatures.

Anjum *et al.* [29] have studied the effects of microwave roasting on the physico-chemical composition and oxidative stability of sunflower seed oil. The results showed that roasting decreased the oil content and no change in the fiber, ash, and protein could be seen. The oil extracted from roasted seeds has shown an increase in density and a decrease in refractive index. The color of the oil was changed from light yellow (5 min of roasting), yellow (10 min of roasting) and brown (15 min of roasting). The free fatty acid contents were increased with increasing roasting time which might be attributed to hydrolysis of triacylglycerol to produce free fatty acid and diacylglycerol.

Mariod *et al.* [30] have studied the effects of roasting and boiling on the chemical composition and oil stability of safflower seeds. The seeds were roasted at 180 °C for 10, 20 and 30 min. In general, the results of peroxide value has displayed that roasting has accelerated the oxidation of oils. This was clearly noticed from study of oxidative stability of raw and roasted oils at 70°C for three days. In another study, Mariod *et al.* [31] have shown that roasting of roselle seeds by oven and microwave has increased fat and protein contents compared to untreated seeds. Additionally, it was found that fatty acids composition of roselle seeds did not change with roasting temperatures and the nutritional value of the seeds was increased.

### **2.8.2 Germination**

Germination does not have only one accepted definition and many definitions were proposed. It can be defined according to seed physiologists as the emergence of the radicle through the seed coat. In addition, seed analysts have defined germination as the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant [32].

Germination is simply carried out by soaking the clean seeds (In some cases, sterilization of the seeds using different methods such as ethanol, bromine water and hydrogen peroxide for short time was reported in the literature) in cold distilled water for varying time (12, 24, and 36 hours as reported in the literature). The soaked seeds are taken out and kept between moist filter papers, sheets of chromatographic paper or thick layers of cotton cloth in a dark place with regular irrigation till germination takes place at room temperature [33-36].

During germination storage triacylglycerols are cleaved by lipases from their glycerol backbone in the oil body, and convert to carbohydrates major as sucrose for transport to the root and shoot axes [37].

Obizoba and Egbuna [35] have studied the effect of germination on the nutritional quality of bambara nut. The results displayed that germination caused a decrease in the protein, carbohydrate and starch and it increases sugar content and had varied effect on the lipids contents of the dry samples. When compared to the ungerminated seeds, germination decreased total carbohydrate (CHO). Similarly, Akpapunam and Dedeh [36] have investigated the effect of germination on physicochemical and antinutritional factors of Jack bean (*Canavalia ensiformis*). They reported that germination decreased the protein, fat and starch contents of the bean. On the other hand, their results showed that germination has increased the ash and soluble sugars of sample.

Badifu [34] has studied the effect of germination of seeds of cucurbitaceae on the proximate, anti-nutritive, and toxic components of the kernels. It was found that the moisture was significantly increased whereas protein was slightly increased from 24 to 48 h germination period. However, there were gradual but insignificant decreases in protein content in subsequent germination periods of 72 and 96 h. There were no appreciable changes in fibre and ash contents and total phenols content was decreased.

In another study, Mariod *et al.* [38] have studied the effect of germination periods and conditions on chemical composition, fatty acids and amino acids of two black cumin seeds. The results were showed that the germination in general increased both oil and protein contents while other constituent were decreased. Additionally, germination at dark showed higher increase in oil content than at light.

## **2.9 Advantages of fixed oils**

Oils and fats are widely used in life. Edible oils and fats are an important source of energy for the human diet. Edible oils are use in salad, cooking, baking and frying. Some vegetable oils is used in animal feed and also used in foodstuffs industry. Non-edible oils are used in many applications such as inks, diesel fuel, paints, lubricants and soap manufacture [15,22].

## **2.10 *Moringa Peregrina* (Forssk) Fiori**

### **2.10.1 Taxonomical profile [39,40]**

<i>Kingdom:</i>	<i>plantae</i>
<i>Division</i>	<i>Embryophyta</i>
<i>Class:</i>	<i>Magnoliopsida</i>
<i>Order:</i>	<i>Brassicales</i>
<i>Family:</i>	<i>Moringaceae</i>
<i>Genus:</i>	<i>Moringa</i>

### **2.10.2 Description and distribution**

*Moringa Peregrina* is a genus of shrubs and trees up to 5 m high, branches whip-like with multi-purpose uses. It is distributed throughout many countries from tropical Africa to the East Indies and bordering the Red Sea to north Somalia and the Dead Sea area. It is distributed in several parts of Sudan which include Red sea hills, Blue Nile, Kordfan, Darfure and North of Sudan [1,41,42].

### **2.10.3 Uses**

The main product derived from *Moringa peregrina* is seed oil, called ‘ben oil’. The use of the oil goes back to antiquity and is already referred to in old Egyptian texts and the Bible. The oil is used for cooking, in cosmetics and in medicine. In Yemen the oil is used as a lubricant for small machinery. The seeds are also used as coagulant to purify water, e.g. in Sudan. *Moringa peregrina* is a bee plant and its leaves are used as fodder. The seeds are used in medicine in the Middle East and Sudan. The oil is used to treat abdominal pain. The tuber of the young plant is eaten in Yemen and Oman. The plant is grown as ornamental in Saudi Arabia and the Middle East. The wood is collected for fuel in the Southern Sinai, but it has now become scarce [3,7,43].

## **2.11 The chemical composition and physical properties of *Moringa Peregrina* seeds and oil**

The previous studies showed that the *Moringa Peregrina* composition from Saudi Arabia, Jordan and Iran appear similar results in proximate characteristics and physicochemical properties.

Somali *et al.* [44] investigated the chemical composition and characteristics of *Moringa Peregrina* seeds and seeds oil from Saudi Arabia. The proximate analysis of seeds showed that they composed of 1.8% moisture, 54.3% oil, 22.1% protein, 3.6% fiber, 15.3%

carbohydrate and 2.5% ash. The fatty acids composition of the oil shows the presence of 14.7% saturated fatty acid and 84.7% unsaturated fatty acid.

Tsaknis [45] has characterized *Moringa Peregrina* seed oil from Saudi Arabia. The oil content of seeds was 49.7%, acidity (as oleic) 0.3%, iodine value 69.6 g of I/100g of oil, saponification number 185 mg of KOH/g of oil, and peroxide value 0.4 meq/Kg. The physical properties showed that the refractive index at 40°C was 1.460, density at 24°C was 0.906 g/cm<sup>3</sup>.

AL-dabbas *et al.* [5] have studied the chemical composition and oil components in seeds of *Moringa Peregrina* from Jordan. The results showed the seeds contain 24.1% crude protein, 53.5% fat, 2.6% ash and 2.4% moisture. The mineral analysis indicated high potassium (630.2 mg/100g) and phosphorus (620.5 mg/100g) content, appreciable quantities of sodium, magnesium and calcium and low amount of iron. The seeds oil showed the presence of a high level of unsaturated fatty acids 83.5% whereas the saturated fatty acids were 16.53%.

Gharibzahedi *et al.* [6] have studied the physicochemical properties of *Moringa Peregrina* seeds and its oil from Iran. The proximate analysis of seeds showed the percentages were 4.4% moisture, 2.7% ash, 55.7% fat, 23.4% protein and 14.8% carbohydrate. The physical properties of oil seed display 0.9092 g/cm<sup>3</sup> and viscosity of 52.05 m Pa.s. The oil extracted from the studied seeds had a low acid value (0.06 % oleic acid). The peroxide value of the oils from samples ranged from 0.62 to 0.70 meqO<sub>2</sub>/Kg, saponification value (169.3 mg KOH/g) and iodine value (69.6 g of I/100g of oil). The mean values of mineral contents of the seeds showed that potassium (43.21 g/100g) was the most abundant element in these seeds, followed by phosphorus (541.0 mg/100g) and zinc (274.19 mg/100g). Comparing physical and chemical properties of *Moringa Peregrina* seed oil with those of other vegetable oils it is similar to olive oil.

## Chapter Three

### Materials and Method

#### 3.1 Sample collection and pretreatments

The *Moringa Peregrina* seeds were collected from Wadi Alkasinger in the Northern State of Sudan in February 2015. The seeds were cleaned from any extraneous materials and dehulled using a hammer. The kernels were stored at 10°C for next steps.

#### 3.2 Chemicals

n-Hexane (density = 0.66 g/mL at 20°C, b.p = 65-70°C, minimum assay = 95%), was purchased from SDFCL. Potassium hydroxide flakes (minimum assay 85%), was purchased from CDH Laboratory Reagent. Sodium Sulphate anhydrous (minimum assay = 99%), was purchased from Techno Pharmchem. Potassium iodide (assay = 99%), was purchased from SCP. Hydrochloric acid pure (wt per mL at 20°C about 1.18g), (assay 35-38%), purchased from Lobe Chemie. Sodium thiosulphate (minimum assay 99%), purchased from Alpha Chemika. Chloroform (density = 1.474-1.480 g/mL at 20°C, assay = 99.5%), was purchased from Alpha Chemika. Sodium hydroxide (assay 98%), purchased from Nice Laboratory reagent. Acetic acid glacial (b.p = 118°C, minimum assay = 99.7%), was purchased from Duksan Reagents. Starch. Ethanol absolute anhydrous (density=0.7892g/mL at 20°C, b.p=78.3-78.8°C), was purchased from CARLO ERBA Reagents. Nitric acid (minimum assay 69-72%, density = 1.41-1.42g/mL at 20 °C), was purchased from Alpha Chemika. Sulfuric acid (minimum assay 90%), was purchased from Loba Chemie. Copper sulfate pentahydrate (minimum assay = 98.5-101%), was purchased from Alpha Chemika.

#### 3.3 Roasting of the kernel seeds

Kernels were arranged as a single layer in an aluminium tray and placed in a roaster oven. The roasting process was carried out at 180°C for 25 minutes and finally the sample was allowed to cool to ambient temperature after and then stored at 10 °C.

#### 3.4 Germination of the seeds

2 Kg of *Moringa peregrina* seeds were thoroughly washed using a 5% solution of sodium chloride and the clean seeds were laid uniformly in a wetted-jute bag and covered with another wetted jute bag and finally with a thin layer of a soil. The contents were kept wet by regular irrigation with water (four to five times a day). Germination has taken five days to

occur and in some cases six to seven days. Germinated seeds were dried, dehulled and stored at 10°C for further analysis.

### **3.5 Proximate analysis**

#### **3.5.1 Determination of moisture content**

2g of sample were weighed and placed in a clean and dry porcelain crucible. The crucible with its content was weighed and transferred into an air oven at 105 °C and left for 18 hours. After completion of the specified time, it was removed from the oven and left to cool in a desiccator and reweighed again. The previous step was repeated till constant weight was obtained. The experiment was repeated three times and the mean and standard deviation were calculated. The moisture was calculated using the following equation:

$$\text{Moisture \%} = (W_1 - W_2) \times 100 / W_0 \quad (3.1)$$

Where  $W_1$  = the weight of sample and crucible before drying,  $W_2$  = the weight of sample and crucible after drying,  $W_0$  = Original weight of the sample.

#### **3.5.2 Determination of ash content**

2 g of the crushed sample were weighed in a clean and dry porcelain crucible. The crucible was placed in a furnace and heated at 550°C for 4 h, then it was left to cool in a desiccator. The crucible was weighed and reignited again for additional half an hour, removed, left to cool and reweighed. The previous steps were repeated several times till constant weight was attained. The experiment was repeated three times and the ash content (mean and standard deviation) was calculated using the following equation:

$$\text{Ash content (\%)} = 100 (A - B) / C \quad (3.2)$$

Where A is the weight of crucible with the ash (g), B is weight of the empty crucible and C is the weight of sample (g).

#### **3.5.3 Determination of fat**

50 g of the crushed sample were weighed and placed in a soxhlet extractor. The oil was extracted using n- hexane at 65°C for 5 hours. The solvent was recovered from the oil by rotary evaporator. The oil was placed in a beaker at ambient conditions for 24 hours until the odor of n-hexane was completely disappeared. The weight of the oil was taken and the result was recorded. The experiment was repeated three times and the mean and standard deviation were calculated. The percentage of the oil was calculated using the following equation:

$$\text{Oil content (\%)} = 100 (A/B) \quad (3.3)$$

Where A is weight of extracted oil and B is weight of the original sample.

#### **3.5.4 Determination of protein content**

0.2g of sample was weighed and placed in a digestion flask (Kjeldahl flask). 3.5 mL of a concentrated sulfuric acid and 0.4g of a mixture of (copper sulfate + sodium sulfate) were added. The flask was placed in a heating unit (digestion apparatus) and heated gently until frothing ceases. Then it was boiled briskly for 3 hours until the solution was clear.

The digested sample was placed in distillation unit and 20 mL of 40% sodium hydroxide was added. 10 mL of boric acid containing 3 drops of a mixture of methyl red and methylene blue indicator was placed in a conical flask. The flask was connected to the distillation bulb on a condenser and returned to heat source. The apparatus was continued working until the volume of solution was become 50mL and the colour in the conical flask was changed from red to greenish-blue. The solution (ammonium borate) was titrated against 0.02 N hydrochloric acid to the end point of the indicator (greenish-blue to red). The experiment was repeated three times and the mean and standard deviation were calculated. The crude protein was calculated using the following equation:

$$N \% = V \times 0.02 \times 14 \times 100/W_t \times 1000 \quad (3.4)$$

$$\text{Crude protein \%} = N \% \times 6.25 \quad (3.5)$$

Where N % = percentage of nitrogen, V = volume of hydrochloric acid, 0.02 = normality of hydrochloric acid, 14 = Nitrogen atomic weight, W<sub>t</sub> = weight of sample.

#### **3.5.5 Determination of total carbohydrate**

The total carbohydrate was calculated using the following equation:

$$\text{Total carbohydrate\%} = 100 - (\text{Ash \%} + \text{Moisture \%} + \text{Fat \%} + \text{Protein \%}) \quad (3.6)$$

### **3.6 Determination of physicochemical characteristics of the oil**

#### **3.6.1 Determination of acid value and free fatty acids**

3.5g of oil were placed into a 250 mL flask and 25 mL of alcohol (previously neutralized by adding 2 drops phenolphthalein solution and enough 0.1 N NaOH to produce faint permanent pink) was added to the flask. The contents were titrated against 0.25 N NaOH, with vigorous shaken until permanent faint pink color appears and persists  $\geq 1$  min. The experiment was

repeated three times and the mean and standard deviation were calculated and the acid value and free fatty acids were determined using the following equations:

$$\text{Acid value (AV)} = (V \times N \times 40) / S \quad (3.7)$$

$$\% \text{ Free fatty acid} = AV \times 0.503 \quad (3.8)$$

Where V is mL of NaOH required by sample, N is Normality of NaOH and S is the weight of the sample.

### 3.6.2 Determination of peroxide value

2.5g of oil were weighed into a 250 mL flask and 15 mL of a mixture of glacial acetic acid and chloroform (3:2) were added and stirred well for complete dissolution. 0.3 mL of saturated KI solution was added to the contents of the flask and shaken well for 1min. 15 mL of distilled water and 0.5 mL of 1% starch were added to flask and titrated against 0.001N  $\text{Na}_2\text{S}_2\text{O}_3$  with vigorous shaking until the blue colour disappears (addition of starch was done immediately because the color of the liberated iodine was already pale yellow color). The experiment was repeated three times and the mean and standard deviation were calculated and the peroxide value was determined using the following equation:

$$\text{Peroxide value (milliequiv peroxide/Kg sample)} = (V \times N \times 1000) / S \quad (3.9)$$

Where V is mL of  $\text{Na}_2\text{S}_2\text{O}_3$  required by the sample, N is normality of  $\text{Na}_2\text{S}_2\text{O}_3$  solution and S is weight of the sample.

### 3.6.3 Saponification value

2 g of the oil were weighed into a 250 mL conical flask and 25 mL of alcoholic potassium hydroxide solution (0.5 N) were added and the flask was connected with air condenser. Heating was conducted on a boiling water bath for 30 min with occasional shaking and after completion of the heating process the flask was left to cool and 3 drops of phenolphthalein indicator were added and titrated against a 0.5 N hydrochloric acid until the pink colour was disappeared. A blank solution was treated using the same procedure above but without oil. The experiment was repeated three times and the mean and standard deviation were calculated and the saponification value was determined using the following equation:

$$SV = 56.1 \times (B-S) \times N / \text{Wt of sample} \quad (3-10)$$

Where B is mL of HCl required by Blank, S is mL of HCl required by Sample and N is normality of  $\text{Na}_2\text{S}_2\text{O}_3$  solution.

### **3.7 Physical properties of the oil**

#### **3.7.1 Determination of relative density**

The relative density of the oil was determined using a clean, dried and pre-weighed empty pycnometer. The pycnometer was filled with distilled water and weighed again. Dry pycnometer was refilled by oil and weighed. The experiment was repeated three times and the results were recorded and the mean and standard deviation were calculated. Density was determined using the following equation:

$$\text{Relative density of oil} = (B - A)/(C - A) \quad (3-11)$$

Where A is weight of pycnometer, B is weight of pycnometer with sample and C is weight of pycnometer with water.

#### **3.7.2 Determination of Colour**

The colour of oil was measured using Lovibond Tintometer (TYPE4D model). The oil was filtered through a filter paper to remove any impurities and traces of moisture. The glass cell was cleaned, dried, filled with the oil and placed in the Tintometer. The color of the oil was matched with the standard slides red, yellow and blue colors. The results were recorded.

#### **3.7.3 Determination of viscosity**

The viscosity of the oil was measured using a Thermo Scientific HAAKE Viscotester 6 plus model. A rotor rotating at a constant speed (200 round /min) was immersed in 60 mL of oil to be tested. The viscosity was recorded.

#### **3.7.4 Determination of refractive index**

The refractive index was determined using a Abbe refractometer. A drop of oil was placed on a surface of the lower prism. The prisms were closed and the mirror and light were adjusted until a dark border line was observed on the cross wire. The refractive index was determined.

### **3.8 Fourier transform infrared spectroscopy (FT-IR)**

FT-IR spectrum of the oil was obtained using an IR 300 model spectrometer (Thermo Nicolet). A drop of oil was placed between a pair of salt plates. The pair of the plates were inserted into a holder that fits into Infrared spectrophotometer. The scanning was done in the range between 4000 and 500  $\text{cm}^{-1}$ . The number of scans was adjusted to 10 scans with resolution of 4  $\text{cm}^{-1}$ .

## Chapter Four

### Results and discussion

#### 4.1 Proximate composition of *Moringa peregrina* seeds

The results of the proximate composition of raw, roasted and germinated seeds of *Moringa peregrina* are shown in table 4.1.

**Table 4.1:** Shows the proximate compositions of raw and processed seeds

Component	Raw	Roasted	Germinated
Moisture %	2.90±0.20	3.32±0.12	3.90±0.02
Ash%	2.60±0.08	2.97±0.01	3.91±0.02
Protein %	30.16±0.41	29.72±0.43	27.25±0.44
Fat %	40.80±0.08	36.81±1.80	40.20±0.60
Total carbohydrate %	23.54	27.18	24.74

As can be noticed from table 4.1, both roasting and germination have affected all the analyzed parameters to varying degrees. In the case of roasting, the slight increase of moisture content, from 2.90 to 3.32%, probably due to formation of a hygroscopic compound as a result of heating the sample up to 180°C. Many articles [46,47] were reported the decrease in moisture content as a result of roasting, this is because roasting removes moisture, facilitates oil extraction from the intact cells and hence forms oily layer which internally covers the seeds components (gives hydrophobic character). This should lead to decrease in moisture content. On the other hand, germination has significantly increased the moisture content from 2.90% for raw sample to 3.90% for germinated sample. The results also shows that roasting process causes marginal decrease in protein content from 30.16% to 29.72% for raw and roasted seeds respectively. This reduction in the protein content of roasted seeds could be attributed to denaturation during roasting process at high temperatures [48]. In germinated seeds, the protein content decreases noticeably from 30.16% for raw seeds to 27.25% for germinated seeds. The decrease in protein may be due to utilization of protein for growth process. Protein is one of the major sources of energy for the developing embryo. Proteins are hydrolyzed to form simple peptides and amino acids due to protease activity and are then transported to the developing axis [35]. Furthermore, compared with raw seeds, the germinated seeds showed slight increase in total carbohydrate from 23.54% to 24.74% whereas roasting increases the total carbohydrate from 23.54% to 27.18%. It is important to mention that the total carbohydrate was calculated by the percentage difference. The results of the ash content (Table 4.1) reveal that roasting slightly increases the ash content from 2.60 to 2.97%, whereas

germination has significantly increased the ash content from 2.60 to 3.91%. The increase of the ash content for the germinated seeds could have come from the tap water used for steeping and rinsing seeds during germination [49]. The oil content, on the other hand, does not change as a result of germination, whereas roasting has led to observable decrease, from 40.80 to 36.81%, in the oil content. In case of germination, it has been reported in the literature [35] that in the early stages of seed development, lipids are virtually devoid of triglycerides. Seeds develop with rapid increase in the synthesis of the triglycerides. In contrast to the previous observations, a decrease in triglycerides during germination was reported in the literature [35]. This was attributed to degradation of lipids during germination to supply energy which is crucial for the growth of the plant. The noticeable decrease in oil content due to roasting was also reported by other studies. Furthermore, it was observed in some cases that roasting did not change the oil content [31,36,48]. This was explained by the decomposition of the triglycerides at high temperatures during roasting.

#### 4.2 physical properties of oil seeds of *Moringa peregrina*

The results of the physical properties of raw, roasted and germinated oil seeds of *Moringa peregrina* are shown in table 4.2. As can be seen from the table, the roasting and the germination of the seeds have significant effects on the color and the viscosity of the extracted oils whereas marginal changes in the density and the refractive index were noticed.

**Table 4.2: Represents the physical characteristics of the raw, roasted and germinated oils**

Physical properties	Oil from raw seeds	Oil from roasted seeds	Oil from germinated seeds
Relative density	0.9069±0.002	0.9008±0.003	0.9114±0.0002
Refractive index	1.465	1.468	1.467
Colour	12.2y, 0.1r	23.2y, 0.3r	32.3y, 1.3r
Viscosity (Cp)	60.5±2.5	50±0.00	70.5±0.5

The intensity of the color is higher for the oil from the germinated seeds (32.3y, 1.3r) followed by the oil from the roasted seeds (23.2y, 0.3r) and finally the oil from the raw ones (12.2y, 0.1r). The change in the color of the oil extracted from the germinated seeds could be due to the increase in the content of chlorophylls and carotenoids which are synthesized during the early stages of germination, after approximately 2 to 3 days, and increases rapidly through the 6<sup>th</sup> day after which there is a decline [50]. In the case of roasting, the increase in the intensity of the oil's color may be due to formation of browning substances during roasting of samples causes by maillard-type non-enzymatic reactions between reducing sugars and free amino acids or amides [51].

On the other hand, the significant variations in viscosities between the three samples could be attributed to the fact that viscosity depends on the fatty acid composition and it decreases with the increase of unsaturation of the fatty acids and increases with increasing saturation [15].

#### 4.3 Chemical properties of seeds oil of *Moringa peregrina*

Table 4.3 shows the chemical characteristics of the oils from raw, roasted, and germinated seeds. As it obvious from the table, germination has considerably changed the chemical characteristics of the oil compared to unprocessed sample. On the other hand, compared to unprocessed sample, roasting has caused noticeable changes in the chemical characteristics of the oil.

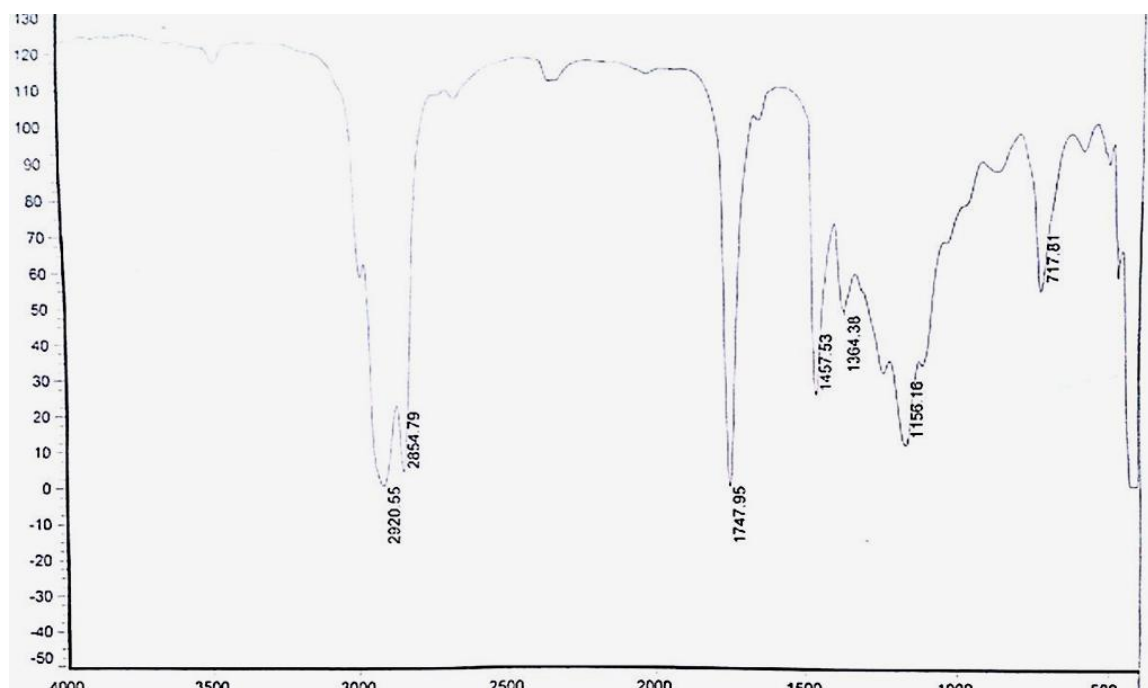
**Table 4.3: Represents the chemical characteristics of oils**

Chemical properties	Oil from raw seeds	Oil from roasted seeds	Oil from germinated seeds
PV (meqO <sub>2</sub> /Kg oil)	0.57±0.04	0.95±0.02	-
AV (mgKOH/g)	0.68±0.04	0.25±0.02	20.03±0.13
%FFA	0.34±0.02	0.12±0.01	10.08±0.07
SV (mg KOH/g oil)	185.40±5.95	174.18±2.7	180.04±5.36

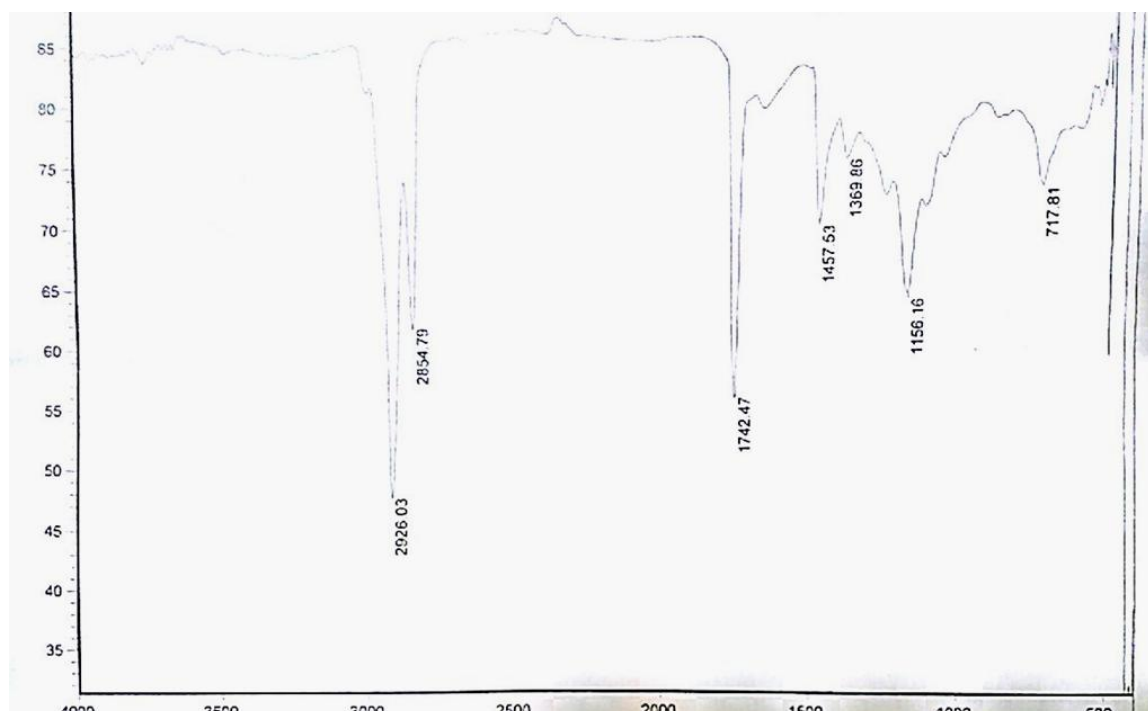
As can be seen from the table, the peroxide value (PV) is slightly increased from 0.57 for the raw sample to 0.95 meqO<sub>2</sub>/Kg oil for the roasted sample whereas nil result was obtained for peroxide value of the oil from the germinated seeds. The increase of tocopherol content during germination was taken as the main reason for inhibition of oil oxidation [52]. Furthermore, roasting has decreased the acid value (AV) and free fatty (FFA) acid from 0.68 mg KOH/g and 0.34% for the raw sample to 0.25 mg KOH/g and 0.12% for the roasted one. On the other hand, germination has drastically increased the acid value and free fatty acids from 0.68 mg KOH/g and 0.34% for the raw sample to 20.03 mg KOH/g and 10.08% for the germinated one. The higher percentage of FFA and acid value for the oil extracted from germinated seeds could be due to the initial hydrolysis (lipolysis) of triacylglycerols by lipases, enzymes that catalyze the three-stage hydrolytic cleavage of the fatty acid ester bonds in triacylglycerols (TAGs), ultimately to yield glycerol and free fatty acids (FFA) [53]. The results have shown that the saponification value (SP) of the raw, roasting, and germination oils were 185.40, 174.18 and 180.04 mg KOH/g oil respectively.

#### 4.4 FT-IR analysis

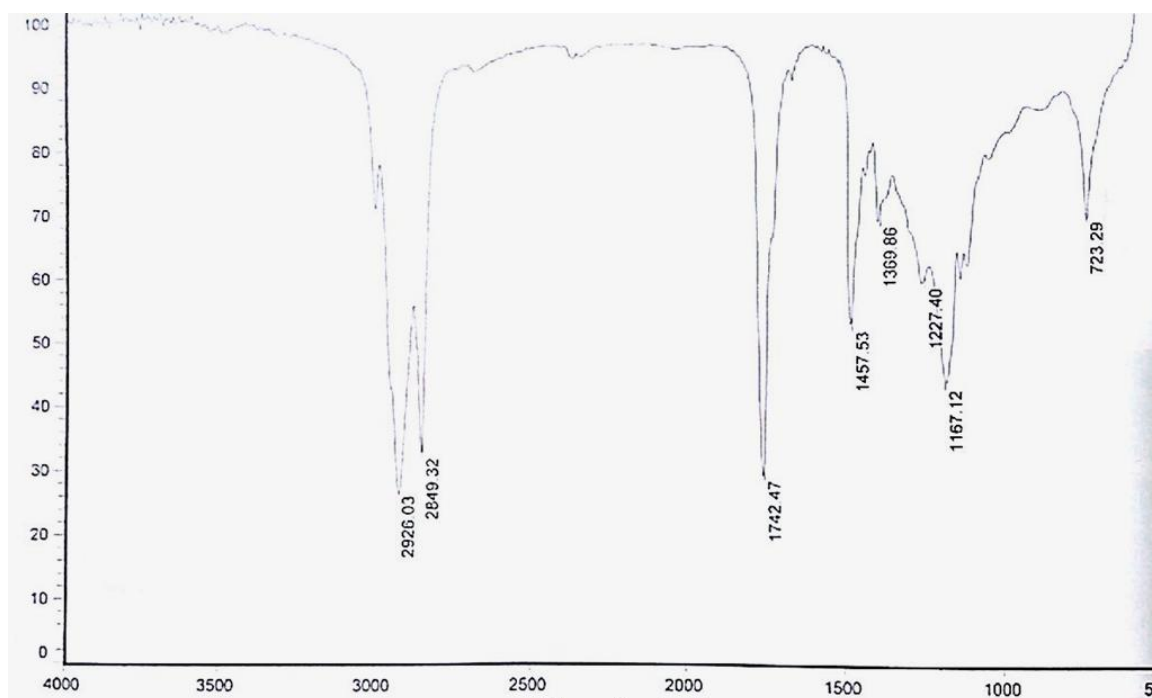
The Fourier transform infrared analysis was used to investigate the structural characteristics of the raw sample and the possible changes in the structural characteristics as a result of processing.



**Figure 4.1:** IR spectrum of *Moringa peregrina* seeds oil



**Figure 4.2:** IR spectrum of *Moringa Peregrina* roasted seeds oil



**Figure 4.3:** IR spectrum of *Moringa Peregrina* germination seeds oil

The characteristics absorption peaks of the raw sample together with their wave numbers are given in table 4.4.

**Table 4.4:** Displays the FT-IR's wavenumber and the functional group which give rise to absorption band for the raw sample (Figure 4.1)

Wavenumber	functional group
About 3006 $\text{cm}^{-1}$	-CH stretching vibration of the <i>cis</i> -double bond (-CH)
2920 $\text{cm}^{-1}$ , 2854 $\text{cm}^{-1}$	C-H asymmetric and symmetric stretching vibrations of the aliphatic $\text{CH}_3$
1747 $\text{cm}^{-1}$	double bond stretching of carbonyl group C= O of ester of the triglycerides
1457 $\text{cm}^{-1}$ , 1364 $\text{cm}^{-1}$	C-H bending vibrations of the $\text{CH}_2$ and $\text{CH}_3$ aliphatic groups
1156 $\text{cm}^{-1}$	Stretching vibration of the C-O ester Group

Careful inspection of FT-IR spectra of the roasted and germinated samples in Figures 4.2 and 4.3 reveal that there are no significant differences in the absorption peaks between these samples and the spectrum of the raw one. Hence processing of the samples does not lead to appreciable changes in the structural characteristics of the oils. However, this does not seem reasonable because the results of the chemical properties have shown significant increase in

the percentage of the free fatty acids which should give rise to characteristics absorption band of the carbonyl group of the acids.

## Conclusion

In this study, the results have shown that both roasting and germination have significantly affected the chemical compositions of the kernels and the physicochemical properties of the extracted oils from seeds of *Moringa peregrina*. Roasting changes ash, moisture, and oil contents to varying degrees while germination has lead to similar variations except in the case of the oil content. The physical characteristics of the extracted oils have shown significant changes in the colors as well as the viscosities of the processed oils. On the other hand, the chemical properties have revealed that only the acid and the peroxide values have changed observably due to processing.

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## Appendix



**Figure A.1:** *Moringa peregrina* seeds



**Figure A.2:** kernels of *Moringa peregrina* seeds



**Figure A.3: Roasted kernels**



**Figure A.4: Germinated seeds**



**Oil from raw seed**



**Oil from roasted seeds**



**Oil from germinated seeds**

**Figure A.5: Oil from raw, roasted and germinated seeds**