



Sudan University of Science and Technology
College of Graduate Studies



Chemical Characterization and Physicochemical Properties of Constituents of Hot pepper Seed Oil

التوصيف الكيمائي و الخصائص الفيزيوكيميائية لمكونات زيت بذور الشطة الحارة

A Thesis Submitted in Partial Fulfillment for the Requirements of Master of
Science in Chemistry

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February, 2019

الإستهلال

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ تَعَالَى:

﴿ * اللَّهُ نُورُ السَّمَوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ ﴿٣٥﴾ ﴾ النور: ٣٥

صدق الله العظيم

Dedication

To my lovely father

To my lovely mother

To my dear brother and dear sisters

Acknowledgment

My praise and thank to Almighty Allah the most Gracious who gave me strength to finish this work.

I would like to express my gratitude to my supervisor Dr. Kamal Mohamed Saeed for his careful supervision, valuable advice and kindness.

I would also like to express my sincere thanks to my big family for their support Thanks are also due to all those who helped and encourage me to do this work.

Abstract

In the present study, the fixed oil of hot pepper (*Capsicum annum*) was extracted from seeds using hot continuous extraction (soxhlet). The oil was analyzed by gas chromatography-mass spectrometry (GC-MS). There are 23 fatty acids were identified the major constituents were: linoleic acid (42.52%), palmitic acid (18.99%), oleic acid (12.74%), stearic acid (10.14%).

The oil was screened for the following physicochemical properties: the viscosity (64.68); specific density (0.9039); refractive index (1.4804); color: yellow(36.3), red(8.5) and blue(0.26); acid value (0.26 mg KOH/g); peroxide value (2.32 mg Eq/kg); saponification value(215.57 mg KOH/g); iodine value (13.72 mg/g); unsaponification value(0.062), ester value(215.31).

المستخلص

استخلص الزيت الثابت لنبات الشطة الحار و درس الزيت بتقنية الكروماتوغرافيا الغازية-طيف الكتلة حيث اتضح وجود 23 من الاحماض الدهنية بالمكونات الرئيسية التالية:

{Linoleic acid (42.52%), palmitic acid (18.99%), oleic acid (12.74%),
stearic acid (10.14%)}

تم اخضاع الزيت لاختبارات فيزوكيميائية و وجد ان اللزوجة (64.68) ،الكثافة 0.9039 ،معامل الانكسار 1.4807 ،درجة اللون الاحمر 8.5 .الاصفر 36.3 .الازرق 0.26 ،رقم الحموضة 0.26 ،رقم البيروكسيد 2.32 ،رقم التصبن 215.57 ،رقم اليودي 13.72 ،رقم الاستر 215.31 ، رقم المواد الغير متصبنة 0.062.

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Chapter One

Introduction

1- Introduction:

Hot peppers (*capsicum*) belong to the *solanoaceae*, they grow widely in various parts of the world especially in the temperate regions. Among hot pepper producing countries, China is the leading producer with above 1,745,000 tons per year followed by Mexico and Turkey (firatliqil-Durmus and Evranuz, 2010). Hot pepper is widely used as a flavoring and for nutritional purposes. Hot pepper seeds are separated from the pods after eating or processing the flesh. Worldwide millions of tons of hot pepper seed from food processing are generated some of them are still discarded without treatment leading to the environment pollution (jarret *et al.*, 2013).

Recently more attention has been focused on the utilization of food processing by-products and wastes, as well as underutilized agricultural product, such utilization would contribute to maximizing available resources and result in the production of various new foods (El-Adawy and taha, 2001). A number of studies report that hot pepper seed are rich in proteins, fats and minerals (Park *et al.*, 2006). These nutritional constituents particularly essential amino acids and essential fatty acids are necessary nutrients for the maintenance of healthy body (Koyuncu *et al.*, 2014). Thus hot pepper seed may be an inexpensive source of dietary proteins, fats and minerals.

Factors like cultivars and environmental condition have influence on the nutritional constituents of hot pepper and hot pepper seeds (Baeer, 2012). In literatures, there are several reports about the chemical composition of the divergent hot pepper seeds grown under different ecological conditions in Egypt, United States and Korea (El-Adawy and Taha, 2001; Jarret *et al.*, 2013; Park *et al.* 2006).

The word “pepper” is used to describe a group of spices associated with two different kinds of plants from two unlike species thus the original pepper used in international trade belong to the genus *piper* (*Piperaceae*), this species being a climbing vine. chilies and other types come from the bushy *capsicum* species (Family-*Solanaceae*) .the work described in this paper is associated with the latter species ,for which four broad categories have been identified. Of particular interest is the variety *Capsicum Annum L.* (also known as scotch Bonnet and Bell peppers).which grows in abundance in the Caribbean. it of particular importance as a food flavouring, the specific flavours being characterized by both the volatile oil and the fixed oil .the volatile oil provides the basic flavour and the fixed oil gives the pepper pungency .in addition, however, the pungency which created by the presence of capsaicinoids (molecular formula $C_{18}H_{27}NO_3$; IUPAC name 8-methyl -N-vanyllyl-6-noneamide). Constitutes the active ingredient in pepper sprays used for personal security.

1.1- Chili Pepper:

The Chili pepper (also chile pepper, chilli pepper, simple chilli) is the fruit of plants from the genus *capsicum*, members of the nightshade family, *solanaceae* Chili pepper are widely used in many cuisines to add spiciness to dishes. The substances that give Chili peppers their intensity when ingested or applied topically are capsaicin and related compounds known as capsaicinoids.

Chili pepper originated in Mexico. After the Columbian exchange, many cultivars of Chili pepper spread across the world, used for both food and traditional medicine.

Worldwide in 2014, 32.3 million tones of green Chili peppers and 3.8 million tones of dried Chili pepper were produced. China is the world's largest producer of green Chilies, providing half of the global total.

1.1.1- History of Chili Pepper:

Chili peppers have been a part of the human diet in the Americas since at least 7500 BCE. The most recent research shows that Chili peppers were domesticated more than 6000 years ago in Mexico. In the region that extends across southern Puebla and northern Oaxaca to southeastern Veracruz, and were one of the first self-pollinating crops cultivated in Mexico, central and parts of south America. Peru is considered the country with the highest cultivated Capsicum diversity because it is a center of diversification where varieties of all five domesticates were introduced, grown and consumed in pre-Columbian times. Bolivia is considered to be the country where the largest diversity of wild Capsicum pepper is consumed. Bolivian consumers distinguish two basic forms: Ulupicas, species with small round fruits including *C. eximium*, *C. cardenasii*, *C. eshbaughii* and *C. caballeroi* landraces; and arivivis with small elongated fruits including *C. baccatum* var. *baccatum* and *C. chacoense* varieties.

1.2- Oils:

An oil is any nonpolar chemical substance that is a viscous liquid at ambient temperatures and is both hydrophobic (does not mix with water, literally "water fearing") and lipophilic (mixes with other oils, literally "fat loving"). Oils have a high carbon and hydrogen content and are usually flammable and surface active. The general definition of oil includes classes of chemical compounds that may be otherwise unrelated in structure, properties and uses. Oil may be animal, vegetable, or petrochemical in origin, and may be volatile or non-volatile. They are used for food (e.g., olive oil), fuel (e.g.,

heating oil), medical purposes (e.g., motor oil), and the manufacture of many types of paints, plastics, and other materials, specially prepared oils are used in some religious ceremonies and rituals as purifying agents.

1.2.1- Fixed oils:

A natural animal or vegetable oil that is not volatile: a mixture of esters of fatty acids, usually triglycerides. Also called fatty oil compares to essential oils.

1.2.1.1- Uses of fixed oils:

Fixed oils and fats are important products used as industrially, pharmaceutically, or nutritionally products.

Industrially:-

-As detergents, soaps, paints, and varnishes and as lubricant.

Pharmaceutically:-

-As vehicle peanut oil, sesam oil is used as solvent in the preparation of certain I/m injection.

-As stimulant, cathartic, purgative e.g. castor oil.

-As emollient for ointments, liniment creams and other preparations e. g. almond oil, coconut oil.

Nutritionally:-

Other like corn oil with its high contents of unsaturated fatty acids is suggested in diet instead of fixed oil or fats having high saturated fatty acids to reduce high blood cholesterol level.

1.2.1.2 - Occurrence of fixed oils:

Vegetable oil and fats may occur in various parts of the plant but a general rule, seeds contain larger quantities of fats and oils than do other plant parts. seeds are the usual source of fixed oils. Few examples are

cottonseed oil linseed oil, sesame oil, hemp seed oil, coconut oil castor beans, almond etc.

Other plants parts also yield considerable amounts of fixed oil e. g. pericarp of the olive, in certain fungi e. g. ergot. Fat is a characteristic food material from animal e. g. lard.

1.2.1.3- Properties of fixed oils:

Fixed oils are obtained from various plants or animals. These are called fixed oils because they cannot be distilled without being decomposed. These are the esters of higher fatty acids such as oleic acid, palmitic acid, and stearic acid.

- They are bland and non-irritant.
- They leave a greasy mark on the paper.
- They form soap with alkalis.
- They are odorless and tasteless.
- They are insoluble in water, soluble in organic solvent and in cold alcohol.
- They are non-volatile.
- They have usually few pharmacological actions.

1.2.2- Essential oils:

Essential oil is a concentrated hydrophobic liquid containing volatile (defined as the tendency of a substance to vaporize) aroma compounds from plants essential oil are also known as volatile oils, ethereal oil, aetherolea or simply as the oil of the plant from which they were extracted such as oil of Clare . An oil is “ essential “ in the sense that it contains the “essence of” the plants fragrance .the characteristic fragrance of the plant from which it is derived the term essential used there does not mean indispensable as with the term essential amino acid or essential fatty acid

1.2.2.1- Uses of essential oils:

Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. Because of their anti depressant, stimulating, detoxifying, antibacterial, antiviral and calming properties, essential oils are recently gaining popularity as a natural, safe and cost-effective therapy for a number of health concerns.

There's no wonder why, considering the high cost of healthcare bills and the side effects of conventional medications, adding essential oils to your personal medicine cabinet can make a world of difference. This is especially true of because essential oils benefits are vast and their uses range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments such as:

- Balance hormones
- Support Digestion
- Boost energy level
- Reduce Emotional stress & Anxiety
- Alleviate Aches and pains
- Boost skin and hair health
- Reduce toxicity

1.2.2.2- Properties of essential oils:

In plants the amount of essential oils is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigment contributes to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods.

Essential oils are good source of several bioactive compounds which possess antioxidant and antimicrobial activities, thereby serving as natural additives in food and food products. They can be used as active compounds in

packaging materials, in which the properties of those material, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils can be improved.

Table 1.1: Difference between Essential oils and Fixed oils

Essential oils	Fixed oils
Volatile	Natural-nonvolatile
Evaporate under room temperature	Not evaporate
They can be extracted by distillation process	Specific techniques for extraction
Unable to undergo saponification	Easily saponified
High refractive index	Low refractive
Optically active	Optically inactive
Mixture of terpenes and sterpenes are termed as volatile oils	Esters of higher fatty acid and glycerin are called fixed oils

1.2.4 -Extraction of oil (Soxhlet):

In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask is heated, and its vapors condense in chamber E. The condensed extract then drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube, liquid contents of chamber E siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of

this method, compared to previously described methods, is that large amounts of oil can be extracted with a much smaller quantity of solvent. This affects tremendous economy in term of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

1.2.5 -Rancidity of oil:

Rancidity is the complete or incomplete oxidation or hydrolysis of fat and oils when exposed to air, light, moisture or by bacterial action, resulting in unpleasant taste and odor. Specifically, it is the hydrolysis or autoxidation of fats into short-chain aldehydes and ketones, which are objectionable in taste and odor. When these processes occur in food, undesirable odors and flavors can result.

In certain cases, however, the flavors can be desirable (as in aged cheeses). In processed meats, these flavors are collectively known as warmed-over flavor. Rancidification can also detract from the nutritional value of food, as some vitamins are sensitive to oxidation similar to rancidification, oxidative degradation also occurs in the hydrocarbons, such as lubricating oils, fuels, and mechanical cutting fluids.

Oxidative rancidity is associated with the degradation by oxygen in the air. The double bond of an unsaturated fatty acid can be cleaved free-radical reactions involving molecular oxygen. This reaction causes the release of malodorous and highly volatile aldehydes and ketones. Because of the nature of free-radical reaction, the reaction is catalyzed by sunlight. Oxidation primarily occurs with unsaturated fats.

1.2.6- Shift time of oils:

Shift work is an employment practice designed to make use of, or provide service across, all 24 hours of the clock each day of the week (often abbreviated as 24/7). The practice typically sees the day divided into shifts, set periods of time during which different groups of workers perform their duties. The term “shift work” includes both long-term night shifts and work schedules in which employees change or rotate shifts.

In medicine and epidemiology, shift work is considered a risk factor for some health problems in some individuals, as disruption to circadian rhythms may increase the probability of developing cardiovascular disease, cognitive impairment, diabetes and obesity, among other conditions. Shift work can also contribute to strain in marital, family, and personal relationships.

1.2.6.1 -Health Effects:

Shift work increases the risk for the development of many disorders. Shift work sleep disorder is a circadian rhythm sleep disorder characterized by insomnia, excessive sleepiness, or both. Shift work is considered essential for the diagnosis.

Shift work also can worsen chronic diseases, including sleep disorders, digestive diseases, heart disease, hypertension, epilepsy, mental disorder, substance abuse, asthma, and any health conditions that are treated with medications affected by the circadian cycle. Artificial lighting may additionally contribute to disturbed homeostasis. Shift work may also increase a person’s risk of smoking.

The health consequences of shift work may depend on chronotype, that is ,being a day person or a night person, and what shift a worker is assigned to

when individual chronotype is opposite of shift timing (day person working night shift), there is a greater risk of circadian rhythms disruption.

1.2.7 -Interesterification:

Transesterification is a term used to describe reactions in which FA esters react with FFA (acidolysis), alcohols (alcoholysis), or other FA esters (interesterification). In food applications, interesterification often refers to the reaction between different oils or fats (esters), with their fatty acyl groups rearranging among the molecules. Interesterification can be conveniently achieved by an alkali methoxide-catalyzed reaction at mild temperatures (20–100 °C). Microbial lipases are also widely used as biocatalysts in enzymatic interesterification. In contrast to the chemical process, the enzymatic process can be more selective if an enzyme with positional specificity is used.

1.2.8 - Saponification of Fats and Oil:

Fats and oils are esters of triglycerols and fatty acids. Hydrolysis can breakdown at fat or oil and release the triglycerol and fatty acids. If the hydrolysis is carried out under alkaline conditions a soap can be made the reaction is known as saponification. A saponification value can be found for individual fats and oils and it can be used to compare their relative molecular masses. It can also be used to compare relative chain length of the fatty acids that make up the fat or oil.

The saponification value of the fat or oil is defined as the number of milligrams of potassium hydroxide that react with 1 gram of the oil or fat.

The oil or fat should be refluxed with potassium hydroxide the unreacted potassium hydroxide should then be titrated against hydrochloric acid to determine the amount of un used potassium hydroxide. Therefore the

amount of potassium hydroxide that has reacted with the fat oil can be determined.

1.3- Physicochemical Characteristics of Oil

Physicochemical characteristics provide a base line for suitability of the oil. The physicochemical properties of the oil determined were: color, odor, density, refractive index , viscosity index , iodine value, saponification value, peroxide value , acid value, and unsaponification value.

1.3.1 - Color Determination:

Color of the respective oils was determined by physical observation in day light and under ultraviolet radiation of 254 and 366nm using ultraviolet chamber.

1.3.2 - Odor Determination:

Odor of the respective oils was determined by organoleptic evaluation

1.3.3 -Acid Value

Is an important physicochemical property index of oil which is used to determine the quality, age edibility and suitability of oil for industrial use such as paint. This value is used to measure the extent of glycerides in the oil ,which have been decomposed by lipase and other physical factors such as light and heat

1.3.4 -Density

Most of the information concerning the physical properties of hot pepper and other vegetable oils was reported many years ago. However, there are recent developments in establishing mathematical models to predict

changes in physical properties with FA composition and temperature. For vegetable oils, it has been shown that density decreases linearly with increase in temperature (Formo 1979): $r = b + mT$ where r is the density, T is the temperature, and b and m are constants. These constants are different for different oils. A widely used method for density prediction of vegetable oils was developed by Lund and discussed by Halverson and co-workers (1993). The Lund relationship is: $sg (15\text{ }^{\circ}\text{C}) = 0.8475 + 0.00030\text{ SV} + 0.00014\text{ IV}$ where sg is the specific gravity of vegetable oil at $15\text{ }^{\circ}\text{C}$, SV is the saponification value, and IV is the iodine value of the oil. This equation can be used for a wide variety of oils. For further details, readers are directed to the paper by Halverson and co-workers (1993). A generalized method of density estimation, which was also developed by Rodebush and coworkers (1999), was designed to predict oil viscosity, thereby relating two key physical properties

1.3.5- Viscosity:

The effect of temperature on the viscosity of various vegetable oils and FA was investigated by Nouredini and co-workers (1992). The relationship was expressed

$$\ln m = A + B/(T + C)$$

in which m is viscosity in centipoises, A , B , and C are constants and T is temperature in Kelvin. For each oil and FA, there is a set of constants that can be used to predict how temperature affects the viscosity of individual oils. The viscosity of fatty systems was also predicted by Rabelo and co-workers (2000) using the same temperature–viscosity relationship. The set

of A, B, and C values for each fatty compound class was then correlated with the number of carbon atoms and double bonds, and rather complicated relationships were established. Wang and Briggs (2002) studied the viscosity of hot pepper oils with modified FA composition. The viscosity was expressed as:

$\eta = Ae^{(Ea/RT)}$; in which R is the universal gas constant, T is temperature in Kelvin and Ea is the energy of activation. The concept of effective carbon number was used to describe acyl chain length and degree of unsaturation, and was correlated with viscosity and Ea. Linear relationships were established, which indicate that the more the saturation or the longer the fatty acyl chains, the more viscous the oil and the faster the viscosity will change with temperature fashion

1.3.6-Refractive index:

The refractive index (RI) is a parameter that relates to molecular weight, FA chain length, degree of unsaturation, and degree of conjugation. A mathematical relationship between RI and IV has been described by Perkins (1995b)

$$n_D^{25} = 1.45765 + 0.0001164 IV$$

The reverse relationship can be used to calculate the iodine value of crude hot pepper oil when the RI is known. RI was shown to increase by 0.000385 for each degree rise in temperature.

1.3.7- Unsaponifiable matter

is a measure of the amount of non-glycerol ester components in the oil (e.g., sterols, tocopherols, pigments, hydrocarbons) and is determined by measuring the mass of the sample taken up in a non-polar solvent after complete saponification of the oil and correction for residual fatty acids. For hot pepper oil, unsaponifiable matter is typically between 0.5% and 0.7% of the oil mass. The saponification value relates to the amount of potassium hydroxide that is needed to saponify a sample. In effect, it is useful for estimating the average molecular weight of the esterified fatty acids in a sample. For pepper seed and most oleic–linoleic-type vegetable oils, the saponification value ranges from 189 to 198 with an average value of 195.

1.3.8- Saponification Number (saponification value):

The saponification number is defined as the weight of potassium hydroxide, in milligrams, needed to saponify one gram of fat. This parameter is inversely proportional to the molecular weight of the fat. In other words, the higher the molecular weight, the lower the molecular weight.

Replacement of long-chain fatty acids such as erucic acid in hot pepper oil by C18 fatty acids increases the saponification numbers from 168–181 to 188–192 due to the reduction in molecular weight.

1.3.9- Iodine Value:

The iodine value (IV) indicates the degree of unsaturation of a fat or oil. It is defined as the number of grams of iodine absorbed by 100 grams of fat. The higher value for canola oil is due in part to the replacement of erucic acid with unsaturated C18 acids, mainly oleic acid, together with a slight

increase in the contribution of linoleic and linolenic acids currently; the iodine value is calculated from fatty acid composition using specific factors for each unsaturated fatty acid (Kyriakidis and Katsiloulis 2000). The calculation of the iodine value provides more accurate data, eliminating the unsaturation present in unsaponifiable compounds. The iodine value is a simple and rapid method that determines the amount of double bonds (average unsaturation) present in a fixed amount of oil. For pepper seed oil, iodine values of 98-118 are typical. Winterized stearin fractions and hydrogenated products have lower iodine values and winterized salad oil has slightly higher iodine values. Iodine value is widely used to monitor the progress of winterization and hydrogenation processes. When exposed to air and heat, vegetable oils tend to oxidize. This tendency is an important property for oils that are to be used for frying or other high-temperature purposes. During the initial stages of oil oxidation, oxygen reacts with the fatty acid double bonds to form hydro peroxides.

1.3.10 -The Peroxide Value:

Provides a measure of these oxidation components in the oil and is generally reported as the number of mill equivalents present per kilogram of oil, which is determined by monitoring the liberation of iodine from potassium iodide. Good-quality pepper seed oil will typically have peroxide levels of <1. The anisidine value is a second measure of oxidation that quantifies the presence of aldehyde groups in the oil formed with more advanced oxidation. Reaction of *p*-anisidine with aldehyde moieties results in yellow-colored products that are detected at 350 nm. Values of <10 are typical of good-quality pepper seed oil. While peroxide and anisidine values are useful for determining the oxidative state of an existing

oil, it is often more important to know the tendency of an oil to oxidize under a given set of conditions. The AOM stability test uses heat and aeration to accelerate oil oxidation, which is followed until the oil reaches a specified peroxide value. Usually, this peroxide value is 100 mill equivalents. RBD pepper seed oils tend have AOM stability times of 15-19 hr.

1.4 -Fatty Acid:

In chemistry, particularly in biochemistry, a fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated, most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms ,from 4to28.fatty acids are usually not found per se in organisms, but instead as three main classes of esters: triglycerides, phospholipids ,and cholesterol esters, in any of these forms, fatty acid are both important dietary sources of fuel for animals and they are important structural components for cells.

1.4.1- Length of Fatty Acids:

Fatty acids differ by length, often categorized as short to very long

- Short-chain fatty acids (SCFA). They are fatty acids with aliphatic tails of five or fewer carbons (e.g.butyric acid)
- medium-chain fatty acids(MCFA) are fatty acids with aliphatic tails of 6 to 12 carbons.which can form medium-chain triglycerides.
- Long-chain fatty acids (LCFA) are fatty acids with aliphatic tails of 13 to 21 carbons.
- Very long chain fatty acids (VLCFA) are fatty acids with aliphatic tails of 22 or more cabons.

1.4.2- Saturated Fatty Acids:

Saturated fatty acids have no carbon- carbon double bonds. They have the same formula $\text{CH}_3 (\text{CH}_2)_n \text{COOH}$, with variations in “n”. An important saturated fatty acid is stearic acid (n=16), which when neutralized with lye is the most common form of soap.

1.4.3 -Unsaturated Fatty Acids

Unsaturated fatty acids have one or more $c = c$ double bonds. The $c = c$ double bonds can give either cis or trans isomers, for example, oleic acid, with one double bond, and linolenic acid, with two double bonds, has more pronounced bend linolenic acid ,with three double bonds ,favors a hooked shape.

1.5 -Benefits of Hot Pepper Oil:

- helps reduce insulin levels
- Protects human heart
- boosts immunity
- helps fight inflammation
- lower blood pressure
- prevents cancer
- relieving pain
- boosting cognition
- preventing chronic disease

1.6 -Hot Pepper Plant:

(*Capsicum annum* L) is a widely grown vegetable crop in Sudan, and was introduced a long time ago. Variable local cultivar 3 are well known in the country, in western Sudan. Some unique and distinct local cultures are very famous. For example, the type called (Dingaba) which is extremely hot, I produced in Darfur state, while the type called (Gabaneeet), which is also very hot is produced in the Nuba mountain in kordofan state. Introduced sweet pepper cultivars like California wonder and yolo wonder are popular in the urban areas.

Taxonomy:

Scientific Name: *Capsicum annum. L*

Kingdom: plantae

Phylum: Tracheophyta

Class: magnoliopsida

Order: *Solanales*

Family: *Solanaceae*

Genus: *Capsicum*

Species: *C. annum*

Chapter two
Materials and Methods

2- Materials and Methods

2.1- Materials:

2.1.1-Plant Material:

Seeds of hot pepper were purchased from the local market-Khartoum and kindly taxonomically authenticated by Yahia Suliman Mohamed at herbarium of Medical and Aromatic Plants and Traditional medicine Research Institute (MATPMRI), Natural centre for research, Khartoum, Sudan.

2.1.2 -Chemicals:

n-hexane (assay :n-isomer95%, all isomer 99.5%,B 67-70°C ,d=0.66,water < 0.02% methanol (assay by GC 99.5% -CH₄O . M .wt 23.04).

Conc. sulphuric acid.

Conc. nitric acid.,

Hydrogen peroxide.

ethanol (absolute-extra pure, assay GC 99.9%)

petroleum ether (60-80CLR,min assay 95%,wt permal=0.67g)

potassium hydroxide(min assay 85.0%, KOH 56.11,max impurates K₂CO₃ 2%-CL 0.03% -SO₄ 0.02%)

glacial acetic acid

chloroform (assay 99.5%,M.wt=119.35 ,non volatile matter 0.002%, acidity 0.01ml N%).

potassium iodide

Sodium thiosuphate (Na₂S₂O₃.2H₂O (99-100.5)%

hydrochloric acid

carbon tetrachloride

10 % potassium iodide

alcoholic NaOH
alcoholic H₂SO₄
acidity 0.003%,
sodium sulphate

2.1.3 Instruments:

GC/MS-QP2010-Ultra

Lovibonod(TINTO meter) – Type D.

Viscometer.

Rotary Evaporator.

Refractometer.

2.2 -Methods:

2.2.1- Determination Color of Oil:

The color intensity was measured using a Lovibondtintometer, units of red, yellow and blue were recorded according to the AOAC (2008). Samples Appropriate cell (2inches cell) was filled with oil and placed in the tin to meter placed nearby the window for light. The instrument was switched on and looked upon through the eye piece. Slides were adjusted until color match was obtained. The readings of the filter, used to make the match (red , yellow, and blue).

2.2.2 - Determination of Refractive Index of Oil:

Refractive index was determined by Abbe-60 refractometer as described by AOAC(200) where a double prism was opened by means of screw head and few drops of sample were placed on the prism. The prism was closed firmly by lighting screw head. The instrument was left to stand

for few minutes before reading in order to equilibrate the sample temperature with that of the instrument. The prism was cleaned between readings wiping off oil with soft cloth, then with pepper moistened with petroleum ether and left to dry. The refractive indices of all samples were determined at 28.9°C.

2.2.3 -Determination of Viscosity of Oil:

The viscosity of the oil samples under investigation were recorded using Ostwald-U-tube viscometer according to Cocks and Van Rede (1966).The viscometer was suspended in the constant temperature water bath so that the capillary was vertical.

Minutes before reading in order to equilibrate the sample temperature with that of the instrument (35°C).

By means of pressure on the respective aim of the tube, the oil moved into the other arm so that the meniscus is (1 cm) above the mark at the top of upper reservoir. The liquid was then allowed to flow freely through the tube and the time required for the meniscus to pass from the mark above the upper reservoir to that at the bottom of the upper reserve was recorded and the viscosity was determined.

2.2.4 - Determination of Specific Density:

The specific gravity of the oil was determined using a density bottle according to the methods described by (AOAC, 2000). The weight of 50 mL empty density bottle (w_0) was recorded and the density bottle filled with water recorded (w_1) . Equivalent quantity of oil was replaced with the water in the same bottle (w_2) and weighed. The specific density of the oil was determined .

2.2.5 - Determination of Acid Value of Oil:

The acid value was determined using titration and the method adopted was described by the British Pharmacopeia (2007 Version 11). 10.00 g of oil to be examined were dissolved, in 50 ml of a mixture of equal volumes of ethanol (98%) and petroleum ether (1:1), then was neutralized with 0.1 M potassium hydroxide, 0.5 ml of phenolphthalein solution was used as indicator. After the mixture dissolved, was titrated with 0.1 M potassium hydroxide until the pink color persists for at least 15 s (n ml of titrant). The acid value (AV) was then calculated:

$$AV = \frac{56.1 \times V \times M}{W}$$

Where;

V: volume in mL of the standard potassium hydroxide.

M: morality of the potassium hydroxide solution.

W: weight in g of the sample.

56.1= molar mass of KOH.

2.2.6 - Determination of Peroxide Value of Oil:

The peroxide value (PV) of oils was determined using titration and the method adopted was described by the British Pharmacopeia (2007 Version 11). About one gm of the sample was weighed into Peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation, extent of secondary oxidation. It is defined as amount of peroxide oxygen per 1 kilogram of fat or oil. 5.00g of oil was placed 250 ml conical flask, 30 ml of mixture a glacial acetic acid/chloroform solution (3:2) were added, and the flask was' swirled until

the sample was dissolved 0.5 ml of saturated potassium iodide was added .The solution was again swirled for one minute, 30 ml of distilled water were added and 0.5 ml of 1% starch solution were also added. The contents of the flask were then titrated with 0.1 N sodium thiosulphate added gradually with constant and vigorous shaking and the titration was continued until the blue color just disappeared. A blank test was carried out. The number of 0.1 N sodium thiosulphate required was recorded. The peroxide value was calculated by;

$$PV = \frac{(V_a - V_p) \times N \times 100}{W}$$

Where;

V_a = volume (mL) of sodium thiosulphate.

V_p = volume (ml)of sodium thiosulphate from blank.

N = (0.1N) sodium thiosulphate.

W = weight of oil sample.

2.2.7 - Determination of Saponification Value of Oil:

The determination of saponification number was carried out according to the British Pharmacopeia (2007 Version 11).2.00gram of oil sample was weighed accurately in to 200 ml conical flask. 25 ml of 0.1N alcoholic KOH solution was added, and the contents of the flask were boiled under reflux for one hour with frequent rotation. one ml of phenolphthalein indicator was added, while the solution was still hot, and the excess alkali was titrated with 0.5N HCL. The numbers of ml of HCL required (a) were noted. The same process was repeated without oil and the numbers of ml of HCL (b) were also recorded. The saponification value (SV) was calculated.

$$SV = \frac{28.05 \times (b-a)}{W}$$

Where;

SV = saponification value (mgkoH/g)

a= ml of Hcl from sample.

b= ml of Hcl from blank.

W= weight of oil gram.

2.2.8 - Determination of Iodine Value of Oil:

The iodine value (I V) of the oils which quantifies their unsaturation level was determined according to AOCS recommended practice (AOCS, 1998). 0.2 g oil sample was dissolved in 15 mL carbon tetrachloride in a conical flask and 25 mL WIJ'S solution was added. The content was mixed vigorously when 20 mL of 10 % potassium iodide solution and 15 mL water were added. A blank was also prepared concurrently, both were placed in a dark room and allowed to stand for at least 1 hr. This was to allow for complete addition reaction between the double bonds of the oil and the liberated iodine to a pale yellow colour. At this point, a few drops of starch indicator solution were added and titrated against standard 0.1N Sodium thiosulphate to a blue end point. iodine value was then determined

The iodine value (I V) of the oils which quantifies their unsaturation level.

The iodine value was then determined from the relationship:

$$IV = \frac{m(b-a) \times 126.9 \times (100/1000)}{W}$$

Where:

126.9 = molar Mass of iodine.

M = molarity of sodium thiosulphate.

a=volume (ml)of sodium thiosulphate used for blank.

b=volume(ml)of thiosulphate used for the test.

100/1000 =Multiplication factor as define for iodine value.

W=weight of oil sample

2.2.9 - Determination of Ester Value:

Ester value = Saponification – Acid value

2.2.10 -Determination of Unsaponifiable Matter:

The full method for determining the unsaponifiable matter in oils in fats is BS 684 :1958, p.52 and in the 1968 BP, p.1269. In routine work, however, the extraction of unsaponifiable matter is done as follows: After the titration of the saponification value, the neutralised liquid is made alkaline again with 1ml of aqueous 3N potassium hydroxide solution, transferred to a separator and washed with water (50ml less the volume of 0.5N hydrochloric acid used). The solution is then extracted three times with 50 ml quantities of diethyl ether. Each ether extract is poured into another separator containing 20ml of water. After the third extract has been added, the combined ether extracts are washed with the first 20ml of washed water and then vigorously with two further 20ml quantities. The ether extract is washed twice with 20ml of aqueous 0.5N potassium hydroxide solution and at least twice with 20ml quantities of water until the washed water is no longer alkaline to phenolphthalein. The ether extract is poured into a weighed flask. The solvent is evaporated off, the residue is dried at not more than 80°C and weighed to constant weight. The unsaponifiable matter is then determined in neutral alcohol

were dissolved and titrated with 0.1N alkali (not more than 0.1ml should be required to neutralize any free fatty acid present).

2.2.11- GC/MS Analysis of Oil:

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was prepared by taking 2ml of sample in test tube, then 7 ml of alcoholic NaOH and 7 ml of alcoholic H₂SO₄ were added. Then the mixture was shake by vortex for 3 minutes and left to over night. 2ml of Add 2ml from supersaturated NaCl Was added to the mixture and with continuous shaking 2ml normal hexane was added and was shake for three minutes and then was collected the hexane layer, 5µL of collected hexane was taken and was diluted with 5ml diethyl ether ,then 1gram from sodium sulphate was added as drying agent, finally the sample was filtered through syringe filter 0.45 µm, the filtrate was Transferred and 1µL directly was injected to the GC-MS. injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 3 minutes hold time , the injection port temperature was 300c, the ion source temperature was 200c and the interface temperature was 250c. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 minutes .Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library ,the National Institute of Standards and Technology (NIST). , results were recorded.

Table (2.3):oven temperature program

Rate	Temperature(C)	Hold Time(min.)
-	60.0	0.00
10.00	300.0	3.00

2.2.12- Extraction of Oil from Seeds of Hot Pepper

Powdered seeds of hot pepper (300g) were exhaustively extracted with n-hexane (soxhlet). The solvent was removed under reduced pressure and the oil was kept in the fridge at $4C^o$ for further manipulation.

Chapter three
Results and Discussion

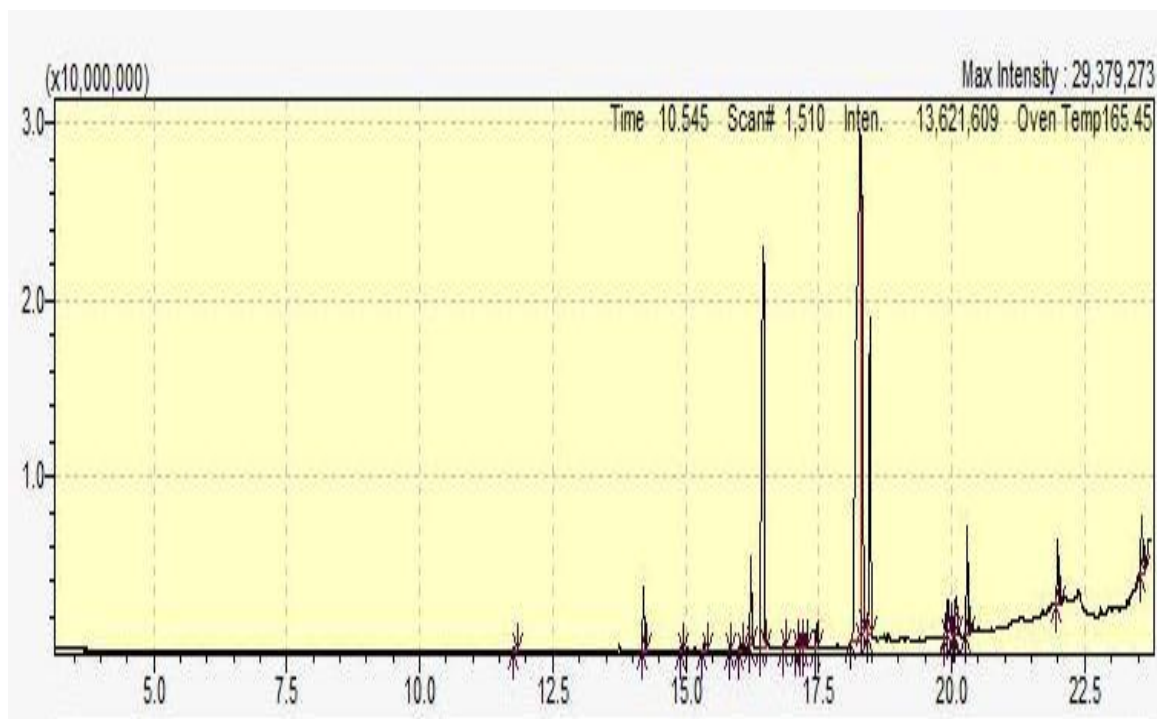
3- Results and Discussion

3.1 The GC-MS analysis of hot pepper fixed oil:

The oil from hot pepper seeds was analyzed by GC-MS and the characterization of the constituents was initially accomplished by comparison with the MS library (NIST) and also confirmed by interpretation of the recorded fragmentation pattern.

Table (3.1): Constituents of Hot Pepper from GC-MS analysis

Peak#	R.Time	Area	Area%	Name
1	11.765	361563	0.09	decanoic acid
2	14.205	6296794	1.57	Myristic acid
3	14.927	620755	0.15	Lauric acid
4	15.341	701388	0.17	Eicosapentaenoic acid
5	15.831	434451	0.11	Palmitoleic acid
6	16.026	851859	0.21	Iso-palmitic methyl ester
7	16.225	8499474	2.11	Methyl pamitioleate
8	16.464	76361225	18.99	Palmitic acid
9	16.854	461833	0.11	Palmitoleic acid
10	17.079	301626	0.08	15-Methyl palmitic acid
11	17.173	1022526	0.25	14-methyl palmitic acid
12	17.240	1445928	0.36	Methyl stearolate
13	17.457	2285654	0.57	Margaric acid
14	18.281	170984213	42.52	Linoleic acid
15	18.308	51237447	12.74	Oleic acid
16	18.467	40756183	10.14	Staric acid
17	19.889	2582773	0.64	Cyclopropanoic acid
18	19.929	4022606	1.00	Dihomo –gamma-linolein acid
19	20.058	3498915	0.87	Eicosadienoic acid
20	20.086	3392006	0.84	Eicosenoic acid
21	20.291	11683072	2.91	Arachidic acid
22	21.993	6798696	1.69	Behenic acid
23	23.572	7507024	1.87	Lignoceric acid
		402108011	100.00	



Fig(3.1): The total ion of chromatograms

3.1.2. Palmitic Acid (18.99):

Palmitic acid $C_{16}H_{32}O_2$ is saturated fatty acid according to the world organization; evidence is that consumption of palmitic acid increase the risk of developing cardiovascular disease. Based on studies indicating that it may increase LDL levels in the blood. Retinyl palmitate is an antioxidant and asouce of vitamin A added to low fat milk replace the vitamin content lost through the removal of milk fat. Rate fed a diet of 19% palmitic acid and 565 carbohydrate for extended periods showed alteration in central nervous system control of insulin section ,palmitic acid Palmitic acid is used to produce soaps, cosmetics(Kingsbury *et al*, 1961).

The mass spectrum of palmitic acid is shown in fig.3.1.1 The peak at m/z 270, which appeared at retention time R.T.16.464 in total ion chromatogram,

corresponds to $M [C_{17}H_{34}O_2]^+$. The peak at m/z 239 corresponds to loss of a meth oxide Group.

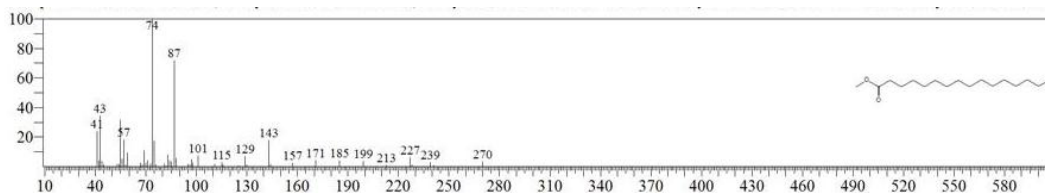
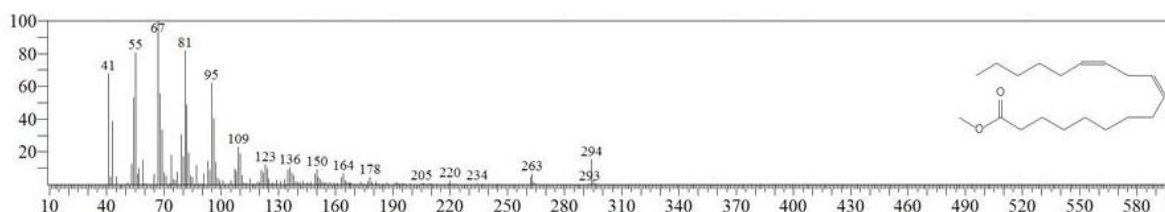


Figure (3.2): Mass spectrum of Palmitic acid

3.1.2 Linoleic Acid (42.52%):

Linoleic acid belongs to one of the two essential fatty acids. Which means that the human body cannot synthesize it from other food components that must be consumed for proper health? A diet deficient in linoleate causes mild skin scaling, hair loss, and poor wound healing in rates, Linoleic acid has become increasingly popular in the beauty products industry because of its beneficial properties on the skin, research points to linoleic acids anti-inflammatory, acne reductive ,skin ,lightening and moisture retentive properties when applied topically on the skin, linoleic acid lipid radicals can be used to show the antioxidant effect of poly phenols and natural phenols, linoleic acid the main polyunsaturated fat found in vegetable oil lower risk of coronary heart disease and improves insulin sensitivity and blood pressure(William and Haxnes, 2016).

The mass spectrum of linoleic acid is shown in fig.3.1.2 the peak at m/z 294, which appeared at retention time R.T. 18.281 in total ion chromatogram, corresponds to $M [C_{19}H_{34}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxyl function.

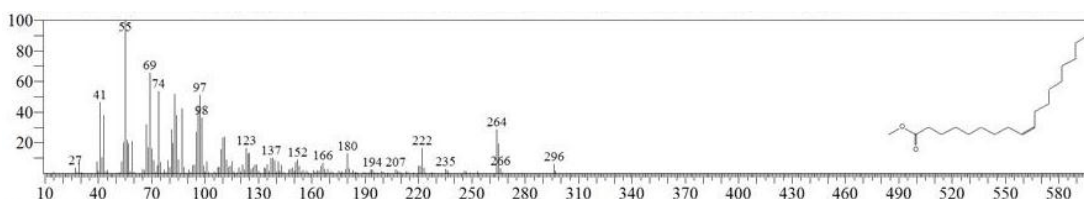


Fig(3.3):Mass spectrum of linoleic acid

3.1.3 Oleic Acid (12.74%):

Oleic acid is the most abundant fatty acid in human adipose tissue, oleic acid undergoes the reaction of carboxylic acid and alkenes, its soluble in aqueous base to give soaps called oleates, oleic acid is a common monounsaturated fat in human diet, monounsaturated fat consumption has been associated with decreased low-density lipoprotein(LDL) cholesterol, its ability to raise HDL is still debated, oleic acid may be responsible for the hypotensive (blood pressure reducing)oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer(Pala *et al*, 2001).

The mass spectrum of oleic acid is shown in fig.3.1.3 The peak at m/z 296, which appeared at retention time R.T.18.308 in total ion chromatogram, corresponds to $M [C_{19}H_{36}O_2]^+$. The peak at m/z 264 corresponds to loss of a methoxyl function.



Fig(3.4): Mass spectrum of oleic acid

3.1.4 Stearic Acid (10.14%):

Stearic acid is used in the production of detergents, soaps, and cosmetics such as shampoos and having cream products, an isotope labeling study in humans concluded that the fraction of dietary stearic acid that oxidatively desaturates to oleic acid is 2.4 time higher than the fraction of palmitic acid analogously converted to palmitoleic acid , stearic acid is less likely to be incorporated in to cholesterol esters, in epidemiologic and clinical studies, stearic acid was found to be associated with lowerd LDL cholesterol in comparison with other saturated fatty acids(Hunter *et al* ,2009).

The mass spectrum of stearic acid is shown in fig. 3.1.4 The peak at m/z 298, which appeared at retention time R.T.18.467 in total ion chromatogram, corresponds to $M [C_{19}H_{38}O_2]^+$.The peak at m/z 267 corresponds to loss of a methoxyl function.

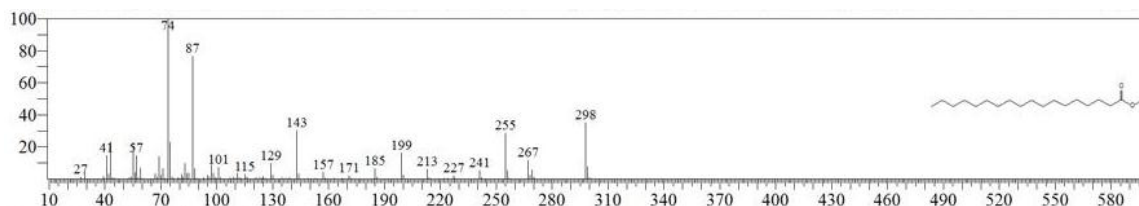


Figure (3.5): Mass spectrum of stearic acid

3.2- Physicochemical properties of oil:

The quality assessment of hot pepper seed oil was analyzed by evaluating physicochemical properties presented in Table (3.2.1). Viscosity was recorded to be 64.68 (poise) and Density was noticed to be 0.9039 which the standard 0.9 - 1.16and Refractive index was found to be 1.4807 which is similar to that standard 1.4677-1.4705 and the color present the yellow 36.3 higher than the red 8.5 and blue 0.26 .and the acid value

0.26(mgkoH/g) is lower than the standard 4 , peroxide value (2.32 mg Eg / kg) is lower than the standard < 10 ,the saponification value 215.57(mgkoH/g) is higher than standard (181.4) ,iodine value 13.72(mg/g) is lower than the standard 80-106, and present the unsaponification value 0.062 is small amount, and ester value 215.31is high amount

Table (3.2): Physicochemical properties of hot pepper oil of the sample and standard

Parameter	Hot pepper oil of sample	FAO/WHO standard
Viscosity	64.68	-
Specific Density	0.9039	0.9-1.16
Refractive index	1.4807	1.4677-1.4705
Color	Yellow36.3-red8.5	-
Acid value	0.26(mgkoH/g)	4
Peroxide value	2.32(mgEg/kg)	<1.0
Saponification value	215.57(mgkoH/g)	181.4
Iodine value	13.72mg/g	80-106
Unsaponification value	0.062	-
Ester value	215.31	-
Free fatty acid	o.13	

Table (3.2) shows the physical properties of oil, present the Density (0.9039), viscosity (64.68), Refractive index (1.4807) and the degree of the color: yellow (36.3) ,Red (8.5), Blue (0.26). This shows a good quality of the standard.

The viscosity of a fat is due to the internal friction between the lipids number of molecules that make up a fat by increasing the degree of unsaturation the viscosity deceases and when the length of the chain increases the fatty acids components also increases the viscosity

The refractive index of substance is defined as the ratio between the speed of light in air and in matter (oil or fat) that is analyzed. Increasing the degree of unsaturation increases the refractive index and when the length of the chain increases. The refractive index also increases. Found the RI (1.4807) value. Density decrease when fats dilate when going from solid to liquid. When a fat melts its volume increases and consequently its density decreases. The density value was found to be (0.9039).

Determination the color is different degree of color, yellow (36.3), red (8.5), blue (0.26).

Table(3.3):Physical properties of hot pepper oil

Sample	Density (g/cm)	Viscosity (poise)	Refractive Index	Color		
				Yellow	Red	Blue
Pepper oil	0.9039	64.68	1.4807	36.3	8.5	0.26

Table (3.3) shows GCMS and identities of the fatty acids present in the oil. The most prominent of the fatty acids are palmitic acid (18.99%), oleic acid (12.74%), linoleic acid (42.52%) and stearic acid (10.14%). The unsaturated fatty acid of hot pepper oil good edible oil. However, oils with high degree of unsaturation are considered good for the heart because it decreases total cholesterol and low density lipoproteins. And saponification value (215.57),is higher sponify and esterification ,however ,low acid value(0.26),and iodine value(13.72) .and peroxide value (2.32).and low unsaponification value(0.062).

The PV of oil is used as measurement of the extent to which rancidity reactions have occurred during storage. This parameter expresses the oxidation in its early stage. Values obtained were low (mean 2.32) compared to the maximum acceptable value 10megkoH/g the low values of PV are

indicative of low levels of oxidation rancidity of oils. The PV increase in short time with the temperature while the temperature is increasing, the change of the partial pressure of the oxygen had less influence on the reaction rate of oxidation, because the oxygen is becoming less soluble in lipids and water

The IV is an indicator of the degree of unsaturation of oils .the IV is the mass of iodine in grams that is consumed by 100grams of oil. IV Provides on overall status of unsaturation of the oils .iodine value increases with increasing of unsaturation of oil. As in the measurement of % FFA, are oil have acceptable value of IV in comparison with the range established by Algerion Official Journal(120-143g /100g oil)and codex STAN210, the high IV indicate a high degree of unsaturation for our oils. Found IV (13.72) lower value indicates low degree of unsaturation.

The saponification number is defined as the number of milligram of potassium hydroxide needed to saponify completely one gram of oil the number of saponification number depeds on the value of the molar mass of oil. The higher value of the molar mass of oil . the lower number of saponification value. Triglycerides that contain long fatty acids have lower number of saponification than the triglycerides that have shorter fatty acids. This is because the lower number of carboxylic functional group per unit mass of oil caused higher molecular weight and less acid per gram of oil hydrolyzed. Found high SV (215.57) that mean shorter fatty acid and lower value of the molar mass

Acid Value is the mass of potassium hydroxide (koH) in milligram that is required to neutralize one gram of chemical substance . the acid number is a measure of the number of carboxylic acid groups in chemical

compound. Low acid value indicates good cleaning by soap. Found AV (0.26) is lower value indicate low number of carboxylic group

The unsaponifiable matter consists of substance present in oil which are not saponifiable by alkali hydroxide and are determined by extraction with an organic solvent of a solution of the saponified substance. Found unaponifiable number (0.062) is lower value

Free Fatty acids (FFA) are produced by the hydrolysis of oils. FFA are less stable than neutral oil .they are more prone to oxidation and to turning rancid. Found FFA(0.13)is lower value

The ester value is the number of mg of potassium hydroxide required to saponify the esters in 1g of the substance. Found the ester value (215.31) is higher

Table(3.4):Chemical properties of hot pepper oil

Sample	Pepper oil
Peroxide value	2.32
Acid value	0.26
Saponification value	215.57
Iodine value	13.72
Unsaponification value	0.062
Free fatty acid	0.013
Palmitic acid	18.99
Linoliec acid	42.52
Oleic acid	12.74

Stearic acid	10.14
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3.3 Conclusions:

Hot pepper seeds oil of Sudanese origin shows 23 constituents of fatty acid. With linoleic acid, palmitic acid, oleic acid, stearic acid as major abundant, followed by lauric acid, arachidic acid and behenic acid. The physiochemical properties of hot pepper seed such as: viscosity, refractive index, density, acid value and iodine value, peroxide value, saponification value present a good amount compared with edible oil but the saponification is higher amount that indicate is a good source in soap industry.

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Appendices



Date 24.4.2018

To whom it may concern

This to certify that the plants materials were taxonomically authenticated Yahya Sulieman Mohamed at herbarium of Medicinal and Aromatic Plants & Traditional Medicine Research Institute (MAPTMRI), National center for Research, Khartoum, Sudan, and voucher herbarium samples were deposited there for further future reference.


Botanical Name:- . *Capsicum annum L.*

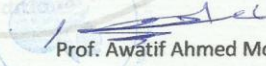
vern.Name:- shata

Family:- Solanaceae

Name:- Zubaida Daud Ali Hassan

Sudan University science and technology


Hanaa Hassab Al Rasoul
Head department of Taxonomy &
Phytochemistry
Institute (MAPRTMI)


Prof. Awatif Ahmed Mohamed
Director of Medicinal and Aromatic
Plants Research & Tradition Medicine

ID#	Name	Ret. Time	Area	Area%
1.	Dodecanoic acid, methyl ester	11.765	361563	0.09
2.	Methyl tetradecanoate	14.205	6296794	1.57
3.	Tridecanoic acid, 12-methyl-, methyl ester	14.927	620755	0.15
4.	Pentadecanoic acid, methyl ester	15.341	701388	0.17
5.	cis-9-Hexadecenoic acid	15.831	434451	0.11
6.	Pentadecanoic acid, 14-methyl-, methyl ester	16.026	851859	0.21
7.	9-Hexadecenoic acid, methyl ester, (Z)-	16.225	8499474	2.11
8.	Hexadecanoic acid, methyl ester	16.464	76361225	18.99
9.	Methyl hexadec-9-enoate	16.854	461833	0.11
10	Hexadecanoic acid, 15-methyl-, methyl ester	17.079	301626	0.08
11	Hexadecanoic acid, 14-methyl-, methyl ester	17.173	1022526	0.25
12	10-Octadecynoic acid, methyl ester	17.240	1445928	0.36
13	Heptadecanoic acid, methyl ester	17.457	2285654	0.57
14	9,12-Octadecadienoic acid, methyl ester	18.281	170984213	42.53
15	9-Octadecenoic acid (Z)-, methyl ester	18.308	51237447	12.74
16	Methyl stearate	18.467	40756183	10.14
17	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	19.889	2582773	0.64
18	8,11,14-Eicosatrienoic acid, methyl ester	19.929	4022606	1.00
19	10,13-Eicosadienoic acid, methyl ester	20.058	3498915	0.87
20	cis-11-Eicosenoic acid, methyl ester	20.086	3392006	0.84
21	Eicosanoic acid, methyl ester	20.291	11683072	2.91
22	Docosanoic acid, methyl ester	21.993	6798696	1.69
23	Tetracosanoic acid, methyl ester	23.572	7507024	1.87