Sudan University of Science and Technology

College of Graduate Studies

Extraction and Determination of Physicochemical Characteristics Oil from *Prosopis juliflora* (*Mesquite*) Seeds

إستخلاص وتحديد الخصائص الفيزيكوكيميائيه لزيت بذور نبات المسكيت

A Thesis Submitted in Partial Fulfillment of the Requirement of the Degree of M.Sc. (Chemistry)

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إستهلال

قال تعالي:

بسم الله الرحمن الرحيم

﴿فَلْيَنْظُرِ الْإِنْسَانُ إِلَى طَعَامِهِ (24) أَنَّا صَبَبْنَا الْمَاءَ صَبّاً (25) ثُمَّ شَقَقْنَا الْأَرْضَ شَقّاً (26) فَأَنْبَتْنَا فِيهَا حَبّاً (27) وَعِنَباً وَقَضْباً (28) وَزَيْتُوناً وَنَخْلاً (29) وَحَدَائِقَ غُلْباً (30) وَفَاكِهَةً وَأَبّاً (31) مَتَاعاً لَكُمْ وَلِأَنْعَامِكُمْ (32)﴾

صدق الله العظيم

[سورة عبس الآيات: 24-32]

Dedication

I dedicate this work to my parents,

Brothers and sisters.

Acknowledgment

I would like to thanks Allah, almighty for giving me the strength to complete this research.

I would like to express my special thanks and gratitude to my supervisor Dr. Elfatih Ahmed Hassan for giving me the opportunity to work with him, for the fruitful discussion and excellent guidance.

I would also like to thank my parents and friends who helped me a lot in finalizing this project within the limited time frame.

Abstract

In this study, *Prosopis juliflora* (*mesquite*) Seeds oil was extracted using normal hexane. The oil content on the seeds was found to be 3% w/w.

The extracted oil was determined physicochemical properties.

Prosopis juliflora oil quality was tested by determining some parameters such as viscosity, density, iodine value, saponification value, peroxide value, and acid value as well as its FTIR spectrum.

The iodine values (IV) was found to be (97.157 I_2 g/100g),

saponification value (SV) was about (186.53 KOH mg/g),

Acid value (AC) was about (0.561NaOH mg/g),

Density of oil was a *Prosopis juliflora* bout (0.8397g/ml),

Viscosity of oil was e *Prosopis juliflora* estimated at

(4.933mm^2/ sec),

And the peroxide value (PV) of *Prosopis juliflora* was found to be (163.285ml/g).

The results derived from the oil physicochemical analysis shed light into the possibility and limitation of the industrial application of the oil.

مستخلص

في هذه الدراسه تم استخلاص زيت بذرة نبات المسكيت باستخدام الهكسان كمذيب.

وجد أن النسبه المئويه لزيت بذرة المسكيت حوالي (3%).

تم تحديد الخصائص الفيزيوكيميائيه للزيت المستخلص.

تم اختبار جودة زيت بذرة المسكيت بتقدير بعض العوامل مثل:

اللزوجه، الكثافه، قيمة اليود، قيمة التصبن، قيمة البروكسيد و قيمة الحمض،

و كذلك طيف الاشعه لجهاز الاشعه تحت الحمراء.

وجد إن قيمة الايودين لزيت بذرة المسكيت حوالي (97.157 I_2 g/100g)،

قيمة التصبن حوالي (186.53 KOH mg/g)،

قيمة الحمضيه حوالي (0.561NaOH mg/g)،

كثافة زيت بذرة المسكيت حوالي (0.8397g/ml)،

لزوجة زيت بذرة المسكيت حوالي (4.933mm^2/ Sec)،

ووجد أن قيمة البروكسيد لزيت بذرة المسكيت حوالي (163.285ml/g).

النتائج المستمده من تحليل الخواص الفيزيكوكيميائيه للزيت تسلط الضوء لإمكانية وحصر التطبيقات الصناعيه للزيت.

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Chapter one

1- Introduction

Prosopis juliflora is one of most economically and ecologically important tree species in arid and semi-arid zones of the world. It is an important species because of its high nitrogen fixing potential in very dry areas and in drought seasons and also because it provides shelter and food to many species of animals on its nectar, pollen, leaves and fruits [25, 7]. The shrubs *prosopis juliflora* (fig. 1) are highly esteemed for windbreak, soil binders, sand stabilizers, living fences, fuel wood, bee plants and animal feed [6]. These uses, together with fast growth [63], drought resistance [21] and salt tolerance [26] have lead its introduction in many arid zones [63 , 26 , 16 , 62]. The genera *prosopis* and acacia contain some of the most widespread and important tree species in the arid and semi-arid zones of the tropical and subtropical world .Species of these two genera have been estimated to occupy some 3.1 million square kilometers [27]. *Prosopis juliflora* grows abundantly in Indian sub-continent [7, 51 , 37 ,5 ,57 ,19] and commonly it is known as mesquite (English), Algarroba (Spanish), Vilayati babul, Vilayati khejra, gando baval, vilayati kikar (India). The genus *prosopis* is thought to be evolved approximately 70 million years ago, before the African and south-American continents separated [46]. *Prosopis* genus cropped up in the American sub-continent with two centers of diversity the Texan -Mexican and the Argentinean center [16] having a large number of sympatric specie Studies using morphological characters isoenzymes [49, 50], seed protein electrophoresis [12] and molecular markers [45] have shown the occurrence of intra- as well as inter- series hybrids in populations of both sections of

Algarroba and Strombocarpa. *Pasiesznik et al* [40]. Suggested grouping of species of section Algarroba into “complexes” or “species groups”

Fig (1): Tree of *prosopis juliflora*

Principally according to their genetic similarity and geographic distribution, but also to their habit and resource characters. In India, original introductions are thought to have been *Prosopis juliflora* from Mexico or Jamaica [47, 36]. Differences in plant morphology may be due to further introductions of seed material of various origins and possible hybridization between them. Five forms of *prosopis juliflora* have been identified in India [44].Considerable amount of literature is present on *prosopis juliflora* however; a synthesis of this information into comprehensive, concise and authoritative review is rare. Here is an attempt to synthesize the information available on *prosopis juliflora*.

1.2 General tree structure

Prosopis species are all trees or shrubs of varying size (rarely sub-shrubs) (Fig. 1) predominantly xerophilous, aculeate, spiny or rarely unarmed [14].

1.2.1 Seeding

Seeds of *prosopis juliflora* are epigeous in germination. The fleshy cotyledons are the first seed leaves, persisting after the first true leaves have formed, being green or somewhat pale-green in colour (Fig. 2). Once germinated, most energy is expended on rapidly developing a root system and locating a water source as soon as possible. In the first month, root length and biomass increases are much greater than shoot biomass leading to a high root: shoot ratio [40].

1.2.2 Leaves and flowers

Leaves are bipinnate, with 1-10 leaves per node and petiole plus rachis 5-20 cm long [18]. Trees are aphyllous or sub-aphyllous, with a rapid turn-over of

leaves. Pubescence varies from entirely glabrous or ciliolate to somewhat pubescent or pubescent [14, 41]. Leaflets are linear-oblong, elliptic-oblong or ovate in shape, with an obtuse to mucronulate or minutely pointed apex,

Figure (2*): Prosopis juliflora* seeds

Nerved below. Leaflets vary greatly in size, 2.5-23mm long and 1-7mm wide (Fig.3).Glands are cupuliform, sessile with an apical pore, present at the junction of the pinne, sometimes also at the junction of the leaflets [14, 22, 18]. While tree are generally evergreen, *prosopis juliflora* is occasionally deciduous, possible due to drought or cold temperature [28]. Flowers are small, 4-6mm long, gathered densely together on cylindrical, spike-like inflorescences known as racemes. They are generally yellow, straw yellow or yellow-white in colour. Flowers are hermaphrodite, sometimes sterile, actinomorphic and pentamorous [14]. The caylx is campanulate, green or greenish-yellow, bell shaped and ciliolate outside, 0.5-1.5mmlong. The corolla is 3.0-3.2mm long, styles 2.0-3.0mm long, petales 2.5-3.0mm long, free and villous within [22]. The five stamens are 4-7mm long, pistils 4-5mm long, and the stipitate, villous ovaries are light green in colour and 1.5-1.8mm long [22]. Anthers have a glandular appendage [17].The pedicelis short, 0.5-0.3mm long [40].

1.2.3 Fruits and seeds

The fruit is an indehiscent legume, straight with an incurved apex, sometimes falcate or sub-falcate, with or without parallel margins. Pods are stipitate and acuminate, compressed to sub- compressed and sub moniliform. They are flattened, rectangular to sub-quadrate in section. Immature pods are green in colour, becoming commonly straw yellow when fully mature [40]. The number of pods produced per inflorescence varies greatly, with 1-16 fruit per inflorescence. Pods also vary greatly in size, 8-40 cm long, 9-18mmwide and 4-10 mm thick [14, 22, 18]. Pods are made up of an exocarp,

afleshly mesocarp, and endocarp segments each containing a single seed,

Fig (3): Leaves and flowers of *Prosopis juliflora*

with up to 30 seed per pod. Exocarps vary in their thickness, external colour and ease of separation from the mesocarp but are generally consistent. The relative proportion of mesocarp in the pod varies greatly, affecting pod thickness and chemical composition, of particular note being the content of sugars and proteins. The fibrous endocarp each contain asingle compressed, ovoid or oblong seed. Seeds are brown in colour, shiny and with a horseshoe-shaped fissural line on both surface of the testa, with the arms pointing towards the hilar end. Seeds are up to 6-5 mm long and weigh

approximately 0.25-0.30 g (25000-3000 seeds/kg). Inside the tegmen is endosperm, which surrounds the yellow cotyledons. The cotyledons are round or elliptical, with a sagitate base and frequently so not cover the upper part of the radicle [40].

1.3 Biology

1.3.1 Species

The genus *Prosopis* Linnaeus emends. *Burkart* is in the family Liguminosae (Fabaceae), sub-family Mimosoideae. The placing of *Prosopis* in the wider taxonomic classification system based on *Elias* [20] *and Lewis and Elias* [35].

Family:	Leguminosae	650 genera, 18,000 species
Sub-family	Mimosoideae	50-60 genera, 650-725 species
Tribe:	Minoseae	5 tribes
Group:	Prosopis	9 groups
Genus:	Prosopis	4 genera

Table (1): *Prosopis juliflora* species classifications

The name *juliflora*, comes from julus, meaning" whip-like", referring to the long inflorescences, and flora being the flower. *Prosopis juliflora* (*swartz*) DC. Has had an array of synonymy since its first description in 1788. Originally known as mimosa *juliflora swartz*, it become both *Algarobia juliflora* (*swartz*) Bentham ex Heynh. And *Neltuma juliflora* (*swartz*) Rafinesque during the last two centuries. *Prosopis juliflora* is used here in its original, restricted and certainly biological sense, re- established by *burkart* [13] and accepted by *Johnston* [28].

1.5 Medicinal uses

With time there has been a shift from synthetic to herbal medicines, which we say "return to nature" [39]. In traditional and ancient therapy various parts of plants are used therapeutically like its fruits, flowers, leaves, bark and roots [24, 55]. Extracts of *prosopis juliflora* seeds and leaves have several in vitro pharmacological effects such as antibacterial [1, 8, 30, 15, 9, 52], antifungal [2, 3, 32], and anti-inflammatory properties [2, 3]. These properties have been attributed to piperidine alkaloids [1, 11]. It's also known for its ethno-medicinal properties, mainly used for boils, rheumatic pain, digestive disturbances [61].All parts of *prosopis juliflora* are used in the preparation of medicinal products to treat human ailments. An astringent decoction is made from boiling wood ships, a bark extract is used as an antiseptic on wounds and gum is used to treat eye infections [60]. *Prosopis juliflora* flour is used as an aphrodisiac, syrup as an expectorant and tea infusion against digestive disturbances and skin lesions [48]. *Prosopis juliflora* is also used to treat sexually transmitted diseases [15]. Anticarcinogenic effects have also been reported. Of the many chemical with effects on human health that have been isolated from *prosopis juliflora*, most work has concentrated on alkaloids, flavonoidsan and tannins. Several groups of piperidine alkaloids have been isolated from *Prosopis* species. The alkaloids juliflorine, juliprosine, juliprosopine, julifloricine and julifloridine have been isolated from *prosopis juliflora*[60]. Diketone, prosopidione and cytotoxin patulitrin have been isolated from *prosopis juliflora* leaves by *Ahmad and Sultana* [4]. Many flavonoids and tannins have also been isolated [48]. Chemical compounds have also been isolated from *Prosopis juliflora* bark, Pods and roots by *Vimal and Tyagi* [60]. Amixture of alkaloids from *prosopis juliflora* has

significant inhibitory effects on gram positive bacteria [8]. Water – soluble mixture of alkaloids from *prosopis juliflora* leaves has found to be more active against gram -positive bacteria than three commercial antibiotics bacitracan, chlormycentin, gentamicin or trimethoprim. The antifungal, antibacterial and general antimicrobial activity of plant extracts of *prosopis juliflora* is well established, but there are concerns as to their toxic effects [40]. Cytotoxic effects were also observed with extracts of *prosopis juliflora* [4] and alkaloids were reported to cause haemolysis of rat and human erythrocytes. Rocha also identified the presence of the toxic of chemical furfural. Tannins present are irritant of internal organs and can also bind protein making them indigestible [48]. Roots and shoots samples of *prosopis juliflora* have been assessed for their heavy metal content by *Varun et al* [58]. To evaluate the species as a green solution to decontaminated soils decontaminated by lead and cadmium. *Prosopis juliflora* has a good phytoremediation potential. It is an effective heavy metal remediator couple with environmental stress. The impact of metal nanoparticles (NPs) on biological systems, especially plants, is still not well understood. *Viezcas et al* [59]. assessed the effects of zinc oxide (ZnO) NPs in velvet mesquite (*prosopis juliflora-velutina*). Zinc concentration in roots, stems and leaves were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). Plant stress was examined by the specific activity of catalase (CAT) and ascorbate peroxidase (APOX); while the biotransformation of ZnO NPs and Zn distribution in tissues was determined by X-ray absorption spectroscopy (XAS) and micro

X- Ray fluorescence (μXRF), respectively. ICP-OES results showed that Zn concentrations in tissues (2102±87, 1135±56,

and 628±130 mg kg^{-1} d wt in roots, stems and leaves, respectively) were found at 2000 mg ZnONPsL-1. Stress tests showed that ZnO NPs increased CAT in roots, stems, and leaves, while APOX increased only in stems and leaves. XANES spectra demonstrated that ZnO NPs were not present in *mesquite* tissues, while Zn was found as Zn (II), resembling the spectra of Zn $(NO_3)_2$. The μXRF analysis confirmed the presence of Zn in the vascular system of roots and leaves in ZnO NP treated plants [59].

1.6 Aim of this research

- Extraction of *prosopis juliflora (mesquite)* seeds oil.
- Determination of physicochemical characteristics of the extracted oil.

Chapter two

2.1 Material and methods:

2.1.1 Plant collection and preparation:

The seed of *prosopis juliflora* plant used for the present study were collected from (Bahri, Eldroshab area). The seed of the test plant were manually separated from the pods and cleaned to remove all foreign matter. After which the separated seed then powdered using grinding mill. The powdered sample were sieved and preserved for the next procedure extraction. At the end of the extraction, the percentage of the extracted oil was determined.

2.1.2 Chemicals of Extraction:

All chemicals used in this research were of analytical grade type and includes; n-hexane, hydrochloric acid, potassium hydroxide, ethanol 95%, phenolphthalein, diethyl ether, sodium hydroxide, iodine trichloride, carbon tetrachloride, glacial acetic acid, sodium thiosulphate, potassium iodide, iodine, chloroform, starch.

2.1.3 Equipments and Apparatus:

Extractor system, FTIR, Ostwald`s viscometer, bycnometer, water bath, sensitive balance, glass rode, pipettes, burettes, volumetric flask, Beakers, funnel, cylinder, rotavaporator.

2.1.4 Extraction of oil:

5 g of the sample was placed in the thimble and about 250 ml of n- hexane was poured into the round bottom flask and was inserted in the centre of the extractor .The extractor system was heated at 65 C^0. when the solvent was

boiling the vapour rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre which contained the solid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube, where it flowed back down into the round bottom flask. This was allowed to continue for 8hours. It was then removed from tube, dried in the oven, cooled in the desiccators and weighed again to determine the amount of oil.

2.2 Analysis of *Prosopis juliflora (mesquite*) seed oil

The following physicochemical parameters were estimated using samples of the oil extracted from *prosopis juliflora* seeds.

2.2.1 Saponification value:

The saponification value of an oil or fat is defined as the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of the sample.

A soap is formed during the saponification, for example:

$$C_3\ H_5\ (C_{17}\ H_{35}\ \mathrm{COO})_3 + 3KOH\ \rightarrow\ C_3H_5(OH)_3\ +\ 3C_{17}H_{35}.COOK$$

$$Stearin \qquad\qquad Glycerol \qquad Potassium\ stearate$$

The esters of the fatty acids of low molecular weight require the most alkali for saponification, so that the saponification value is inversely proportional to the mean of the molecular weights of the fatty acids in the glycerides present. As many oils have somewhat similar values (e.g. those in the olive oil series fall within the range 188-196), the saponification value is not, in general, as useful for identification purpose as the iodine value.The

saponification value is most use for detecting the presence of coconut oil (SV225), Palm – Kernel oil (SV247), and butter fat (SV 225), which contain a high proportion of the lower fatty acids, paraffin, which, of course, gives a negligible saponification value, can also be detected and estimated if present as an adulterant.

2.2.1.2 Alcoholic solution of potassium hydroxide

4g of the potassium hydroxide was dissolved in 2ml water, diluted to 100ml with ethanol 95%. Allowed to stand overnight and decant off the clear liquid.

2.2.1.3 Determination of saponification value:

The indicator method was used as specified by ISO 3657 (1988). 2g of the sample was weighed into a conical flask; 25 ml of 0.1N ethanoic potassium hydroxide was then added. Then content which was constantly stirred was allowed to boil gently for 60 min. A reflux condenser was placed on the flask containing the mixture and a few drops of phenolphthalein indicator was added to the warm solution and then titrated with 0.5M HCl (titrated= Vi) to the end point until the pink colour of the indicator just disappeared. The same procedure was used for a blank at the same time($titrated = Vo$). The expression for saponification value (SV) was given by:

$$\mathrm{SV} = \frac{(\mathrm{Vo} - \mathrm{Vi}) \times \mathrm{N} \times 56.1}{Wt\ of\ sample}$$

Where:

Vo: The volume of the solution used for the blank test.

Vi: The volume of the solution used for the determination.

N: actual normality of HCl used.

Wt: weigh of sample.

2.2.2 Acid value or free fatty acids (FFA)

The acid value of an oil or fat is defined as the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the sample. The result is often expressed as the percentage of free acidity. The acid value is a measure of the extent to which the glycerides in the oil have been decomposed by lipase action. The decomposition is accelerated by heat and light. As rancidity is usually accompanied by free fatty acid formation, the determination is often used as general indication of the condition and edibility of oils.

The FFT figure is usually calculated as oleic acid ($1\,ml\,0.1N \equiv 0.0282g\ \ oleic\ acid$), in which case the acid value = $2 \times FFT$.

BS 684 however recommends that the FFT in the following oils should be calculated as the acid which is more appropriate to that present:

Palm oil as palmitic acid($1ml\ 0.1\ N\ \equiv 0.0256\ g$).

Palm – kernel, coconut and similar oils as lauric acid

($1ml\ 0.1N\ \equiv 0.0200g$).

With most oils acidity begins to be noticeable to the palate when the FFT calculated as oleic acid is about $0.5 - 1.5\%$. Occasionally however some quite rancid oils show only minimal acidities. (See below). Baker and Bains, Rao and Bhatia (ibid., P. 831) have show that the FFT of vegetable oil can be determined calorimetrically by shaking a benzene extract with copper acetate solution .the fatty acids react to form copper salts, the blue colour of which in organic layer can be measured at $640 - 690\, m\mu$ and compared with the results obtained using solutions containing known amounts of oleic acid.

2.2.2.1 Determination of acid value or (FFA)

25 ml of diethyl ether and 25 ml of ethanol was mixed in a 250 ml beaker. The resulting mixture was added to 2 g of sample oil in a 250 ml conical flask and a few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1N NaOH to the end point with consistent shaking for which a dark pink colour was observed and the volume of 0.1N NaOH (Vo) was noted.

$$\text{Av} = \frac{\text{volume} \times \text{N} \times 56.1}{\text{Wt of sample}}$$

Where:

N: actual normality of sodium hydroxide.

Wt: weigh of sample.

Free Fatty acid (FFT) was calculated as Vo/Wo = 82-100 where 100 ml of 0.1 N NaOH = 2.83 g of oleic acid , Wo = sample weight ; then acid value = $2 \times FFT$ (Laboratory handbook, 1997).

2.2.3 Iodine value (IV)

The iodine value of an oil or far is defined as the weigh of iodine absorbed by 100 parts by weight of the sample , the glycerides of the unsaturated fatty acids presents (particularly of the oleic acid series) unite with a definite amount of halogen and the iodine value is therefore a measure of the degree of unsaturation.

The ranges of figures for the iodine values of the various groups of oils and fats are as follows in table (2):

Group	Examples	Ranges of iodine values
Wax	–	Very small
Animal fats	Butter, dripping , lard	30 - 70
Non-drying oils	Olive oil , a rachis oil , almond oil	80 - 110
Semi-drying oils	soya oil Cotton seed oil , sesame oil	80 - 140
Drying oils	Linseed oil , sunflower oil	125 - 200

The iodine value is often the most useful figure for identifying an oil or at least placing it into a particular group. It should also be noted that the less unsaturated fats with low iodine values are solid at room temperature, or

conversely, oils that are more highly unsaturated are liquids. (Showing there is a relationship between the melting point and the iodine values). A further point of interest is that, in general, the greater the degree of unsaturation (i.e. the higher the iodine value), the greater is liability of the oil or fat to go rancid by oxidation. The iodine value is usually determined Wijs method;

2.2.3.1 Wijs solution

2 g iodine trichloride was dissolved in 50 ml glacial acetic acid. 2 g iodine was dissolved in 75 ml carbon tetrachloride. The two solutions was mixed and diluted to 250 ml with glacial acetic acid. A method for checking the iodine, chlorine ratio of wijs solution has been described by *Groupner* and *Aluis* Method.

2.2.3.2 Determination of iodine value

The method specified by ISO 3961(1989) was used. 2 g of the sample was weighed into a conical flask and 10 ml of carbon tetra chloride was added to dissolve the oil. Then 20 ml of wijs solution was added to the flask using a safety pipette. A stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 30 min. At the end of this period, 15 ml of 10% aqueous potassium iodide and 100 ml of water were added using a measuring cylinder. The content was titrated with 0.1N sodium thiosulphate solution until the yellow colour almost disappeared. A few drop of 1% starch indicator were added and the titration continued by adding thiosulphate drop- wise until blue coloration disappeared after vigorous shaking(Vi) .The same procedure was used for the blank test(Vo) . The iodine value (IV) is given by the expression:

$$IV = \frac{(Vo - Vi) \times N \times 126.9)}{Wt\ (in\ g) of\ sample}$$

Where:

V0: the volume of the solution used for the blank test.

VI: the volume of the solution used for the determination.

N: actual normality of thiosulphate used.

Wt (in g): mass of the sample.

The *Hanus* method for determining iodine value employing iodine monobromide gives similar result to the Wijs method and is stated as an alternative procedure in BS 684. For the determination of the iodine value of the glycerides of halibut – liver oil the BP prescribe the rapid pyridine bromide method *of rasenmund* and *Kuhnhenn* .

Toms (Analyst, land, 1928, 53, 69) has devised a navel method in which the bromine absorbed by 0.02 – 0.03 oil on a heated microscope slide is weighed. The percentage increase in weigh gives the bromine value from which an equivalent iodine value can be calculated by multiplying by 1.588.

Tom's method causes complete halogenations of oil containing conjugated systems of double bond in much shorter time than when Wijs method is employed. A method for determining the iodine value using

N- Bromosuccinimide has been described by *jovtscheff* (Analyt. *Albert*, 1960, 7, 4007).

2.2.4 Peroxide value (PV)

The peroxide value is a measure of the peroxides contained in the oil. During storage, peroxide formation is slow at first during an induction period which may vary from a few weeks to several months according to the particular oil or fat, the temperature, etc, and this must be borne in mind when interpreting quantitative result. The peroxide value is usually determined volumetrically by methods which have been largely developed by Lea.

These depend on the reaction of (KI) in acid solution which the bound oxygen followed by titration of the liberated iodine with sodium thiosulphate. Chloroform is normally used as solvent; the following method gives rapid results.

2.2.4.1 Determination of peroxide value

To 1 g of the oil sample, 1 g of potassium iodide and 20 ml of solvent mixture were added and the mixture was boiled for one minute. The hot solution was poured into flask containing 20 ml of 5% potassium iodide and 20 ml water. A few drops starch solution were added to the mixture and titrated with 0.15N sodium thiosulphate and the peroxide value was determined as follows:

(Glacial acetic acid $/_{chloroform}$, $^{2}/_{1}$ $by\ volume$)

$$PV = \frac{S \times N \times 10^3}{W}$$

Where:

S: ml of sodium thiosulphate,

N: Normality of sodium thiosulphate,

W: Weigh of oil sample (g).

Fresh oils usually have peroxide values well below 5ml 0.002N sodium thiosulphate per (g). A rancid taste often begins to be noticeable when the peroxide value is between 10 and 20. In interpreting such figures, however, it is necessary to take into account the particular oil or fat involved.

2.2.5 Density measurement

Density of the sample is directly proportional to unsaturation and inversely to molecular weight .firstly; the empty bycnometer was weighed, then filled with water and weighed again, finally it was weighed after filled with the sample.

2.2.6 Viscosity measurement

Viscosity is a measure of internal friction and resistance of flow. Viscosity of the sample was measured using Ostwald's viscometer in which the sample was allowed to flow from the etched mark (X-Y) through the capillary of the viscometer.

Viscosity was calculated as:

$$\frac{n_1}{n_2} = \frac{d_1 t_1}{d_2 t_2}$$

Where: d1 is the density of sample, d2 the density of water, t1 the time of flow for sample, t2 the time flow for water, n1 the viscosity of sample,

n2 the viscosity of water.

2.2.7 FTIR measurement

A small drop of the sample was placed on one of the KBr plates, the other plate was placed on top to make quarter turn to obtain a nice even film, then the plates were placed into the sample holder and the spectrum was run.

Chapter three

3.1 Results and discussion

Some physical and chemical properties of oils/fats extracted from seeds are shown in table (3):

Parameters	*Prosopis (mesquite)oil*	Unit
Oil content (%)	3.246	%
Density	0.8397	g/ml
Viscosity	4.933	mm^2 /sec
Acid value	0.561	NaOH mg/g
Iodine value	97.157	I_2 g/100g
Saponification value	186.5325	KOH mg/g
Peroxide value	163.285	ml/g

Prosopis juliflora with low percentage content of (3.246%).

The density of *prosopis juliflora* oil (0.8397g/ml) was found to be high,

Viscosity of the oil obtained was ($4.933 mm^2 /sec$),

The acid values content are lower than 1% of the crude oil is (0.561mg/g),

The saponification value (SV) is much high (186.5325mg/g), this oil may be used for soap making. The peroxide values (PV) of *prosopis juliflora* is (163.285ml/g), the high value of (PV) is indicative of high level of oxidative rancidity of the oil and also suggest absence or lower level of antioxidant.

The iodine value for the *prosopis juliflora* oil is very high (97.157g⁄100g), resulting high unsaturation of the oil. The limitation of unsaturation of fatty

acid is vital due to the fact that heating of highly unsaturated fatty acid result in polymerization of glycerides which could lead to deposits formation.

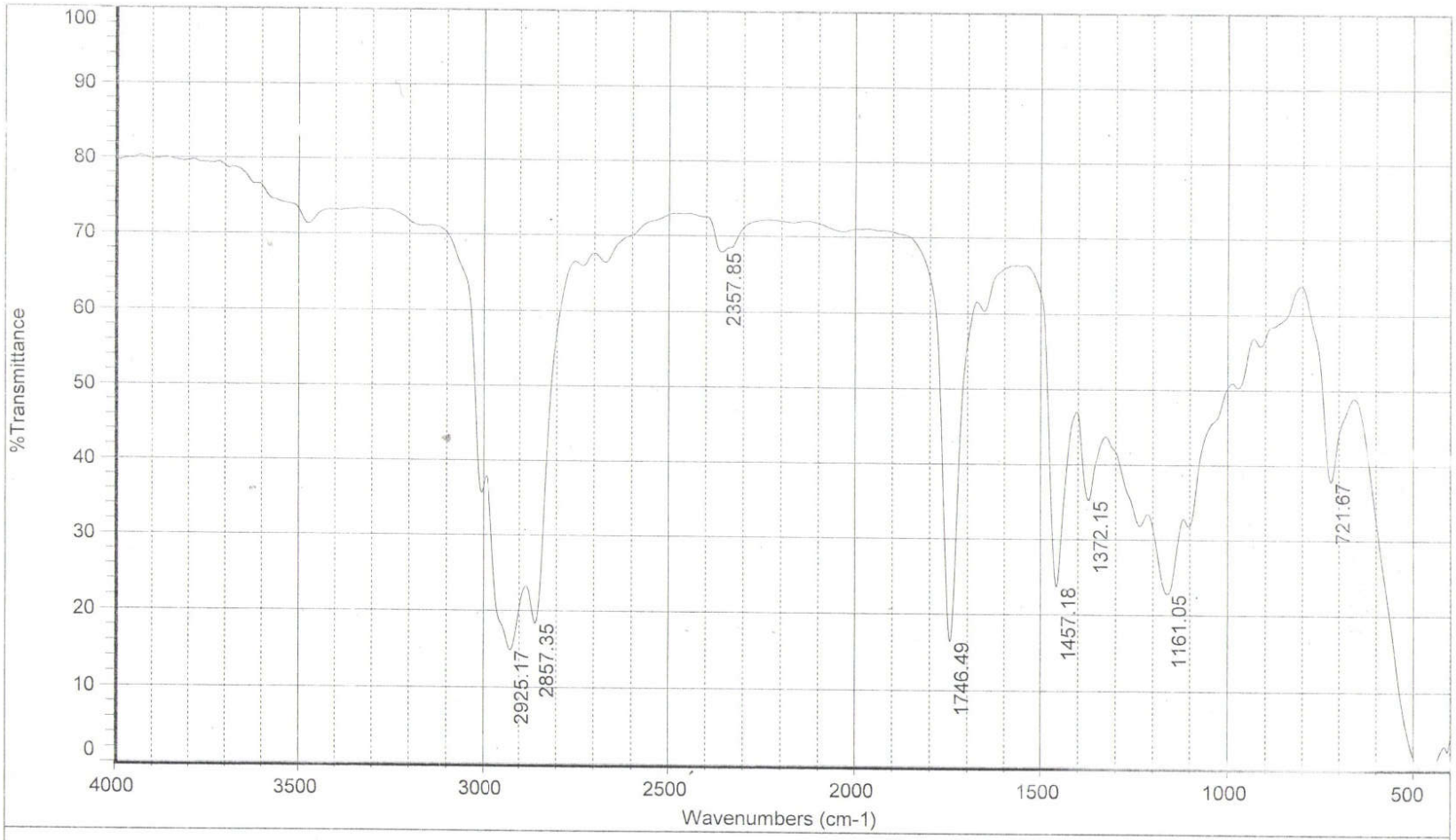
In the figure (4) the IR spectra of crude *prosopis juliflora (mesquite)* oil show in graph. The peak analysis of spectra show affect of the ester group.

IR represents long chains of hydrocarbons appears at (2925, 2857, 2357) cm^{-1}. Additional chain representing oleic acid, stearic and palimatic are visible in spectra with the $-CH_2$ hydrocarbon part. The chain of alkyl ester group to methyl ester has the strongest impact in the IR spectra. The inductive effect in carbonyl group needs more energy to get it into vibration.

The ester group is commonly describe as R_1-C (OR) =O in *prosopis juliflora* oil. The strong broad signal at $1146cm^{-1}$in edible oil is separated into concrete signal at $1457cm^{-1}$, and $1372cm^{-1}$.

3.2 Conclusion

- The seeds examined in this work have been shown to contain oil in low levels, which it was about 3% oil (w/w).
- Results showed that it contain mainly saturated fatty acid judging by their low iodine value which does not exceed (97g/100g) and are therefore not suitable as alkyl resin for paint formulation.
- They may however be useful for other purpose such as medicinal and pharmaceutical applications.



Date: Tue Nov 01 14:44:23 2016 (GMT-08:00) Sample Oil

Scans: 8

Resolution: 4.000

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