Introduction

1.1 Oil

An oil is any neutral, nonpolarchemical substance that is a viscousliquid at ambient temperatures and is both hydrophobic (immiscible with water, literally "water fearing") and lipophilic (miscible with other oils, literally "fat loving"). Oils have a high carbon and hydrogen content and are usually flammable and slippery.

The general definition of oil includes classes of chemical compounds that may be otherwise unrelated in structure, properties, and uses. Oils may be animal, vegetable, or petrochemical in origin, and may be volatile or non-volatile.[1] They are used for food, fuel, lubrication, and the manufacture of paints, plastics, and other materials. Specially prepared oils are used in some religious ceremonies as purifying agents.

1.1.1 Types

1.1.1.1 Organic oils:

Organic oils are produced in remarkable diversity by plants, animals, and other organisms through natural metabolic processes. *Lipid* is the scientific term for the fatty acids, steroids and similar chemicals often found in the oils produced by living things, while oil refers to an overall mixture of chemicals. Organic oils may also contain chemicals other than lipids, including proteins, waxes (class of compounds with oil-like properties that are solid at common temperatures) and alkaloids.

Lipids can be classified by the way that they are made by an organism, their chemical structure and their limited solubility in water compared to oils. They have a high carbon and hydrogen content and are considerably lacking in oxygen compared to other organic compounds and minerals; they tend to be relatively nonpolar molecules, but may include both polar and nonpolar regions as in the case of phospholipids and steroids.[2]

1.1.1.2 Mineral oils:

Crude oil, or petroleum, and its refined components, collectively termed *petrochemicals*, are crucial resources in the modern economy. Crude oil originates from ancient fossilizedorganic materials, such as zooplankton and algae, which geochemical processes convert into oil.[3] The name "mineral oil" is a misnomer, in that minerals are not the source of the oil ancient plants and animals are, Mineral oil is organic. However, it is classified as "mineral oil" instead of as "organic oil" because its organic origin is remote (and was unknown at the time of its discovery), and because it is obtained in the vicinity of rocks, underground traps, and sands. *Mineral oil* also refers to several specific distillates of crude oil.

1.1.2 Oils Properties:

1.1.2.1 Free Fatty Acid:

Fatty acids, esterified to glycerol, are the main constituents of oils and fats. The industrial exploitation of oils and fats, both for food and oleochemical products, is based on chemical modification of both the carboxyl and unsaturated groups present in fatty acids.

1.1.2.2 Saponification value and iodine value:

Oils and fats are now characterized mainly by their fatty acid composition determined by gas chromatography, replacing the titrimetric and gravimetric assays used previously. However, the saponification value (SV) or equivalent (SE) and iodine value (IV) are still used in specifications and to monitor processes. SE, expressed as grams of fat saponified by one mole of potassium hydroxide, is an indication of the average molecular weight and hence chain length, whereas the IV, expressed as the weight percent of iodine consumed by the fat in a reaction with iodine monochloride, is an index of unsaturation. Standard analytical methods are available, but these parameters are now often calculated from the fatty acid composition, assuming that the sample is all triacylglycerol. Indirect measurements of IV and SV using FTIR spectroscopy have been developed for real-time process monitoring.[4]

1.1.2.3 Acid value:

The acid value is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. [5]

1.1.2.4 Peroxide number:

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. The double bonds found in fats and oils.

The double bonds found in fats and oils play a role in autoxidation oil with high degree of instauration are most susceptible to autoxidation. The best rest for autoxidation of the peroxide value.[6]

1.1.3 Applications

1.1.3.1 Cooking:

Several edible vegetable and animal oils, and also fats, are used for various purposes in cooking and food preparation. In particular, many foods are fried in oil much hotter than boiling water. Oils are also used for flavoring and for modifying the texture of foods (e.g. Stir Fry).

Cooking oils are derived either from animal fat, as butter, lard and other types, or plant oils from the olive, maize, sunflower and many other species.

1.1.3.2 Cosmetics:

Oils are applied to hair to give it a lustrous look, to prevent tangles and roughness and to stabilize the hair to promote growth. See hair conditioner.

1.1.3.3 Cultural uses:

Oil has been used throughout history as a religious medium. It is often considered a spiritually purifying agent and is used for anointing purposes. As a particular example, holy anointing oil has been an important ritual liquid for Judaism and Christianity.

1.1.3.4 Painting:

Color pigments are easily suspended in oil, making it suitable as a supporting medium for paints. The oldest known extant oil paintings date from 650 AD.[7]

1.1.3.5 Heat transfer:

Oils are used as coolants in oil cooling, for instance in electric transformers. Heat transfer oils are used both as coolants, for heating (e.g. in oil heaters) and in other applications of heat transfer.

1.1.3.6 Lubrication:

Given that they are non-polar, oils do not easily adhere to other substances. This makes them useful as lubricants for various engineering purposes. Mineral oils are more commonly used as machine lubricants than biological oils are. Whale oil is preferred for lubricating clocks, because it does not evaporate, leaving dust, although its use was banned in 1980.[8]

It is a long-running myth that spermaceti from whales has still been used in NASA projects such as the Hubble Telescope and the Voyager probe because of its extremely low freezing temperature. Spermaceti is not actually oil, but a mixture mostly of wax esters, and there is no evidence that NASA has used whale oil.[9]

1.1.3.7 Fuel:

Some oils burn in liquid or aerosol form, generating light, and heat which can be used directly or converted into other forms of energy such as electricity or mechanical work. To obtain many fuel oils, crude oil is pumped from the ground and is shipped via oil tanker or a pipeline to an oil refinery. There, it is converted

from crude oil to diesel fuel (petro diesel), ethane (and other short-chain alkanes), fuel oils (heaviest of commercial fuels, used in ships/furnaces), gasoline (petrol), jet fuel, kerosene, benzene (historically), and liquefied petroleum gas. A 42-gallon barrel (U.S.) of crude oil produces approximately 10 gallons of diesel, 4 gallons of jet fuel, 19 gallons of gasoline, 7 gallons of other products, 3 gallons split between heavy fuel oil and liquefied petroleum gases, and 2 gallons of heating oil. The total production of a barrel of crude into various products results in an increase to 45 gallons. Not all oils used as fuels are mineral oils, see biodiesel and vegetable oil fuel. [10]

In the 18th and 19th centuries, whale oil was commonly used for lamps, which was replaced with natural gas and then electricity. [11]

1.2 Biodiesel

Biodiesel is an alternative to petroleum-based fuels derived from vegetable oils, animal fats, and used waste cooking oil including triglycerides. Since the petroleum crises in 1970s, the rapidly increasing prices and uncertainties concerning petroleum availability, a growing concern of the environment and the effect of greenhouse gases during the last decades, has revived more and more interests in the use of vegetable oils as a substitute of fossil fuel. [12]

The production and use of biodiesel creates 78% less carbon dioxide emissions than conventional diesel fuel. Carbon dioxide is a greenhouse gas that contributes to global warming by preventing some of the sun's radiation from escaping the Earth. Burning biodiesel fuel also effectively eliminates sulfur oxide and sulfate emissions, which are major contributors to acid rain. That's because, unlike

petroleum-based diesel fuel, biodiesel is free of sulfur impurities. Combustion of biodiesel additionally provides a 56% reduction in hydrocarbon emissions and yield significant reductions in carbon monoxide and soot particles compared to petroleum-based diesel fuel. Also, biodiesel can reduce the carcinogenic properties of diesel fuel by 94%. [13]

Biodiesel in its pure form is known as “neat biodiesel” or B100, but it can also be blended with conventional diesel, most commonly as B5 (5 percent biodiesel and 95 percent diesel) and B20 (20 percent biodiesel and 80 percent diesel). Biodiesel is registered with the U.S. Environment Protection Agency (EPA) and is legal for use at any blend level in both highway and non-road diesel vehicles.

Most diesel engines can run on biodiesel without needing any special equipment. If it is interested to use biodiesel in a vehicle or equipment, manufacturer recommendations and information regarding engine warranties must be consulted. In addition, once proper blend the vehicle has been determined, fuel must be purchase from a reputable dealer selling commercial grade biodiesel. [14]

1.2.1 Advantages

- B100can be produced from renewable, domestic resources.
- B100 is energy efficient. (The total fossil fuel energy efficiency of biodiesel is 320% vs. 83% for petroleum diesel) (National biodiesel board, 1998)
- B100 can be used directly in most diesel engine applications.
- B100 can reduce global warming and tailpipe emissions (-41%) (Hill, Nelson, Tilman, Polasky, &Tiffany, 2006).

- B100 is nontoxic and biodegradable.
- B100 is a good solvent and may clean out fuel line and tank sediments. (Note that this may result in fuel filter clogging during initial use.)

1.2.2 Limitations

- B100 contains approximately 8% less energy per gallon.
- B100 generally has a higher cloud and pour point (will freeze at a higher temp) than conventional diesel.
- B100 is not compatible with some hose and gasket materials, which may cause them to soften, degrade, and rupture.
- B100 is not compatible with some metals and plastics.
- B100 may increase nitrogen oxide emission.

The most common method used to overcome the limitations of B100 is called "blending". Here biodiesel is mixed with petroleum diesel in varying to proportion starting from 5% and reaching 20% mixture. [15]

1.2.3 Making Biodiesel: Transesterification

Transesterification of natural glycerides with methanol to methyl esters is a technically important reaction that has been used extensively in the soap and detergent manufacturing industry worldwide for many years. Almost all biodiesel is produced in a similar chemical process using base catalyzed transesterification as it is the most economical process, requiring only low temperatures and pressures while producing a 98% conversion yield. The transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and

glycerol. A triglyceride has a glycerin molecule as its base with three long chain fatty acids attached. The characteristics of the fat are determined by the nature of the fatty acids attached to the glycerin. The nature of the fatty acids can, in turn, affect the characteristics of the biodiesel.

During the esterificiation process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkaline like sodium hydroxide. The alcohol reacts with the fatty acids to form the mono-alkyl ester, or biodiesel, and crude glycerol. In most production, methanol or ethanol is the alcohol used (methanol produces methyl esters, ethanol produces ethyl esters) and is base catalyzed by either potassium or sodium hydroxide. Potassium hydroxide has been found more suitable for the ethyl ester biodiesel production, but either base can be used for methyl ester production.

The figure below shows the chemical process for methyl ester biodiesel. The reaction between the fat or oil and the alcohol is a reversible reaction, so the alcohol must be added in excess to drive the reaction towards the right and ensure complete conversion.[16]

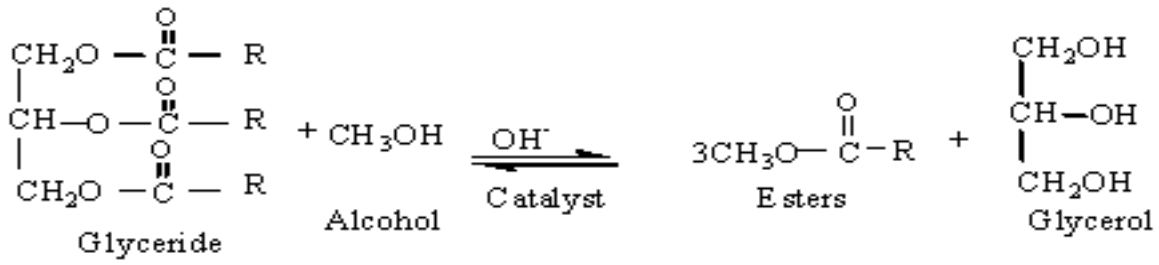


Figure 1: Transesterification reaction

High Free Fatty Acid oil

In the case of using oil with high free fatty acid as a feedstock, free fatty acids (FFA's) may pose a problem. A free fatty acid is one that has already separated from the glycerol molecule. This is usually the result of the oil breaking down after many cycles of use. FFA's create major problems.

- More catalyst will need to be used leading to higher cost.

- Soap (fatty acid salt) is formed, making washing the finished product more difficult.

- Water is formed which will retard the main reaction

- The FFA's are not converted into fuel, reducing the yield.[17]

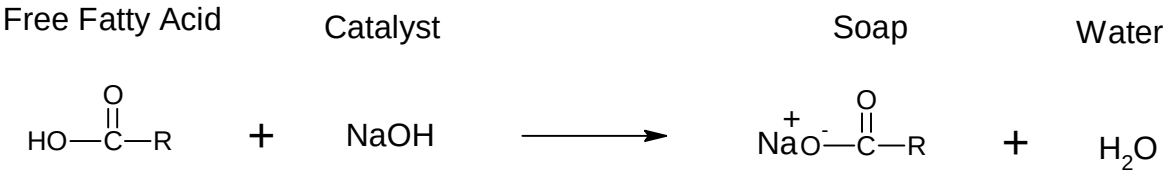


Figure 2: shows the reaction of FFA's and the catalyst NaOH.

1.3 Methods of extraction:

Several approaches can be employed to extract the plant material.

Although water is used as an extractant in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various solubility's of plant constituents.

1.3.1 Maceration:

This simple widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container .simple maceration is performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio : 1:5 or 1:10) and leaving the mixture for several days with occasional shaking or stirring. The extract is then repeated from the plant particles by straining . The process is repeated for once or twice with fresh solvent .Finally the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge.kinetic macerationdiffere from simple one by continous stirring.[18]

- The method is suitable for both initial and bulk extraction.
- The main disadvantage of maceration is that the process can be quite time-consuming, taking from a few hours up to several weeks.

1.3.2 Ultrasound-assisted solvent extraction:

This is a modified maceration method where the extraction is facilitated by the use of ultrasound . The plant powder is placed in a vial. The vial is placed in an ultrasonic bath, and ultrasound is used to induce a mechanical stress on the cells through the production of cavitations in the sample. The cellular breakdown increases the solubilization of metabolites in the solvent and improves extractionyields.[19]

- it is mostly used for the initial extraction of a small amount of material.

1.3.3 Percolation:

the powdered plant material is soaked initially in a solventin a percolator.

Additional solvent is then poured on top of the plantmaterial and allowed to percolate slowly (dropwise) out of the bottom of the percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator.[20]

- Percolation is adequate for both initial and large-scale extraction.
- The main disadvantages are:
  - fine powders and materials such as resins and plants that swell excessively (e.g., those containing mucilages) can clog the percolator.
  - if the material is not distributed homogenously in the container, the solvent may not reach all areasand the extraction will be incomplete.

1.3.4 pressurized solvent extraction:

The powdered plant material is loaded into an extraction cell, which is placed in an oven.

The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time.

The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilize the remaining components. A final purge with nitrogen gas is performed to dry the material.[21]

- offers a more economical and environment-friendly alternative to conventional approaches.

1.3.5 Extraction under reflux and steam distillation:

plant material is immersed in a solvent in a round-bottomed flask, which is connected to a condenser. The solvent is heated until it reaches its boiling point. As the vapor is condensed, the solvent

is recycled to the flask.

- It is commonly applied to the extraction of plant essential oils.
- The main disadvantage is that thermolabile components risk being degraded.[22]

1.3.6 Extraction with supercritical fluids:

Supercritical fluids (SCFs) are increasingly replacing organic solvents,

e.g., n-hexane, dichloromethane, chloroform, and so on, that

are conventionally used in industrial extraction operations because of regulatory and environmental pressures on hydrocarbon and ozone-depleting emissions.

Most of the currently available Solvent FreeExtraction systems utilize CO_2, which is generally considered as safe for solvent-free extraction processes. The fundamental steps involved in SFE are as follows:

1- liquid CO_2 is forced into supercritical state by regulating its temperature and pressure.

2- Supercritical CO_2 has solvent power and extracts predominantly lipophilic and volatile compounds.

3- Gaseous CO_2 returns to CO_2 tank. After a full round, the new extraction starts with circulating CO_2 .[23]

1.3.7 Countercurrent extraction:

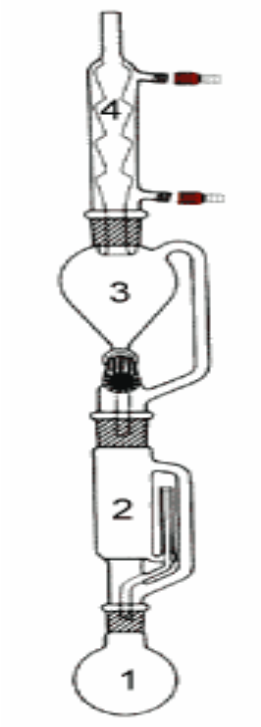
This is a continuous process in which the plant material moves against the solvent. It is suitable procedure for production of large amounts of extracts on an industrial scale. Several types of extractors are available .In the screw extractor the plant material is transported by a screw through a tube and meets the solvent which is pumped in the opposite direction. [24]

1.3.8 Soxhlet extraction:

In this method the sample is dried, ground into small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber (2), which is suspended above a flask containing the solvent (1) and below a condenser (4). The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask containing the solvent and lipid is removed. In some device a funnel (3) allows to recover the solvent at the end of the extraction after closing a stopcock between the funnel and the extraction chamber. The solvent in the flask (1) is then evaporated and the mass of the remaining lipid is measured. The percentage of lipid in the initial sample can then be calculated.

Despite disadvantages of this procedure (poor extraction of polar lipids, long time involved, large volumes of solvents, hazards of boiling solvents), several methods involving automatic solvent extraction were described. [25]

- The main advantage of Soxhlet extraction is that it is a continuous process.



Firgure 3: Soxhlet[26]

1.4 *BalanitesAcacia Seyal*

*Acacia Seyal*Delile (family Leguminosae, subfamily Mimosoideae) is one of over 60 African acacias referred to die Uniseriae group of subgenus *Acacia*. The species usually reaches 9-10 m in height at maturity and in well-formed individuals a flat-topped crown develops. There are two varieties, differing primarily in whether or not pseudo-galls ("ant galls") develop and in bark color. In var. *seyal* there are no

pseudo-galls and a reddish bark color prevails, although periodic bark exfoliation exposes a pale powdery surface which darkens slowly. In var. fistula pseudo-galls are present and the powdery bark typically remains whitish or greenish-yellow. Both varieties have paired, straight, strong, pale-colored, Stipular spines up to 8 cm long which in var. fistula are often fused at the base into the inflated pseudo-gall. The leaves are bipinnate - usually with 4-8 pairs of pinnae, each of which bears 10-20 pairs of close-set, obscurely veined leaflets. Individual leaflets are 1-15mm wide and about 5-8mm long. Small bundles of up to 5 pedunculatecapitates inflorescences arise in axillary positions on the young parts of shoots. Each inflorescence is bright yellow in color, about 15 mm in diameter, and is borne on a peduncle 34 mm long. The dehiscent pods are flat and somewhat curved, brown and up to about 20 cm long and 5-10 mm wide when ripe, with slight constrictions between the seeds. In a well-developed pod 6-10 seeds are present, each 6-9 mm long, 4-5 mm wide and about 2 mm thick - in 1 kg there are 20,000-25,000 seeds. The chromosome number of 2n = 52 suggests tetraploidy. [27]

1.4.1 Taxonomy and nomenclature

Family: Fabaceae (Mimosoideae)

Synonyms: A. stenocarpa A. Rich.; A. hockii De Wild.

Vernacular/common names:*thal*, *white thorn*, *whistling thorn* (Eng.); *soffa*(Arabic); *epineux, seyal* (French); *bulki (Fula); fullai* (Somali); *dushekerafi* (Hausa); *mgunga*(Swahili).

Two varieties are recognised, var. *seyal* and var. *fistula*.

1.4.2 Distribution and habitat

Widespread in the semi-arid zone of tropical Africa from Senegal eastwards to Somalia and the Red Sea, and from the Nile valley south to Zambia. The range of the two varieties is quite distinct, var. seyal extending north- and westwards from central Sudan, var. fistula south of latitude 10°S. Normally found in areas with 500-1200 mm rain/yr. and a distinct dry season. The upper elevation limit is about 2000 m. Lowest temperature within its natural range is 5-10°C occasionally below 5°C at high altitudes but the pattern of distribution indicates a frost-sensitive species. Grows well on deep, heavy soils with high pH (6-8) and especially var. fistula is tolerant to waterlogging.

1.4.3 Uses

Fuel :*Var.seyal*, especially, is an important source of rural energy as both fuel wood and charcoal. Stands managed on a 10-15 year rotation yield 10-35 m3 ha-1 of fuel wood.

Fodder: Both varieties of A. seyal are viewed favorable as forage. Dry matter net energy contents are high: 6-8 MJ kg-1 (foliage) and 4-7 MJ kg-1 (fruits). The associated digestible protein levels are also high: 100-150 g kg-1 in the foliage, and higher in the fruits. For both foliage and fruits, analyses indicate a well-balanced supply of minerals and very favorable qualities in terms of proximate fractions (e.g. crude fiber 10-20%-, ether extract <7%). The foliage of var. *seyal* has been shown to contain secondary metabolites but experience suggests that levels are not a matter of serious concern.

Gum *talha* has not been toxicologically evaluated and is not listed as an approved food additive. It contrasts with gum arabic in several significant respects, being strongly dexatorotatory, of high molecular weight and low in nitrogen (0.06-0.24%) and rhamnose (<4% sugar composition). Ash contents of cobalt, copper, iron, nickel and, especially, aluminum (>6000 ppm) are high and tannin is present (2%), restricting acceptable use to such applications as a binder for foundry molding and a sizing agent in the texile industry. [28]

1.4.4 Botanical description

Variable species. Tree up to 9 m tall, sometimes reaching 17 m, with a flattened, spreading crown. The slash is bright red, exuding yellowish gum. In var. fistula the powdery bark is normally white or greenish-yellow while var. seyal has reddish bark.

Both varieties have straight thorns in pairs, up to 8 cm long but only var. fistula bears whistling thorns (antgalls) that are fused at the base. Leaves are bipinnate with 2-12 pairs of pinnae each with 10-22 pairs of leaflets. Flowers bright yellow, in large round heads 2-3 flower heads together in the leaf axils.[29]

Figure 4: *Acacia Seyal trees*

Figure 5: *Acacia seyal leaves*

1.4.5 Fruit and seed description. [30]

Fruit: the dehiscent pods are light brown, slightly curved, 7-22 cm long, with fine longitudinal veins and slightly constricted between the seeds. There are 6-10 seeds/pod.

Seed: light brown, 6-9 mm long, 4-5 mm wide. There are 20,000-25,000 seeds/kg.

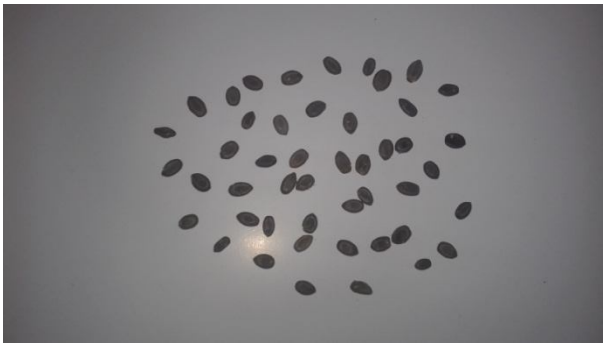


Figure 6: *Acacia Sayal* seeds

1.4.6 Flowering and fruiting habit

Flowering is concentrated in the middle of the dry season and ripe fruits are available about 4 months later.

1.4.7 Harvest

Full-sized pods are harvested from the tree before they open.

1.4.8 Processing and handling

The pods are dried in the sun until they open and the seeds are released.

1.4.9 Storage and viability

Seeds are orthodox and can be stored for several years if they are well dried and kept cool and free of insects. Moisture content for storage should be 4. 59%. [31]

1.4.10 Dormancy and pretreatment

There are various reports on the need for pretreatment, possibly due to differences in provenance. Most agree, however, that pretreatment accelerates the germination rate. The most common method is scarification.

1.4.11 Sowing and germination

Germination is rather slow, normally about 30% in 7 days. The seeds can be pregerminated on moist filter paper to allow rapid identification of viable, non-dormant seed. The germinated seeds are transferred to containers filled with a silt-rich medium. Seedlings require shade until the second leaf expands and watering at intervals of 1-3 days as necessary to keep the medium moist but not waterlogged. Direct seeding in prepared planting spots is practised with success in

Sudan; at early stages of development *A. Seyal* was capable of competing with weeds. Nodulation occurs in natural populations. In artificial regeneration it has been achieved by pelleting seed with culture of bacterial isolates, sowing into an infected medium or germinating in unsterilized soil. Uninfected seedlings have been inoculated successfully by treatment with a suspension of a symbiont. Rhizobium strains from *A. mellifera* and *A. Senegal* and Bradyrhizobium from the latter have proved to be effective symbionts. [32]

1.4.12 Phytosanitary problems

Over 40 species of insects are reported associated with *A. Seyal*. These include 10 species of bruchid beetles, which may damage large proportions of stored seeds. [33]

1.5 Objective of this study

This study aim to:

- Extraction of *Acacia Seyal* seeds oil using solvent extraction techniques.
- Test some of physicochemical properties of oil such as free fatty acid content, density, color, moisture content and refractive index.
- Test the Acid value, Iodine value, Saponification value and ester value
- Subject the oil to spectroscopic analysis (IR and GC analysis).

Materials and methods

2.1 Materials

2.1.1 Sample

Fruits of *Acacia Seyal* were collected from Alkawwa. The seed kernels were released and crushed, it was 2500g.

2.2 Methods

2.2.1 Oil extraction

The ground seeds kernels was Soxhlet extracted with normal hexane for a period of 8 hours. The solvent removed by using a rotary evaporator.

Figure 7: *Acacia Seyal* seeds oil

2.2.2 Oil percentage

The weight of oil extracted from 446.50g of seeds was weighted to determined percentage of oil in the seed (W/W). According to the formula

$\%oil = \frac{w1}{w2} \times 100\%$

Where:

w1 = weight of oil

w2 =weight of ousted seeds pulp

2.2.3 Acid value:

5.013 g of oil was dissolved in 50 ml of a mixture of equal volumes of 96 % ethanol and light petroleum, previously neutralized with 0.1 M potassium hydroxide and titrate with 0.1 M potassium hydroxide until the pink color persists for 15 second then the titrant volume was taken and Acid value was calculated as followed:

Acid value = 5.561 * V / W

Where:

V= volume of sodium hydroxide

W= weight of sample

2.2.4 Determination Free fatty acid

0.931g of oil sample was weighted into a conical flask. 100ml from isopropanol was added and warmed until the oil dissolved. 3drobs of phenolphthalein

indicator were added and titrated with standard sodium hydroxide until a pink color appearance persists for half a minute.

%free fatty acid as oleic acid = $\frac{28.2*N*V}{W}$

Where:

V = volume of sodium hydroxide solution used (ml)

N = normality of sodium hydroxide solution used

W = weight of sample taken (g)

2.2.5 Saponification Number

The number of milligrams of potassium hydroxide required to saponify 1g of fat or oil under conditions specified. It is a measure of the average molecular weight or chain length of all the fatty acid present. As most of the mass of a fat/tri ester is in the 3 fatty acid, it allows for comparison of the average fatty acid chain length. Long chain fatty acids found in fats have low saponification value because they have relatively fewer numbers of carboxylic functional groups per unit's mass of the fat as compared to short chain fatty acids.

2.023g of oil was weighted into a 200-ml flask, 25.0 ml of the ethanolic solution of potassium hydroxide was added and boiled under a reflux condenser for 1 hour and rotated the contents frequently. While the solution is still hot, the excess of alkali was titrated with 0.5M hydrochloric acid using 1ml of phenolphthalein solution as indicator. Operation was repeated without the substance being examined.

Saponification value was calculated from the expression

Saponification value = 28.05 * V /W

Where:

V= difference in volume between the titrations.

W=weight of oil.

2.2.6 Ester value:

Ester value is number of milligrams of potassium hydroxide required to sapoonify the fatty acid esters in one gram a fat, wax, or oil.

Ester value= Saponification value – Acid value

2.2.7 Iodine Number:

0.252 g of oil was dissolved in 10 ml of dichloromethane in a dry iodine flask. 20 ml of iodine monochloride solution was added, stopper, which previously moistened with dilute potassium iodide solution inserted, and allowed to stand in the dark at 15° to 25° for 30 minutes. 15 ml of dilute potassium iodide solution placed in the top cup, carefully stopper removed, the stopper and the sides of the flask were rinsed with 100 ml of water, shacked and titrated vs.0.1M sodium thiosulphate using starch mucilage, added towards the end of the titration, as indicator.

I= 1.269 *V /W

Where:

I= iodine value

V= different between volume

W= weight of sample

2.2.8 Peroxide number:

2g of oil was weighted into a conical flask, a mixture of chloroform and glacial acetic acid (2:1) was added to the oil to dissolve it, 1g of saturated potassium iodide was added and the final mixture was kept in dark for 5min then the content of the conical flask was titrated vs. sodium thiosulphate 0.1M.

The blank solution was prepared and titrated also vs. sodium thiosulphate. The peroxide number was calculated as follow:

P= V*M*1000/W

V= V1-V2

Where:

P= Peroxide value

V= the actual volume.

V1= the volume of sodium thiosulphate titrated vs. oil.

V2= the volume of sodium thiosulphate titrated vs. blank solution.

M= molarity of sodium thiosulphate.

W= weight of sample.

2.2.9 Density

The density of the oil was determined using density bottle 50ml. The density bottle was filled with oil and weight. The density was calculated as:

$$d=\frac{m}{v}$$

Where d is density, m is mass of the oil, and v is the volume of the density bottle.

2.2.10 Color

The color of the oil was determined by using Lovibond comparator colorimeter.

2.2.11 Moisture content

The moisture content of oil is expressed as percentage weight loss when the oil is dried to a constant weight at 110°C. A dry crucible was weighed and the dried oil (5 g) was poured into it. The crucible and content were dried in an oven at 110°C and cooled in a desiccator and weighed.

2.2.12 Refractive index

The refractive index of oil is a function of molecular structure and impurity. The refractometer was used. Placed drops of the oil on the prism, and closed by another one after cleaned the both prisms. The prisms were tightened firmly with the screw head. The instrument was adjusted and lighted and the refractive index was determined.

2.3 Testing of oil

2.3.1 Infra-red analysis

The sample was put directly in the KBR windows, introduced into the FT IR and scanned at 400-4000 cm^{-1}.The IR spectrum was recorded.

2.3.2 Gas chromatography analysis

Gas chromatography (GC) is a technique for the analysis and quantification of organic volatile and semi-volatile compounds.

(GC) is used to separates mixtures into individual components using a temperature-controlled capillary column.

The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different moleculesin a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

Results and discussion

3.1 *Acacia Seyal*seeds oil analysis

3.1.1 Physicochemical analysis

Oil content of *Acacia Seyal* seeds found to be 3% while the oil content of *Acacia Senegal* was 20%.

Moisture content is amount of water in the seeds. A small change in seeds moisture content has a large effect on the storage life of the seeds also of the seeds oil. Therefore it is important to know the moisture content in order to make a reasonably accurate prediction of the possible storage life of each accession. Moisture content of *Acacia Seyal* seeds oil found to be in range of *Sunflower* seeds oil and*Groundnut* seeds oil but it was very high comparing with *Acacia Senegal* seeds oil that mains *Acacia Seyal* seeds oil more susceptible to oxidation than *Acacia Senegal* seeds oil.

Free fatty acid is play a major rule in characterization of oil either high or low value. It found for *Acacia Seyal* seeds oil over the range of *Sunflowe*r seeds oil and *Groundnut* seeds oil, which indicate that the *Acacia Seyal* seeds oil was not edible oil so it cannot be used for eating or food as oil. Also it was high comparing with *Acacia Senegal* seeds oil. This result is not good to complete the process of esterification to biodiesel.

Peroxide value was evidence of rancidity. It found at maximum range of edible oils (*Sunflower* seeds oil and *Groundnut* seeds oil). Comparing *Acacia Seyal* seeds

oil with *Acacia Senegal* seeds oil it was very high that increase the rancidities of *Acacia Seyal* seeds oil more than the other oil.

Saponification value and ester value were in range of edible oil and also Iodine value. Comparing iodine value of *Acacia Seyal* seeds oil with *Acacia Senegal* seeds oil we found that *A.seyal* seeds oil was high than *A.senegal*. The higher the iodine value the higher carbon carbon double bonds percent in the oil.

Comparison physicochemical properties between *Acacia Seyal* seeds oil and *Acacia Senegal* seeds oil had shown clear differences.

Table 1: Test of physicochemical properties of oil of *Acacia Seyal* seeds

Physicochemical property	**Results of *A.Seyal***
Acid value	6.266
Free fatty acid %(w/w)	5.75
Saponification value	171.93
Ester value	165.66
Iodine value	95.67
Peroxide value(g)	15
Density(g/cm^3)	0.891
Color (red/ Yellow)	33.2/6.8
Moisture %(w/w)	6.58
Refractive index	1.47

Table2: comparison between physicochemical properties of *Acacia Seyal* seed oil and Sunflower oil and groundnut oil:

Test	*A.Seyal*	*Sunflowr*	*Groundnut*
Density	0.891	0.918-0.923	0.912-0.920
Refractive index	1.469	1.461-1.469	1.460-1.465
Color(red/ Yellow)	6.8/33.2	1-9/10-70	1-9/10-70
Moiture content	6.58	1-10	0-10
Free fatty acid	5.75	0.2-3	0.2-3
Saponification value	171.93	188-194	188-194
Peroxide value	15	1-15	1-15
Iodiene value	95.67	110-143	80-106
Ester value	165.66	100	100

Table3: comparison between physicochemical properties of *Acacia Seyal* seeds oil and *Acacia Senegal:*

Test	*A.Seyal*	*A.Senegal*
Density	0.891	0.915
Refractive index	1.469	1.4628
Color (red/ Yellow)	6.8/33.2	1.5/29.04
Moiture content	6.58	0.8
Free fatty acid	5.75	3.8
Saponification value	171.93	848.4
Peroxide value	15	6
Iodiene value	95.67	89.144
Ester value	165.66	844.6

3.1.2 IR analysis

The peak at 2925.81 cm^{-1} appear due to (—CH—) sp^3 saturated. The twp peaks at 2854.45 cm^{-1} and 3008.75 cm^{-1} appear due to (—CH—) sp^3 in different environments, this means these two peaks effected by carbonyl (α,β and ɣ). The peak at 1745.46 cm^{-1} appears due to ($\overset{O}{\overset{\|}{C}}$) carbonyl of ester. The peak at 1163.00 cm^{-1} appears due to C—O (str).

The peak at 1458.08 cm^{-1} appears due to ($—CH_2—$) bending. The peak at 721.33 cm^{-1} appears due to the presence of halide C—X (may be chloride).

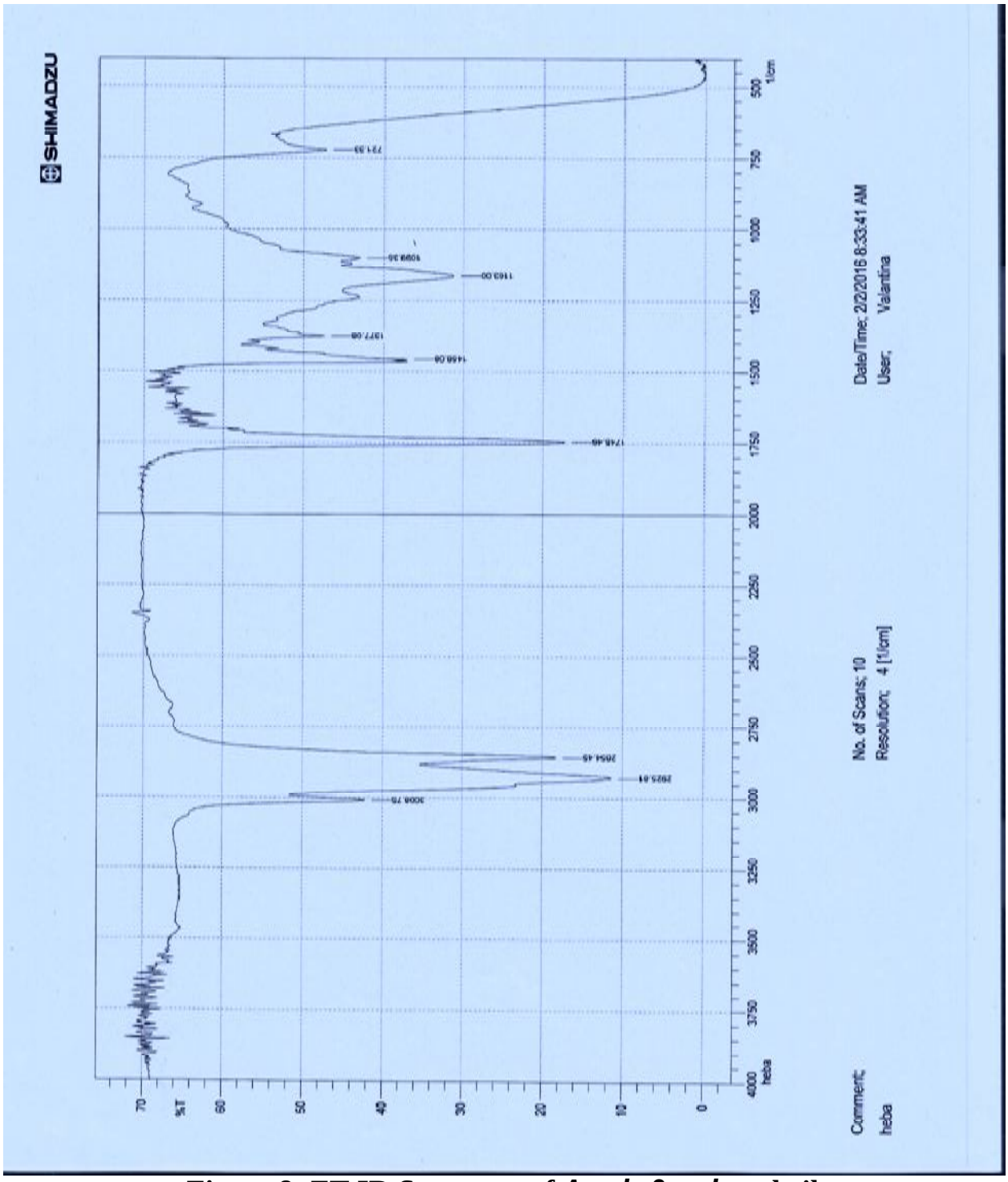


Figure 8: FT IR Spectrum of *Acacia Seyal* seed oil

3.1.3 GC analysis

Fatty acid profile of the *Acacia Seyal* seeds oil was determined by using GC analysis. The individual peaks of the chromatogram shown in figure-9 were analyzed and the fatty acids were identified. Relative percentage of fatty acid esters was calculated by computerized integrator and results are presented in table 3.

The unsaturated fatty acid percentage was found to be 41.59% for Lnoleic acid and 23.97% for Oleic acid; it was more than the saturated fatty acid which was found to be 8.730% for Palmatic acid and 17.34% for Stearic acid.

Table 4: Component of *Acacia Seyal*seeds oil

Component name	Method	Area	Area%
Palmatic acid	UOP-915-92	79084	8.730
Lnoleic acid		376767	41.59
Oleic acid		217119	23.97
Stearic acid		157042	17.34

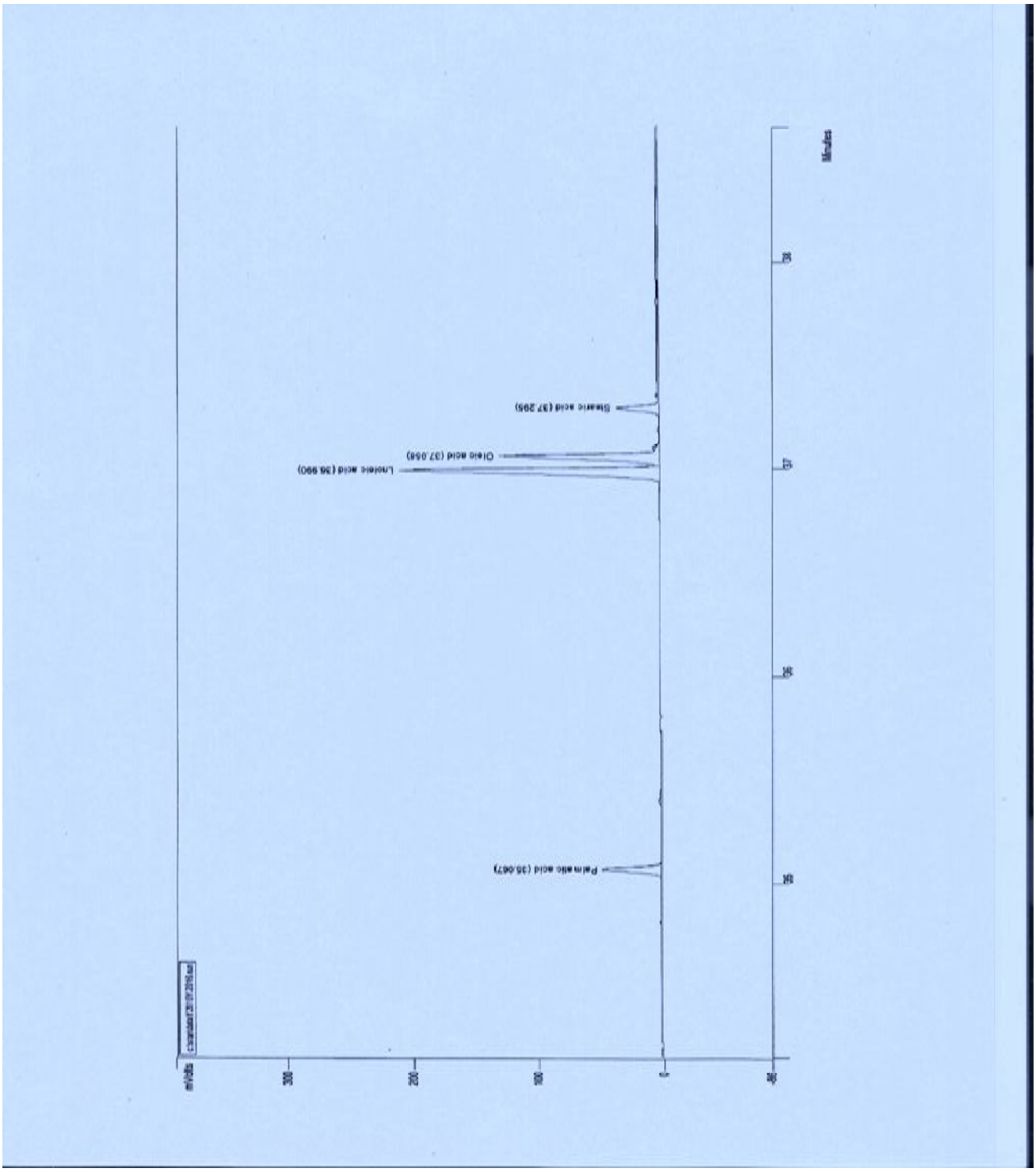


Figure9: Chromatogram of *Acacia Seyal* seed oil showing the major components

3.2 Conclusion:

The oil of *Acacia Seyal* is non edible due to its high fatty acid content thatexceeds the permissible for range of the edible oils.

Acacia Seyal seeds oil is not suitable for biodiesel production due to high free fatty acids percentage, high peroxide value and high ester value. The low oil content in the *A.Seyal* seeds may hinder its economical viability to be used in biodiesel production.

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