University of Benghazi

Faculty of Science

Chemistry Department

Title of thesis

Chromatographic analysis of some constituents of Libyan olive oil

M.Sc. Thesis

By

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Supervised by

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Submitted in Partial Fulfillment for the Requirements for the

Master Science degree in Chemistry

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Dedication

I dedicate this humble work to my wonderful wife *RANDA ABDULHAMID* and my dear daughter *MARAM*. I tell them: Thanks for supporting me during my studies and urging me on it.

And to my father, who did not spare the days something.

And to my mother (rahmha allah), who provided me tenderness and love.

And to all my brothers, sisters and my family.

And to my colleagues and my friends.

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Lastly, I offer sincere thanks to my family members for their endless support, encouragement and love.

Abstract

Olive oil is differ from other plant oils in its high phenolic and unsaturated oil contents. Because it has high level in unsaturated fatty acids (Oleic acid) olive oil resistance to oxidation and the protection against some diseases has been linked to these components of olive oil.

In this work, to determine the fatty acids compositions and phenolic contents (phenolic acids) of Libyan olive oil, eighteen Libyan olive oil samples were collected from different agricultural in Libya, from Benghazi in the east to Nalute in the west of the country, about 1150 Km in length of the Mediterranean coast.

The fatty acids compositions in the Libyan olive oil were separated by liquid-liquid extraction before measured by gas-liquid chromatography, and the phenolic acid were separated by solid phase extraction before measured by reversed-phase HPLC .

The oleic acid values in oil samples were found to be within the optimum levels (55-83%) with the exception of Wadi Ekaam sample.

The type of phenolic acids found in the samples were: caffeic acid, catechol, ferulic acid, p-counaric acid, p-hydroxy benzoic acid, vanillic acid, resorcinol, synergenic acid, sinapic acid, except that collected from Benwaleed area. The range of phenolic acids for all samples were found to be between (1.22-39.4 ppm). the acidity, saponification value, absorbency in ultra-violet at K232 and K270, density, and refractive index were also measured for quality of olive oil. these methods used to determine the olive oil quality and its resistance to oxidative deterioration. It is concluded from this study that most Libyan olive oil samples confided to be good quality , and most samples were virgin grade.

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List of Abbreviations

AOAC	Association of Official Analytical Chemists
ASVD	Arteriosclerotic vascular disease
ASTM	American Society for Testing Materials
BC	Before Christ
CAN	Carbon Atom Number
DAG	Diacylglycerols
DHT	Dihydrotestosterone
EEC	European Economic Community
EC	European Community
EVOO	Extra virgin olive oil
EU	European Union
FAMEs	Fatty Acid Methyl Esters
FFA	Free fatty acids
FID	Flame Ionization Detector
HDL	High density lipoprotein
HPLC	High Performance Liquid Chromatography
GLC	Gas Liquid Chromatography
IOOC	International olive oil council
LDL	Low density lipoprotein
LOX	Lipoxygenase pathway
MAG	Monoacylglycerols
MUFAs	Monounsaturated fatty acids
PV	Peroxide Value

PUFAs	Poly Unsaturated Fatty Acids
TAG	Triacylglycerols
VOO	Virgin Olive Oil
UV	Ultraviolate
RI	Refractive Index
RRT	Relative Retention Time
SFAs	Saturated fatty acids
SPE	Solid Phase Extraction
ONAOO	National organization of olive oil tasters

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CHAPTER I INTRODUCTION

1.1 Introduction

Olive oil is one of the oldest known vegetable oils mainly produced in the countries surrounding the Mediterranean Sea. It is a natural fruit juice, obtained from the fruit of the tree "Olea europea", with a unique composition and quality [1].

Olive (Olea europaea) is an evergreen species, widely cultivated in the Mediterranean basin and its oil is a predominate component of the worldwide known "Mediterranean diet" [2] .

Olive "Olea europaea " tree, which belongs to the olive family, comprises some 400 species, and thrives in temperate and tropical climates. Of the 35 species in the genus Olea, mainly of African, Indian, and Australian origin, Olea europaea is the only Mediterranean species. Although its origin is not known, one theory is that it originated in ancient Iran and Turkestan, spreading westward to Anatolia, Syria, and Palestine along commercial and migratory [3].

Olive oil is an important component in the diet of Mediterranean people, due to its beneficial effects on human health. Some of these effect are associated with olive oil content. Olive oil is considered as one of the best sources of fatty acids and the natural antioxidants. e.g. polyphenols, tocopherols. The nutritional properties, the excellent taste and aroma of olive oil are high valued for their positive effect on human health [4]. Olive oil is not obtained by using solvents, re-esterification processes, or mixed with other vegetable oils qualifies under this description. By obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvant having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds (seed or nut oils).

The oil is produced by grinding whole olive and extracting the oil by mechanical or chemical means. Olive oil is used throughout the world. especially in the Mediterranean countries. It is commonly used in cooking, cosmetics, pharmaceuticals, soaps and as a

fuel for traditional oil lamps. The importance of virgin olive oil is related to its high levels of monounsaturated fatty acids (mainly oleic acid), and several antioxidants [5].

Virgin olive oil quality depends on many factors related to olive tree cultivation and to the harvesting, storage and olive processing steps and time [6]. Of particular importance for olive oil quality are the olive cultivar, the climatic conditions of cultivation, as well as the pruning, fertilization and irrigation of olive trees. Harvest timing can have a significant effect on oil quality as well as on yield, oil stability and sensory characteristics. In order to obtain a characteristically fragrant and delicately flavored olive oil, it is imperative that it is properly extracted from undamaged fruits at its best degree of ripeness. This illustrates the need to determine the quality of olive oil from a range of harvest times and cultivars to establish an optimum harvest time [7].

The fruit of olive tree contains about 35-70 percent oil and the pulp contains more than 75 percent oil. The fruit of the olive tree is an egg-shaped drupe, consisting of a pericarp and an endocarp. The pericarp include sanepicarp (skin) of variable thickness according to the variety, and a mesocarp (pulp) surrounding the endocarp (woody pit) in which the seed is enclosed [8, 9].

The yield per hectare of fruit is about 2.45 tons. Oil yield per 100 kg of fruit is 19.6 kg (based on yields in Italy during the past 10 years). The oil is obtained by pressing. The oil extracted by first pressing is of the highest grade, called "virgin" olive oil and can be used without refining. With successive pressing the grade become low and require subsequent processing like refining by solvent extraction, deodorization and other treatments. The oil obtained from the last pressing is called "olive foots" or "sulfur olive oil" as it is refined by carbon disulfide. But this solvent is now completely displaced. "olive foots" is inedible and it is used for soap making and other industrial purposes.

1.2 History of olive tree

The olive tree has played an important role within history and has appeared so frequently within ancient mythology that even today, despite our advancements in farming and production, we are still not certain of its exact story [10].

Scholars have argued that the formal cultivation of olive trees for first occurred around 6000 years ago on the Mediterranean coasts of modern days of Syria and Palestine. In those days the oil extracted would have been used as a skin emollient and as fuel for lighting. For the banks of the eastern Mediterranean, the olive tree then moved west, taking root on the island of Cyprus as well as in Anatolia, Crete, and Egypt [11].

In the 6th century BC olive tree could be found in Tunis, Tripoli, Sicily, and southern Italy. In North Africa, the Berbers were known to have developed the cultivation of wild olives throughout the territories they occupied, and the Romans continued expansion in using them as a peaceful weapon in order to settle groups of people throughout their empire.

By the 16th century BC, the Phoenicians had begun to spread the olive through the Greek isles where it gained is importance to the extent that Solon, the great Athenian statesman, would later issue decrees regulating their planting and laws would make the destruction of the olive tree punishable by death.

In modern times the olive tree has moved well beyond the Mediterranean and can be found in countries as far from its origins as Australia, China, Unites States and Argentina. The trees we know today with their elongated leaves and fleshy oil rich fruit were probably derived from a cross between different species and bear little resemblance to its wild, bush-like ancestor known to civilizations all those years ago.

1.3 Concepts about olive tree and olive oil

Historians date the first olive trees in Palestine to 4,000 BC. The fruit of the olive tree is called the olive. Olives can be eaten whole after they are cured and they also produce olive oil when crushed.

Olive trees can live up to 600 years or more. Olives were traditionally harvested by hand picking or beating the tree with sticks. Modern harvesting methods include tree shakers and harvesters, which vibrate or 'comb' the tree to collect the fruit

Green olives harvested early produce a green oil, while ripe olives harvested later in the season produce a more yellow oil. Olive oil contains a wide range of important antioxidants that are not found in other oils.

Of all the olive oil produced in the world it is estimated that only 10 percent is 'extra virgin'. This is the best grade of olive oil available and is the healthiest olive oil you can eat.

Olive oil contains no cholesterol and is a good source of Vitamin E. It contains more monounsaturated fatty acids than any other fat or oil. Using olive oil in place of saturated fats as the main fat in your diet will reduce cholesterol levels.

Olive oil is the healthy substitute for any fat or oil used in any recipe. It uses in baking and frying. It also contains anti-oxidants and can be reused many times more than other fats and oils before being discarded.

1.4 World production and consumption of olive oil currently

More than 95% of the world's olive trees grow in the Mediterranean Basin. About 81% of total olive production comes from the European Community (EC) (Spain, Italy, Greece, Portugal, and France), with the near east contributing 7% and North Africa supplying about 11%. The remaining 1% is of American origin, chiefly from Argentina, Mexico, Peru, and the United States [12].

This heavy concentration of olive oil production in these countries is explained by the very demanding climatic requirements of the olive tree and the fact that virtually all olive trees are grown in a Mediterranean-type climate [8,9].

The main producer countries are also the main consuming countries, such as Spain, Italy and Greece (Figure 1.1). 71% of world consumption is concentrated in European Union countries. Mediterranean basin countries represent 77% of world consumption. United States, Australia and Japan can also be counted among the other consuming countries [9].

Table 1.1 shows The main producing and consuming countries of olive oil (metric tons):

Table 1.1: the main producing and consuming countries [13]

Country	Production in tons (2009)	Production % (2009)	Consumption (2005)
World	1,911,115	100%	100%
Spain	1,199,200	41.2%	32.1%
Italy	587,700	20.2%	26.3%
Greece	332,600	11.4%	15.1%
Syria	168,163	5.8%	4.8%
Tunisia	150,000	5.2%	4.8%
Turkey	143,600	4.9%	4.5%
Morocco	95,300	3.3%	2.0%
Algeria	56,000	1.9%	1.4%
Portugal	53,300	1.8%	1.2%
Argentina	22.700	0.8%	0.8%
Lebanon	19,700	0.7%	0.3%
Jordan	16,760	0.6%	0.7%
Libya	15,000	0.5%	0.3%

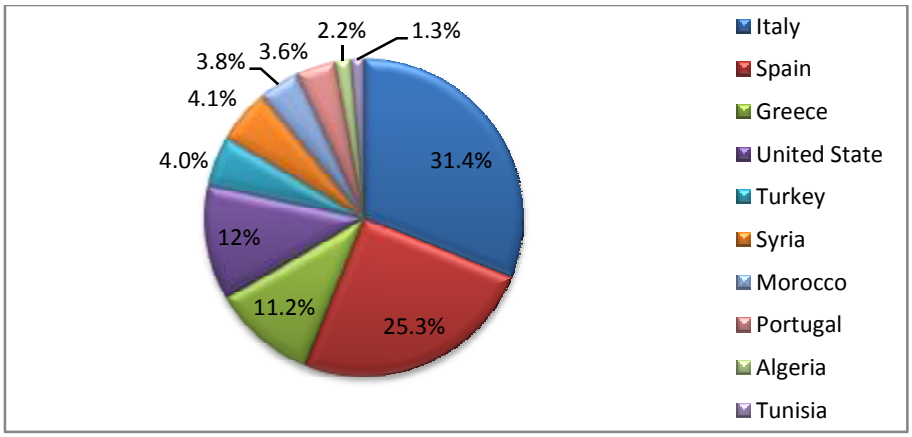


Figure 1.1: world olive oil consummation in 2009 [14]

1.5 Olive oil category

According to the international olive oil council and Turkish food codex, the designation and categorization of olive oils and olive–pomace oils are explained below. free acidity is expressed as % oleic acid [15].

1.5.1 Virgin olive oils

Obtained solely by mechanical or physical means under thermal conditions that do not lead to alterations in the oil; using only treatments such as washing, decantation, centrifugation, and filtration. Those fit for human consumption are as follows:

1.5.1.1 Extra virgin olive oil

This oil, as evaluated numerically by the mean of a certified taste panel, contains zero defects and greater than zero positive attributes. In other words, more than half of the tasters indicated that it is not defective and has some fruitiness. Extra virgin oil also must have a free acidity percentage of less than 0.8% and conform to all the standards listed in its category. This is the highest quality rating for an olive oil. Extra virgin olive oil should have clear flavor characteristics that reflect the fruit from which it was made. In relation to the complex matrix of variety, fruit maturity, growing region, and extraction technique, extra virgin olive oils can be very different from one another.

1.5.1.2 Virgin olive oil

This is oil with a sensory analysis rating of the mean of tasters, having defects from 0 to less than 2.5, a free acidity of less than 2%, and conforms to all the other standards in its category. These are oils with analytical and sensory indices that reflect slightly lower quality than extra virgin olive oil.

1.5.1.3 Ordinary virgin olive oil

Oil with a lower organoleptic rating (defects from the mean of tasters 2.5 to less than 6.0), a free acidity of less than 3.3%, and conformity within its category for all other standards. This is inferior oil with notable defects that is not permitted to be bottled under European Union (EU) laws, so it is sent for refining. The EU has eliminated this category and other regulating agencies are likely to follow. It will simply be absorbed into the lampante category.

1.5.2 Virgin olive oil (not fit for human consumption (lampante))

Oil with severe defects (greater than 6.0) or free acidity of greater than 3.3%, and which conforms to the other standards within its category. It is not fit for human consumption and must be refined. These oils come from bad fruit or from improper handling and processing. This grade is designated as not fit for human consumption.

1.5.3 Refined olive oil (not fit for human consumption)

Oil obtained from virgin oils by refining methods that do not alter the initial glyceride structure. It has a free acidity of less than 0.3 and must conform to the other standards within its category. Refined olive oil must not come from the solvent extraction of pomace. The refining process usually consists of treating virgin oil/ lamp ante with sodium hydroxide to neutralize the free acidity, washing, drying, odor removal, color removal, and filtration. In the process, the oil can be heated to as high as 430^{o}F (220^{o}C) under a vacuum to remove all of the volatile components. Refined olive oil is usually odorless, tasteless, and colorless. It is designated as not fit for human consumption.

1.5.4 Olive pomace oil

Obtained by treating olive pomace with solvents. It does not include oils obtained in the reesterification processes or any mixture with oils of other kinds (seed or nut oils).

1.5.4.1 Crude olive pomace oil (not fit for human consumption)

This is the solvent extracted crude oil product as it comes out of the pomace extractor after distillation to separate and recover most of the solvent. EU law also defines any oil containing 300-350mg/kg of waxes and aliphatic alcohols above 350 mg/kg to be crude pomace oil. It is designated as not fit for human consumption, but is intended for refining.

1.5.4.2 Refined olive pomace oil (not fit for human consumption)

Oil obtained from crude pomace oil by refining methods that do not alter the initial glyceride structure. It has a free acidity of not more than 0.3% and its other characteristics must conform to the standard in its category. Refining includes the same methods used for "refined olive oil" except that the source of the raw product comes from pomace by means of solvent extraction. It is designated as not fit for human consumption.

1.5.4.3 Olive pomace oil

A blend of refined olive-pomace oil and virgin olive oil that is fit for human consumption. Must has a free acidity not exceeding 1% and must conform to the other standards within its category. In no case shall this blend be called "olive oil."

1.6 Olive oil composition

The olive fruit is a drupe, oval in shape and composed of two basic parts; the pericarp and the endocarp (the pit or kernel). The pericarp is composed of the epicarp (skin) and the mesocarp (pulp). The pericarp contains 96% to 98% of the total amount of oil, with the remaining 2% to 4% in the kernel [16].

Olive oil can be divided into major and minor fractions with regard to its chemical composition. The major components that include Triacylglycerols (TAG) and the group

of Glyceridic compounds made up of Free Fatty Acids (FFA) and Mono-Acylglycerols (MAG) and Diacylglycerols (DAG), represent more than 98% of the total oil weight. Minor components, that amount to about 2% of the total oil weight, include more than 230 chemical compounds such as Phospholipids, Waxes, Aliphatic and Triterpenic Alcohols, Esters of Sterols, Hydrocarbons, Volatile Compounds, Carotenoids, Chlorophylls And Antioxidants [17].

1.6.1 Major components

1.6.1.1 Triglyceride

Triglyceride is three fatty acids attached to a glycerol backbone. Technically it is a type of glycerolipid. Triacylglycerols are the major energy reserve for plants and animals. Chemically speaking, these molecules are derived from the natural esterification of three fatty acid molecules with a glycerol molecule. The glycerol molecule can simplistically be seen as an "E-shaped" molecule, with the fatty acids in turn resembling longish hydrocarbon chains, varying (in the case of olive oil) from about 14 to 24 carbon atoms in length. Figure 1.2 shows the triglyceride molecule.

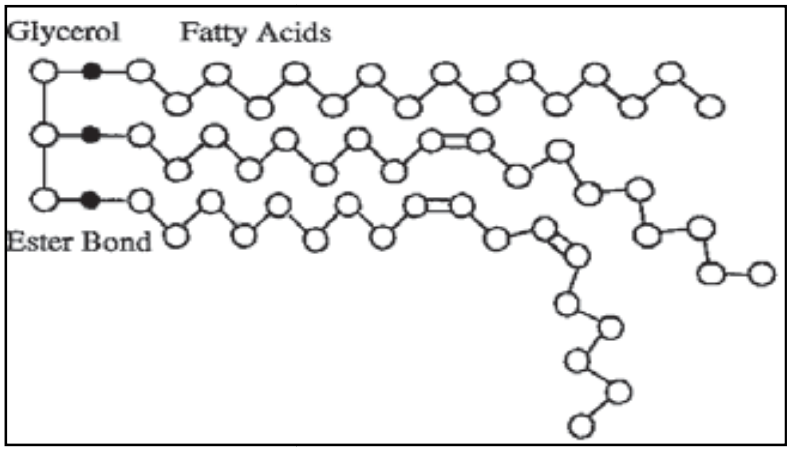


Figure 1.2: Triacylglycerol (oil) molecule with three different fatty acids attached [15]

1.6.1.2 Fatty acids

In the fatty acids molecules carbon atoms normally numbering between 4 and 30 (Figure 1.3). These carbon atoms are linked in a kind of chain. When two carbon atoms

are linked together with two links, a double bond is said to be present . If there are no double bonds, the acid is said to be saturated (e.g. palmitic acid, stearic acid). If a double bond is present, it is said to be monounsaturated (e.g. Oleic acid); if there are two or more double bonds, the fatty acid is said to be polyunsaturated (e.g. linoleic acid, linolenic acid). In oils, fatty acids are mainly combined in the triglycerides (see glycerides), whereas the free ones confer acidity. If two double bonds are separated by only one single bond, there will be two double conjugated bonds (conjugated diene). If three double bonds alternated with single bonds are present, there will be three double conjugated bonds (conjugated triene). Symbols such as C18:3 refer to a fatty acid with 18 carbon atoms and 3 double bonds [18].

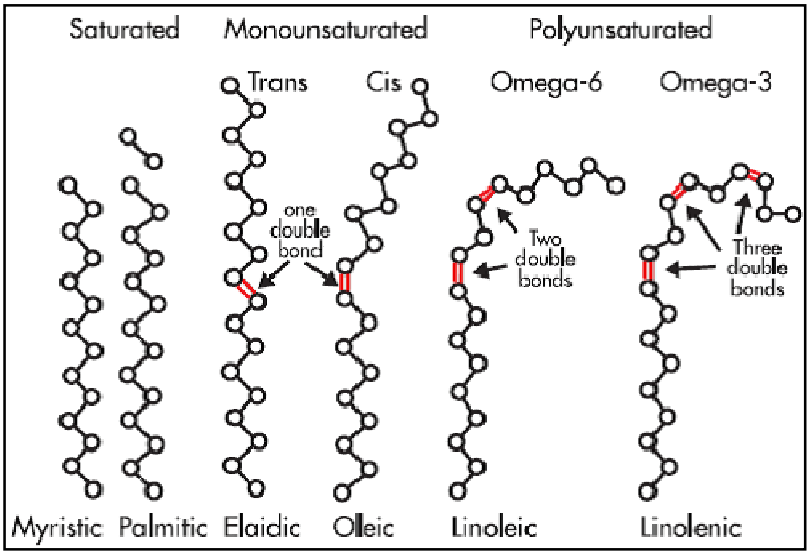


Figure 1.3: Forms of some fatty acids in olive oil [15]

- The olive oil is made of primary fatty acids ,oleic and linoleic acid with a small amount of linolenic acid.
- Olive oil has no trans fatty acids, because it has not been partially hydrogenated in a factory to make it solid at room temperature like margarine has. When an oil is partially hydrogenated it can be in the cis or trans conformation which refers

to which side of the fatty acid double bond the hydrogen is on. Trans fat is created by bubbling hydrogen through 250 to 400 degree hot vegetable oil in the presence of a metal catalyst, usually nickel or platinum. The process can take several hours. You cannot accidentally make trans fatty acids at home on your range when heating olive oil or other oil.

- Regarding to the poly-unsaturated fatty acids (PUFAs) there is a wide range acceptable for EVOO, however the linolenic has to be less than 0.9% (IOOC). There is no problem if the levels are higher than 1.5% regarding to the olive oils nutritional value. But the IOOC linolenic acid level is used to establish the authenticity of the olive oil. Seed oils like Canola have higher levels of linolenic acid. Also the higher the level of unsaturation; i.e. more PUFAs leads to a less stable oil. This has to be counterbalanced by the levels of antioxidants that protect the oil. These will also vary by similar factors to the fatty acid profile as well as stress drought. A higher linolenic than the IOOC may actually be of benefit nutritionally for reasons other than those associated with oleic acid.
- Omega-3 and Omega-6 fatty acids: Olive oil contains both omega-3 and omega-6 fatty acids. Omega-3 fatty acids are important in preventing cardiovascular disease and are particularly high in oily fish such as salmon. While olive oil is not terribly high in omega-3, the ratio of the two omega acids has been found to be more important and olive oil has a great ratio.

a. Level of the fatty acids in olive oil

As shown in Table 1.2 many fatty acids were detected in the virgin olive oils, refined olive oil and olive-pomace oil. palmitic and oleic acids were considered as major fatty acids while palmitoleic, stearic, linolenic and linoleic acids were low.

Table 1.2. Fatty acid percentage composition in the olive oil [15]

Fatty Acid	CAN*	Virgin Olive Oils %	Refined Olive Oils %	Olive-Pomace Oils %
Trans Linoleic acid	C18:1	0.0-0.05	0.0-0.2	0.0-0.4
Trans Linolenic acid	C18:2	0.0-0.05	0.0-0.3	0.0-0.35
Myristic	C14:0	0.0-0.05		
Palmitic acid	C16:0	7.5-20.0		
Palmitoleic acid	C16:1	0.3-3.5		
Heptadecanic	C17:0	0.0-0.3		
Heptadecenic	C17:1	0.0-0.3		
Stearic acid	C18:0	0.5-5.0		
Oleic acid	C18:1	55.0-83.0		
Linoleic acid	C18:2	3.5-21.0		
Arachidic acid	C20:0	0.0-0.6		
Gadoleic acid	C20:1	0.0-0.4		
Behenic acid	C22:0	0.0-0.2		
Lignoceric acid	C24:0	0.0-0.2		

*** CAN** Carbon atom number

b. Health aspects linked to fatty acid in olive oil

- Fatty acid percentage composition of the olive oil is shown in table 1.2; In this Table the olive oil has a high percentage of Oleic acid (55.0%-83.0%), so the olive oil (the unsaturated fat in the diet) is linked with a reduces in the risk of coronary heart disease.
- Unlike saturated fat, olive oil lowers the bad cholesterol (LDL) in the blood. It is known to lower blood sugar and blood pressure. This because olive oil contains high monounsaturated fatty acid (oleic acid) [19].

1.6.2 Minor components

1.6.2.1 Sterols

Sterols are complex compounds which carry out biochemical functions within cellular membranes. Their composition is typical of the botanic species from which the oil originates. The concentration of sterols in the oil is about 2600 mg / kg. They have a characteristic composition and may be used to classify olive oils from different regions. From a nutritional point of view, the most significant is the β-sitosterol which helps to reduce the absorption of cholesterol in mammals. The main sterols of olive oil are Campesterol, Stigmasterol, Clerosterol, B-Sitosterol, Sitostanol, and D-5-Avenasterol. These are accompanied by small amounts of Cholesterol (max. 0.5%), Brassicasterol (max. 0.1%), 24- Methylene- Cholesterol (max. 0.5%), Campestanol (max. 0.5%),D-5,24-Stigmastadienol (max. 1%), D-7-Stigmastenol (max. 0.5%), and D-7-Avenasterol (max. 1.1%). Analysis of the sterol fraction isolated from the unsaponifiable fraction is very important, for determining the authenticity of the oil. The triterpenes and sterols are present both as free alcohols and as fatty acid esters [20] .

1.6.2.2 Hydrocarbons

Hydrocarbons, such as Squalene Figure 1.4, is the major olive oil hydrocarbon and makes more than 90% of the hydrocarbon fraction which ranging from 200 to 7500 mg/kg oil or even higher (800−12000 mg/kg oil). Squalene is regarded as partially responsible for the beneficial effects of olive oil against certain cancers [21].

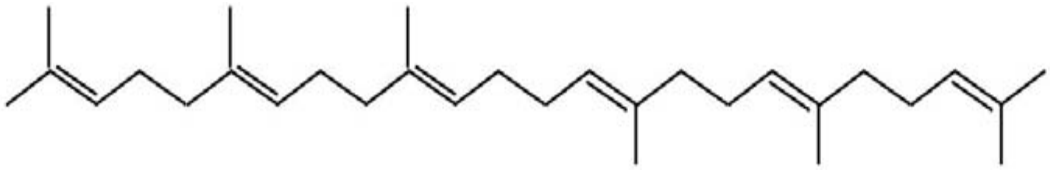


Figure. 1.4: Squalene $C_{30}H_{50}$

1.6.2.3. Tocopherols

These compounds (Figure1.5) are present in olive oil in concentrations of about 150 – 250 mg/Kg, Normally present are the forms α, β, γ and δ of which α (vitamin E)

is the most abundant (90 – 95%). Tocopherols carry out an anti-oxydant action in oils exposed to light (especially ultraviolet radiation) [22].

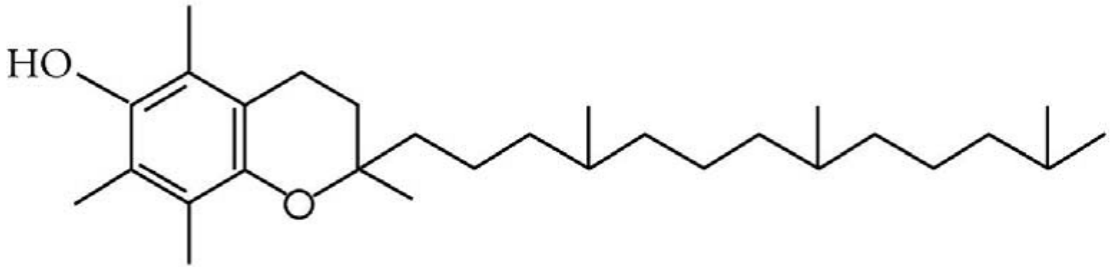


Figure 1.5: α-Tocopherol $C_{29}H_{50}O_2$

1.6.2.4 Phospholipids

The amount of phospholipids in olive oils changes between 40-135 mg/kg. Phosphatidyl -choline, phospatidylethanolamine, phosphatidylinositol, and Phosphatidylserine, phosphatidylglycerol, phosphatidic acid are the main phospholipids detected in olive oils. Their presence in the olive oils may affect their oxidative stability or the physicochemical state of cloudy (veiled) olive oil. The antioxidant functions of phospholipids based on an amino group that has the capacity to chelate metals and keep them in an active form. They can act as synergists with phenolic compounds and tocopherols contributing to enhance their antioxidant activity [23].

1.6.2.5 Phenolic compounds

The phenolic compounds are natural antioxidants present in virgin olive oils (VOO) but not in refined ones. They have a significant importance for their nutritional and technological properties. Their antioxidant function and anti-inflammatory effect have been related to the preventive action on certain diseases such as arteriosclerotic vascular disease (ASVD) and cancer . Polyphenols also represent an important technological value due to their influence on sensory characteristics and the shelf life of virgin olive oil [24]. Phenolic compounds are define these substances which possess a benzene ring bearing one or more hydroxyl groups, including functional derivatives (esters, methyl esters, glycosides, etc.) [25]. Phenolic compounds have much influence on the stability,

sensory and nutritional characteristics of the product and may prevent deterioration through quenching of radical reactions responsible for lipid oxidation. Factors influencing the antioxidant activity of phenolic compounds include position and number of hydroxyl groups, polarity, solubility and stability of phenolic compounds during processing [26]. The prevalent classes of hydrophilic phenols found in VOO are phenolic alcohols, phenolic acids, flavonoids, lignans and secoiridoids. Secoiridoids including aglycon derivatives of oleuropein, demethyloleuropein and ligstroside, that are present in olive fruit, are the most abundant phenolic antioxidants of VOO [27].

These compounds present in olive oil in appreciable quantities (50 – 500 mg/Kg) have an important role in the stability of the oil to oxidation. Moreover some of them confer the bitter-pungent.. Phenolic composition varies from one oil to another and varies in the course of time according to the conditions of preservation. These compounds
partition into the oil. Black olive pericarp extract (from the outer layer of the black olive) has a higher concentration of phenolic compounds and a higher antioxidant capacity than green olive pericarp extract [22].

a. Phenolic acid

Phenolic acids with the basic chemical structure of C6-C1 (benzoic acids) and C6-C3 (cinnamic acids), such as caffeic, vanillic, syringic, p-coumaric, o-coumaric, protocatechuic, sinapic, and p-hydroxybenzoic acid, were the first group of phenols observed in VOO [28].

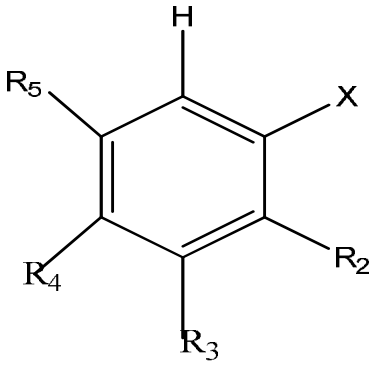
The name “phenolic acids”, in general, describes phenols that possess one carboxylic acid functionality. However, when describing plant metabolites, it refers to a distinct group of organic acids as shown in Table1.3.These naturally occurring phenolic acids contain two distinguishing constitutive carbon frameworks: the hydroxyl cinnamic (Xa) and hydroxyl benzoic (Xb) structures. Although the basic skeleton remains the same, the numbers and positions of the hydroxyl groups on the aromatic ring create the variety. In many cases, aldehyde analogues (Xc) are also grouped in with, and referred to as, phenolic acids (e.g., vanillin). Caffeic, p-coumaric, vanillic, ferulic, and

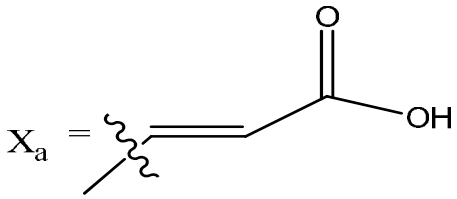
protocatechuic are acids present in nearly all plants. Other acids are found in selected foods or plants (e.g., gentisic, syringic) [29].

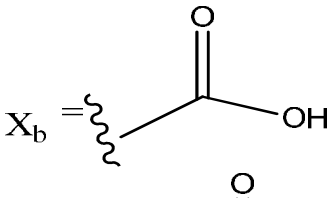
Table 1.3: Major classes of phenolic compounds in virgin olive oil [27]

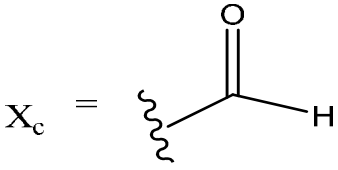
Common Name	X	R2	R3	R4	R5
cinnamic acid	a	-H	-H	-H	-H
ferulic acid	a	-H	$-OCH_3$	-OH	-H
sinapic acid	a	-H	$-OCH_3$	-OH	$-OCH_3$
caffeic acid	a	-H	-OH	-OH	-H
benzoic acid	b	-H	-H	-H	-H
salicylic acid	b	-OH	-H	-H	-H
p−hydroxybenzoic acid	b	-H	-H	-OH	-H
vanillic acid	b	-H	$-OCH_3$	-OH	-H
syringic acid	b	-H	$-OCH_3$	-OH	$-OCH_3$
gallic acid	b	-OH	-OH	-OH	-OH
vanillin	c	-H	$-OCH_3$	-OH	-H

*









b. Roles of phenolic acids in human health.

Studies of the Mediterranean diet suggest that oil phenolic acid deliver key health benefits as following:-

i- Phenolic acid absorb free radicals and have a positive impact on cardiovascular disease and certain forms of cancer.

ii- They also act as anti inflammatory, as confirmed in clinical studies [30].

iii- Olive oil is rich in phenolic acid, which are a chemical compound with powerful antioxidant characteristics, Prevent the oxidation of other molecules. Because oxidation of molecules in body can produce free radical that are damaging to cells [31].

Note: Levels of individual phenols are difficult to establish due to natural variability and strong dependence on oil age and history after production. Free phenols are mainly found in stored oils whereas fresh oils contain more complex forms of secoiridoid glycons [31].

1.6.2.6 Volatile compounds

Volatile compounds are low molecular weight compounds which vaporize readily at room temperatures. Characteristic aroma and in particular green and fruity features of olive oil originates from many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase (LOX) pathway (Figure 1.6) which takes place during the oil extraction process [32].

Some of the volatile compounds found in virgin olive oil are present in the intact tissue of the fruit, and others are formed during disruption of cell structure during the virgin olive oil production due to enzymatic reactions in the presence of oxygen [33].

The major volatile compounds of olive oil (Table 1.4) which contribute for the positive attributes of olive oil aroma (fruity, pungent and bitter) include hexanal, (*E)*-hex-2-enal, hexan-1-ol and 3- methylbutan-1-ol. Their concentrations, except for (*E*)-hex-2- enal, varying widely, are generally very low reaching minimum levels of ppb. Thus, volatile compounds, which are responsible for most sensory properties of olive

oils, play a significant role on the evaluation of the overall oil quality having a decisive influence on acceptability [33].

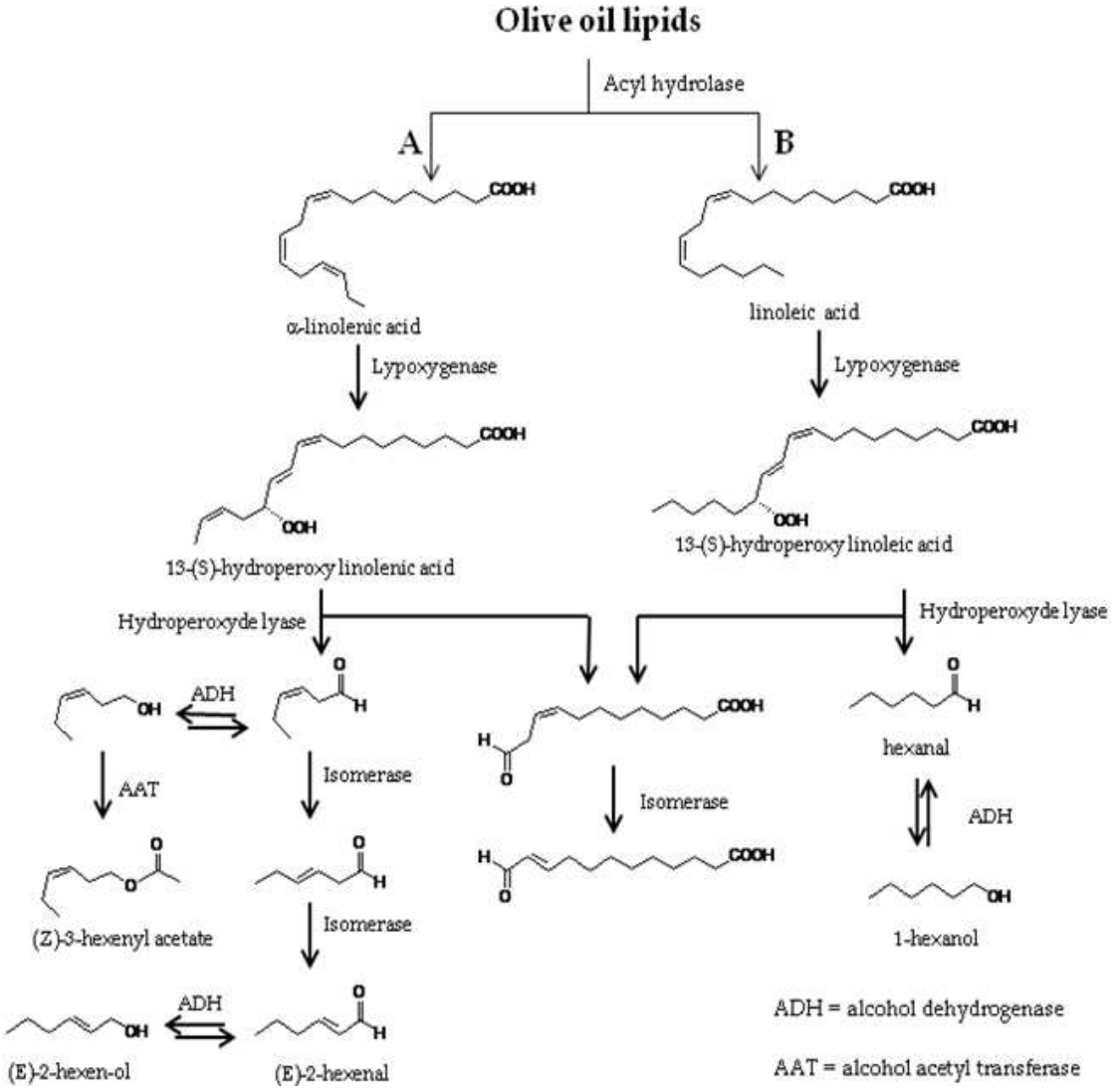


Figure 1.6: Lypoxygenase pathway for the formation of major volatile compounds [34].

Table 1.4.:The major volatile compounds of olive oil [33]

Aldehydes	Alcohols	Esters	Hydrocarbons	Ketones	Furans
3-Methylbutanal	Methanol	Methyl acetate	Octane	2-butanone	Ethyl furan
(E)-2-Pentanal	Ethanol	Ethyl acetate	2-Methyl butane	3-Pentanone	
(Z)-2-Pentanal	1-Hexanol	Butyl acetate	Nonane	1-Penten-3-one	
Hexanal	1-Penten-3-ol	Hexyl acetate	Hexane	2-Octanone	
(E)-2-Hexenal	(E)-2-Hexen-1-ol	(Z)-3-Hexenyl-acetate			
(Z)-2-Hexenal	(Z)-3-Hexen-1-ol	Ethyl propanoate			
Heptanal	1-Octanen-3-ol				
2,4,-Heptdienal	terpineol				
Octanal	3-Methyl butan-1-ol				
Nonanal					
2,4-Nonadienal					
2,4-Decadienal					
(E)-2-Undecenal					

1.7 Olive oil quality: nutritional and sensorial quality

The International Olive Oil Council (IOOC), European Commission (EC) and Codex Alimentarius have defined the quality of olive oil based on several parameters, such as free fatty acid content, peroxide value, spectrophotometric observances in the UV region, halogenated solvents and sensory attributes . In order to evaluate olive oil quality, the Codex Alimentarius and IOOC include also the insoluble impurities, some metals and unsaponifiable matter determinations [33].

The nutritional value of olive oil arises from high levels of oleic acid and minor components, such as phenolic compounds. It is well recognized that the consumption of some natural antioxidant phenolic compounds produce beneficial health effects. These substances possess strong radical scavenging capacities and can play a relevant role in protecting against oxidative damages and cellular aging. Together with their bioactivity, olive oil phenols have a significant role on the flavor and the bitter taste of olive oil.

Olive oil possesses a highly distinctive taste and flavor due to specific volatile organic

compounds, belonging to several chemical classes, namely aliphatic and aromatic hydrocarbons, aliphatic and triterpenic alcohols, aldehydes, ketones, ethers, esters and furan and thiophene derivatives [35].

1.7.1 Acidity determination (free fatty acid content) (FFA)

Olives contain endogenous lipase enzymes which hydrolyze triacylglycerides (oil molecules) to release free fatty acids (hydrolysis). Although isolated from the oil in intact fruit, if the fruit is damaged prior to harvesting (pests, disease) or stored for extended periods prior to processing, the enzymes react with the triacylglycerols, causing the production of free fatty acids.

Acidity determination is mainly accomplished by titration using potassium hydroxide. The method determines the amount of free fatty acids (FFA) present in the oil, which is expressed as percentage of oleic acid. The free acidity is a measure of the quality of the oil, and reflects the care taken in producing and storage processes of the oil**.** As well,

acidity values are used as a basic criterion for classifying the different categories of olive oil.

Maximum levels Table 1.5 have been fixed by Regulations to establish the category, since it is tightly related to the quality of raw material.

Table 1.5: Limits of free fatty acid, as oleic acid percent, fixed by the international organizations for each olive oil category. [14]

Category	IOOC	Codex Alimentarius	EC
Extra virgin olive oil	≤ 0.8		≤ 0.8
Virgin olive oil	≤ 2.0		≤ 2.0
Ordinary virgin olive oil	≤ 3.3		-
Refined olive oil	≤ 0.3		≤ 0.3
Olive oil	≤ 1.0		≤ 1.0
Crude olive residue oil	nl		-
Refined olive residue oil	≤ 0.3		≤ 0.3
Olive residue oil	≤ 1.0		≤ 1.0

nl = no limit

1.7.2 Peroxide value

Oxidation, and the formation of peroxides, occurs during oil extraction and processing and can continue after bottling and during storage. Peroxides are intermediate oxidation products of oil which lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, hydrocarbons, alcohols and esters responsible for the deterioration of olive flavours. Peroxides have been shown to occur when oil is exposed to oxygen and/or light, particularly at elevated temperatures [27].

1.7.3 Ultraviolet absorption

Fatty acids absorb light at particular wavelengths in the UV region and this may be used to determine olive oil quality. Refining causes a change in the configuration of fatty acids and the formation of conjugated dienes and trienes. Increased values of K232 and

K268 in olive oil usually indicate the presence of refined oils. Autoxidation reactions are also associated with conjugation, due to the formation of either carbon-carbon bonds or carbon-oxygen bonds which cause an increase of absorption in the region between 225 and 325nm [32]. Table 1.6 summarizes specific absorbencies at 232 and 270 nm and ΔK value for each olive oil category.

Table 1.6: Limits of the absorbencies at 232 and 270 nm and ΔK value for each olive oil category fixed by the different International Organizations.[34]

Category	**IOOC**			**Codex Alimentarius**		**EC**		
	K_{232}	K_{270}	ΔK	K_{270}	ΔK	K_{232}	K_{270}	ΔK
Extra virgin olive oil	≤ 2.50	≤ 0.22	≤ 0.01	≤ 0.22	≤ 0.01	≤ 2.50	≤ 0.22	≤ 0.01
Virgin olive oil	≤ 2.60	≤ 0.25	≤ 0.01	≤ 0.25	≤ 0.01	≤ 2.60	≤ 0.25	≤ 0.01
Ordinary virgin olive oil	nl	≤ 0.30	≤ 0.01	≤ 0.30	≤ 0.01	nl	-	-
Refined olive oil	nl	≤ 1.10	≤ 0.16	≤ 1.10	≤ 0.16	nl	≤ 1.10	≤ 0.16
Olive oil	nl	≤ 0.90	≤ 0.15	≤ 0.90	≤ 0.15	nl	≤ 0.90	≤ 0.15
Crude olive residue oil	nl	nl	nl	-	-	nl		
Refined olive residue oil	nl	≤ 2.00	≤ 0.20	≤ 2.00	≤ 0.20	nl	≤ 2.00	≤ 0.20
Olive residue oil	nl	≤ 1.70	≤ 1.80	≤ 1.70	≤ 0.18	nl	≤ 1.70	≤ 0.18

nl = no limit

1.8 Olive oil storage

Proper storage techniques for olive oil are very important, not only to preserve the delicate taste of the oil, but also to ensure that it does not spoil and become rancid, which will have a negative effect on its nutritional profile. Olive oil can be kept longer than any other edible oil, and if stored properly it will take years before it becomes rancid. Even though olive oil's monounsaturated fats are more stable and heat-resistant than the polyunsaturated fats that predominate in other oils (especially the easily damaged omega-3 fatty acids found in flax seed oil, which should always be refrigerated and never heated), olive oil should be stored properly and used within a few months to ensure its healthy phytonutrients remain intact and available. Virgin olive oil is more stable than other edible oils because of its high content of phenolic compounds,

tocopherol, carotenoids and monounsaturated fatty acids. On the other hand, high offer; they can also give a shelf life that is considerably longer. The most significant factors affecting the olive oil quality after processing and during storage are environmental, temperature, exposure to light and contact with oxygen. Light is an initiator of reactions that ultimately result in deterioration of the oil. Olive oil should be stored in a cool**,** dark place in consideration of accelerated oxidation effect of light factor [36].

Olive oil should be stored at normal room temperature (21-25°C) if olive oil is kept in a dark area where the temperature remains fairly constant. The stability of olive oil usually ranges from 9 to more than 18 months, assuming that it is properly stored. The best containers for storage are glass (especially tinted glass), ceramic, porcelain, or non-reactive metals such as stainless steel. Olive oil should not be stored in containers made of reactive metals such as copper or iron for the reason of the chemical reaction between the olive oil and the metal will damage the oil and may produce toxins [37].

1.9 Olive oil benefits

Olive oil contains essential fatty acids similar to that found in human milk. It is highly recommended for the elderly as it aids in the assimilation of minerals and vitamins. It stimulates bone mineralization, thus preventing calcium loss. Results of a study of people living in southern Greece suggest that eating hearty amounts of olive oil and cooked vegetables may reduce the risk of developing rheumatoid arthritis [37].

Olive oil is a rich diet may also help to prevent or delay the onset of diabetes by preventing insulin resistance and ensuring better blood sugar level control. Phenolic compounds found in olive oil may help explain the cardiovascular health benefits associated with the Mediterranean diet. Polyunsaturated and mono-unsaturated fats can lower blood cholesterol levels, which in turn can prevent arteriosclerosis. Olive oils are rich in anti-oxidants, oil-based vitamins, and are associated with reduced cardiovascular events. Mono-unsaturated fats increase the HDL or healthy cholesterol, while a good balance of products rich in both omega-3 and omega-6 fatty acids provide some anti-clotting benefits and appear to reduce the likelihood of cardiac arrhythmias as well as second heart attacks. Certain plant oils called plant sterols reduce LDL cholesterol and can be used as a supplement or added to spreads to actually improve cholesterol levels.

Olive oil is very well tolerated by the stomach. In fact, olive oil's protective function has a beneficial effect on ulcers and gastritis. Olive oil activates the secretion of bile and pancreatic hormones much more naturally than prescribed drugs. Consequently, it lowers the incidence of gall stone formation [38].

1.9.1 Olive oil and heart disease

Olive oil is part of the healthiest diet on earth. Olive oil make your arteries more elastic two tablespoons daily makes you more resistant to strokes and heart attack. It reduces bad cholesterol levels. Olive oil contains polyphenols, which help to keep your levels of LDL cholesterol within healthy ranges. Olive oil reduces the risk of stroke in the elderly through yet another mechanism.

Olive oil Lowers the risk of coronary heart disease in women. Mediterranean cultures have long revered the olive and its oil, with good reason.[10] An Italian study found that a diet that included olive oil along with plenty of leafy vegetables and fruit resulted in reduced rates of coronary heart disease in women enrolled in the study [39].

Olive oil protects red blood cells and therefore the heart. Over time, cells oxidize, leading to the common effects of aging. A specific polyphenol in olive oil is especially effective at protecting red blood cells from oxidation. Olive oil lowers blood pressure. Researchers have some theories as to why it works. The reduction in blood pressure by olive oil in one of these theories attributed to capability of olive oil to reduce nitric acid which has been proven to increase blood pressure. However no one is sure why olive oil helps to lower blood pressure..

1.9.2 Olive oil and cholesterol

Olive oil has always served a central role in the Mediterranean diet, providing a strong source of mono-unsaturated and poly-unsaturated fats. These contributions to an overall healthy diet can have positive effects on cholesterol levels by helping to maintain a better balance between HDL (“good”) cholesterol and LDL (“bad”) cholesterol.

Further studies have revealed that other than its high mono-unsaturated content, unprocessed (such as extra-virgin) olive oil contains non-fat components such as certain

phenolic compounds that have a wide range of health benefits including positive effects on cholesterol (both “good” and “bad”) levels and LDL oxidation [37].

1.9.3 Olive oil and skin

The antioxidants in olive oil could help prevent cancer in humans too. Sunlight damages DNA and creates free radicals that cause oxidative damage. Olive oil has polyphenols and other natural antioxidants that could prevent the type of damage that leads to cancer [39].

As explained earlier, olive oil is abundant in antioxidants. These antioxidants work really well as moisturizers. One of the main reasons why olive oil works really well as a moisturizer is the hydrophilic properties of these antioxidants which allow it to form a protective barrier trapping moisture on your skin. Lots of skin products used various synthetic chemical that do moisturizer but they clog your pores and are carcinogenic in some cases. For this reason, olive oil can be applied on your face. In addition to that, its deep penetrating properties not only moisturize your skin but also help brighten your skin. olive oil helps exfoliating skin, helps remove clogged pores and dead skin cells.

1.9.4 Olive oil and hair

One of the most shocking benefits of olive oil is that it can prevent and even cure hair loss. When people lose hair, it is due to a hormone responsible for the shrinkage of the hair follicle shaft. However, the production of that hormone, called DHT (Dihydrotestosterone), is hampered when olive oil is applied to the scalp. Olive oil also has antibacterial properties and antifungal properties (high levels of mono-unsaturated fatty acids ,vitamin E and an antioxidant) that fight off common scalp and hair problems.

1.10 Chemical study of olive oil

1.10.1 Quality Properties

Najafzadeh et al. reported the physicochemical properties of two varieties of olive oil in Iran and their study was summarized in Table 1.7 [40].

Table 1.7: Physicochemical properties of two varieties of olive oil in Iran

Cultivars	Free fatty acid (% oleic acid)	Saponification number (mgKOH/g)	Refractive index	Density (g/ml)
Zard	1.43±0.04	178.94±0.24	1.46±0	0.9127±0
Roghani	1.43±0.04	177.88±0.62	1.46±0	0.9147±0

Refractive index of olive oil at temperature of 25 C^o for oils produced at some Arabic countries were recorded by Al-Ghamdy, Fatimah Bint Saeed Mohammed (2008) to be in range of 1.4628 to 1.4941 [41].

Manel Issaoui et al (2010), reported the K232 and K270 of virgin olive oils from the main Tunisian cultivars, grown in two different locations, north and south Tunisia, to be as in Table 1.8 [42].

Table 1.8: The mean values of the absorbance measurements at 232 and 270 nm of virgin olive oils of Chemlali and Chétoui cultivars from two locations in Tunisia

	Chemlali Southern	Chemlali Northern	Chétoui Southern	Chétoui Northern
K232	1.9	1.4	2.1	2.2
K270	0.2	0.2	0.3	0.2

1.10.2 Fatty acid composition

Abouzar Hashempour et al (2010) studied the fatty acid composition of five varieties of olive oil in Iran. They noticed that the lowest palmitic acid was in 'Frangivento' olive oil (11.37%) and the highest value of palmitic acid was in 'Beledy' (16.03 %). Oleic acid, the major monounsaturated fatty acid, showed also a wide variability depending on the cultivars. The highest content of oleic acid was observed in 'Coratina', (80.72%),

while it was the lowest in 'Beledy', (76.08%). 'Arbequina', 'Frangivento' and 'Zard' showed relatively high levels (76.81, 80.42 and 79.24, respectively). The lowest percentage of linoleic acid was observed in 'Beledy' (2.3%) whereas the highest value was observed in 'Coratina' (3.41%). Beledy' oil was rich in total saturated fatty acids (SFAs) (18.30 %), 'Coratina' cultivar had the highest level of monounsaturated fatty acids(MUFAs) (81.35%) [43].

Tawalbeh (2005) reported that oleic acid content in olive oil obtained from Baladi and Romi cultivars in Jordan were 75.7% and 76.5%, respectively [44].

Al-Rousan (2004). Reported that oleic acid content in olive oil extracted from Nabali Muhasan cultivar grown in north of Jordan contains 69.9% oleic acid, 14.1% palmitic acid, 2.8% stearic acid, and 8.7% lenoleic acid [45].

1.10.3 Phenolic acid in olive oil

Mohammed D. N. (2007). record the phenolic acids in Syrian olive oil to be as described in Table 1.9 below [46].

Table 1.9. Some phenolic acids range of Syrian olive oil

Phenolic acid	Concentration (ppm)
Caffeic Acid	1.1-5.2
Ferulic Acid	0.1-4.2
Syringic acid	0.4-2.2
Vanillic Acid	0.7-4.5
P-Coumaric Acid	10.4-70.9

1.11 Aim of the study

Olive oil is a key component of the traditional Mediterranean diet, which is
believed to be associated with a good health., is a functional food which besides having a high level of monounsaturated fatty acid contains several minor components with biological properties. For some olive oil the minor components, were phenolic compounds. So analysis of monounsaturated fatty acid and antioxidant phenolic acid is one of the most important analyses for olive oil.

The international olive oil council (IOOC) and European communities legislation (EC) define the identity characteristics of olive oil and olive-pomace oil, by specify analytical methods and standard limit values of the quality parameters such as acidity, and UV absorbance values (K232 and K270). The aim of this research is to evaluate the quality of Libyan olive oil represented by samples collected from eighteen different place on the country.

CHPTARE II

MATERIALS and METHODS

2.1 Materials

2.1.1 Olive Oil Samples

The eighteen samples of olive oil that investigated in this study were collected from different places of Libya, during March 2012. These samples were stored in brown glass bottles and placed in dark area, as recommended by ISO.661:1989. Table 2.1 below shows the code and sites of the studied samples. For geographical classification, samples arranged from west to east region from Nalute to Benghazi .(Figure 2.1)

Table 2.1: The code and sample sites for study samples

Sample number	Sample sites	Longitude	Sample code
1	Nalute	10 59 00	Nu
2	Ajjmayl	12 20 00	Aj
3	Beer-ghanam	12 34 10	Bg
4	Azzawiyah	12 43 40	Az
5	Gharian	13 01 00	Gr
6	Tajoora	13 20 24	Tj
7	Alnowahi-4-	13 25 00	An
8	Tarhoona	13 38 00	Th
9	Tarhoona (Spanish)	13 38 00	Ts
10	Benwaleed	13 59 00	Bw
11	Misalata	14 00 00	Mi
12	Ghamata	14 05 00	Gm
13	Wadi Ekaam	14 13 00	Ek
14	Alkhoms	14 15 52	Kh
15	Zleetin	14 34 00	Zl
16	Alwashka	16 35 00	Aw
17	Gharife	16 35 00	Gr
18	Benghazi	20 04 00	Bn

Figure 2.1: Olive oil samples from study region

2.1.2 Chemical reagents and solvent

The chemical reagents used for determination of acidity, seponification value, UV spectroscopy, fatty acid analysis by gas chromatography, and phenolic acids analysis by HPLC are given in Table 2.2.

Table 2.2:. The regents used in the study

No.	Chemical name	company	purity	methods
1	Ethanol	Hayman limited	99.9%	Acid value determination ISO 660 1996
2	Diethyl ether	Arabian medical scietitic lab	99.7%	
3	Pottasium hydroxide	Scharlua merck	98.0%	
4	phenolphthalein	BDH chemicals ltd	-	Saponification value ASTM D 5558
5	Hydrochloric acid	BDH-AnalaR	-	
6	Sodium carbonate	Surechem products LTD	99.5%	
7	Iso-octane	Hayman limited	99.0%	UV EEC 2568/91
8	Methanol	SIGMA-ALDRICH	99.0 %	HPLC and GC
9	Sulphuric acid	BDH-AnalaR	98.0%	
10	n-Hexane	Surechem products LTD	99.5%	

Table 2.2:. The regents used in the study (continued)

No.	Chemical name	company	purity	methods
11	Nitrogen gas	Trithin products limited	99.99%	
12	Sodium chloride	Surechem products LTD	99%	
13	De ionized water	-	-	Cleaning and dilution
14	Acetone	BDH-prolabo	99.8%	
15	Chloroform	Chemical reagent co.	99.0%	

2.2 Methods and instrumentations

2.2.1 Determination of olive oil density

The mass of substance per unit volume density (d) is expressed in units of g/cm^3. It is defined as the ratio of its mass (m) to its volume (v)

$$d = \frac{m}{v} \qquad (1)$$

Liquid samples at ordinary temperature proceed by the method described in the test for density and specific gravity.(AOAC Method 930.17). Density determination by pycnometer is a very precise method. The pycnometer Figure 2.2 is a glass flask with a close-fitting ground glass stopper with a capillary hole through it. This fine hole releases a spare liquid after closing a top-filled pycnometer and allows for obtaining a given volume of measured and/or working liquid with a high accuracy [47].

Figure 2.2: The pycnometer using to determination of density

First we fill pycnometer with distilled water. According to equation above, the volume of water that is filling the pycnometer and the stopper is

$$v = \frac{m}{d} \qquad (2)$$

Where m_{H2O} is experimentally determined weight of water (empty pycnometer weight subtracted). We repeat the procedure for the liquid with unknown density d_l and determine its weight m_l (measured weight minus weight of empty pycnometer). Volume (v) obtained in (2), It follows alternated equation

$$v = \frac{\quad}{\quad} \qquad (3)$$

Combining equations (2) and (3)

$$\frac{\quad}{\quad} = \frac{\quad}{\quad} \qquad (4)$$

yields a relation that provides the density of measured liquid

$$= \frac{\quad}{\quad} \times d\,H\;O. \qquad (5)$$

2.2.2 Determination of refractive index of olive oil samples

The refractive index (n) of an oil is defined as ratio of the speed of light in air to speed of light in the oil [47]. Measurement by means of a suitable refractmeter (Abbe type), of the refractive index of the liquid Sample. Refractometer able to measure the refractive index to ±0.0001 within the range of 1.3000—1.7000.

$$n = \frac{c}{v} \qquad (6)$$

Where: **c**- speed of light in air.

v- speed of light in oil.

2.2.3 Determination of acid value and acidity

The international standard specifies three methods (two titrimetric and one potentiometric) to determine acid value and acidity of oil or olive oil. In this study the method ISO-660:1996 (E) (titrimetric method) was chosen [48].

2.2.3.1 Acid value

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids present in 1g of sample. Acid value is expressed in (mg KOH/ g sample).

2.2.3.2 Acidtiy

Content of free fatty acids determined according to the procedure specified in this international standard. Acidity is expressed as percentage by mass of free acid to mass of sample.

Notes

1- If the results of the determination is reported as acidity without further explanation, this is by convention the acidity expressed based on oleic acid.
2- If the sample contains mineral acids, the acidity determined as fatty acids.

a. The procedure:

Weight accurately (1-10) g of sample, in a glass-stopper, 250-ml flask, add 100 ml of mixture of diethyl ether and ethanol (1:1) as solvent. Then , add a few drops of phenolphthalein as indicator, titrate with 0.1N Ethanolic potassium hydroxide until the solution develops a light red color which persists for 30 seconds [48].

Note: To avoid turbidity, titration should be done in warm medium.

Calculation:

$$\text{Acid value} = \frac{\text{consumed volume(ml)of 0.1N KOH(alc)} \times 56.11}{\text{weight of sample(g)}} \quad (7)$$

$$\text{Acidit} = \frac{\text{base normality } \frac{\text{meq}}{\text{ml}} \times \text{ml base} \times \text{meqw of oleic acide } \frac{\text{mg}}{\text{meq}}}{\text{weight of sample(mg)}} \times 100 \quad (8)$$

b. Preparation of solution

I- Preparation of 0.1 N of Ethanolic Potassium Hydroxide

5.611g of potassium hydroxide powder was dissolved in sufficient quantity of ethanol, transferred to 1L volumetric flask, then filled to the mark with ethanol.

II- Preparation of phenolphthalein indicator

small quantity (about 0.1 g) of solid phenolphthalein was dissolved in a mixture of 20 ml H_2O + 80 ml ethanol [49].

The acidity is mainly determinated by titration using potassium hydroxide. The method determines the amount of free fatty acids (FFA) present in the oil, which is expressed as percentage of oleic acid. Acidity values are used as a basic criterion for classifying the different categories of olive oil. However, according to Kiritsakis , acidity is not considered as the best criterion for evaluating olive oil quality, since one oil with relatively high acidity may have a good aroma while another one with low acidity may not have so good taste and aroma [48]. For extra virgin olive oil the maximum acidity is 1%, increasing as the quality category of the oil decreases.

2.2.4 Determination of saponification value

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters and to neutralize the free acid in 1g of the sample. The test was done according to (ISO 3657:2002) [48].

a. The Procedure:

Accurately 1 to 2 g of the sample, transferred weighed to a 200 mL flask, and exactly 25 mL of 0.5 N potassium hydroxide-ethanol was added. A short reflux condenser was attached to the neck of the flask, and heated gently in a water bath for one hour with

frequent shaking. The solution was cooled,1mL of phenolphthalein was added, and the excess potassium hydroxide was titrated with 0.5 N of hydrochloric acid [48].

Note: some samples solution was turbid at low temperature, so they were titrated while they are warm.

Calculation:

$$\text{Saponi ication value} = \frac{(\quad - \quad) \qquad 56.1}{} \qquad (\quad)$$

Where

A- HCl for blank ml

B- HCl for sample ml

w- weight of sample (dry basis), g

N- normality HCl solution

2.2.5 Olive oil oxidation test by UV- spectroscopy

The evaluation of the degree of olive oil oxidation can be done by means of the measurements of extinctions on oil sample diluted in an Isooctane solvent (ISO 3656:2002). Specific absorbences, conventionally indicated as K, are measured in the UV region at the wavelengths corresponding to the maximum absorption of the conjugated dienes and trienes, respectively at about 232 and 270 nm. The conjugated dienes and trienes are formed in the aut-oxidation process from the Hydro-peroxides of unsaturated fatty acids and their fragmentation products. Absorption around 270 nm could also be caused by substances formed during earth treatment in the refining process. K232 evaluation is considered optional by IOOC trade standards. In addition to K232 and K270, often, especially in trade negotiations ΔK value is considered useful, calculated according to the following equation (10):

$$\Delta K = Kmax - [1/2(Kmax+4 + Kmax-4)] \qquad (10)$$

Where Kmax is the maximum absorbance near 270 nm.

The evaluation of the UV spectrometric analysis is based on K values. The value K232 (for extra olive oil K232 < 2.5) provides evidence of hydro peroxides and conjugated di- and tri-fatty acids). Conjugated tri-fatty acids, which are characterized by the K270 value (for extra olive oil K270 < 0.22) [50].

a. The Procedure:

250 mg of olive oil was weighed in 25 mL volumetric flask filled to the mark with isooctane (spectro-photometric grade). Solution was placed into a quartz cuvette. Absorbance at 232 and 270 nm was determined in a spectrophotometer (UV -Aquarius - 2450 UV-Visible Spectrophotometer, Japan), using pure isooctane as a blank.

2.2.6.Fatty acid composition

The fatty acid composition of the oils was determined by gas chromatography (GC) as fatty acid methyl esters (FAMEs) according to (EEC 2568/91). Methyl esters are formed by trans-esterification with methanolic potassium hydroxide as an intermediate stage before saponification takes place.

Sample Preparation Method: European Official Methods of Analysis (EEC,1991 A) was used for the preparation of methyl esters. 100 mg oil sample was weighed in 20 mL test tube. The sample was dissolved in 10 mL n-hexane and 100 µL 2 N potassium hydroxide in methanol was added (2.8 g in 25 mL). The sample solution was vortexes for 30 seconds and centrifuged for 15 seconds. After centrifugation, decant the upper layer containing the methyl esters.

The method EEC,1991 A was applied directly to samples of this study. Method EEC,1991 B was recommended to be applied for olive oil that is greater in acidity than 3.3% [21], which is not required in this study.

a. Preparation of 2N methanolic potassium hydroxide solution:

This is prepared by dissolving 11.2 g of potassium hydroxide in100 ml of methanol.

2.2.6.1 Gas liquid chromatography (GLC) conditions

Analysis of fatty acids was carried out by gas liquid chromatography (GLC), using TECHCOMP gas chromatography GC-7900) equipped with flame ionization detector (FID), the conditions of GLC used in this study is shown in Table 2.3.

Table 2.3: Chromatographic method for the analysis of fatty acid methyl esters

Chromatographic system	TECHCOMP GC-7900
Detector	FID
Column	fused-silica capillary column (30 m × 0.53µm i.d. × 1.5 µm film thickness, (J&W scientific).
Oven temperature	115 ^{o}C (1min)-200C(1min) at 10 ^{o}C /min -240 ^{o}C (2min) at 5 ^{o}C /min -260 (3 min) at 3.5 ^{o}C /min.
Carrier gas	Helium
Injection volume	1µL
Inlet temperature	300 ^{o}C
Detector temperature	230 ^{o}C
Inlet	Split/spitless
Split ratio	(1:100).

Fatty acid evaluation was performed on the olive oils following the usual product analyses. GC applied to the olive oil samples allowed for the identification of the following fatty acids: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) acids.

2.2.7 Phenolic acid determination

A colorimetric determination of the amount of total phenols present in olive oil was performed by measuring the absorbance at 725 nm using the Folin-Ciocalteu reagent (phosphomolybdic, phosphotungstic acid complexes) against a suitable blank. Results were expressed as mg of caffeic acid equivalents per kg of olive oil.

Individual phenolic compounds were analyzed by HPLC techniques after extracting them from the oil by solid phase extraction using proper organic solvents (methanol/water (50:50v/v) .Various isolation systems have been proposed by different authors, depending on the aim of the particular study. The systems do not vary only in solvents and/or solid-phase cartridges used, but also in the amount of the sample taken for analysis and volumes of solvents.[1].

2.2.7.1 Extraction of phenolic acid

Solid-phase extraction (SPE) is a commonly used technique to fractionate as well as to remove unwanted components from the sample, by eluting with solvent. The phenolic acid was extracted from the oil by a solid phase extraction method according to Tsimidou (1992). A solution of the oil in Hexane was applied to a pre-conditioned reversed-phase cartridge which was washed with hexane cyclo hexane mixtures to remove the non-polar lipid fraction. Phenols were then eluted with Methanol. The polar fraction of olive oil has been partitioned into aqueous methanol from a hexane solution. (eluted from Sep Pak C18 with methanol/water (50:50v/v)) contains only simple phenols and phenolic acids, and then analysis by HPLC [4, 51].

2.2.7.2 HPLC conditions

Analysis of phenolic acids was carried out by high performance liquid chromatography (HPLC) shimadzu LC-9A model HPLC, equipped with uv/vis detector , under the conditions shown in Table 2.4

Table 10chromatographic method for the analysis of phenolic acid

Chromatographic system	shimadzu LC-9A
Detector	GBC LC 1210 UV/vis
Pump	GBC LC1110 hplc pump
Flow rate	1 ml/min.
Eluent	85% Acetonitrile+ 15% Water
Column	Hypercarb 5µ 100*4.6 nm
Detection	254nm

In order to minimize the error analysis, the separation of the phenolic compounds of virgin olive oil using, tyrosol, vanillic acid, syringic acid and o-coumaric acid as external standards, was applied. The phenolic acids detected in the Libyan olive oil were (caffeic acid, catechol, ferulic acid, p-counaric acid, p-hydroxy benzoic acid, vanillic acid, resorcinol, synergenic acid, and sinapic acid) [1]. Some of phenolic acids and their relative retention times is shown in Table 2.5.

Table 2.5: Maximum absorbance (max uv abs) values and relative retention times (RRT) of some phenolic acids

Phenolic acid	RRT*	Max. UV abs. nm
Vanillic acid	0.96	260
Caffeic acid	0.99	325
Syringic acid	1.00	280
Vanillin	1.10	310
Para-coumaric acid	1.12	310
Ferulic acid	1.26	325

*RRT- The relative retention time is calculated with respect to the retention time of syringic acid.

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CHAPTER III

RESULTS and DISCUSSION

3.1. Quality parameters of olive oil sample

The quality parameters data (free acidity, Saponification value**,** Density and Refractive index) of the studied samples were shown in Table 3.1.

Table 11 Quality parameters of some Libyan olive oils samples

No.	Sample site	Acidity (wt/wt) % mean ± σ	Saponification value(mg/g)	Density (g/ml)	Refractive index(n) mean ± σ
1	Nalute	1.28±0.04	196.4	0.9115	1.4677±0.01
2	Ajjmayl	1.58±0.02	182.3	0.9100	1.4678±0.00
3	Beer-ghanam	1.25±0.02	189.3	0.9121	1.4679±0.01
4	Azzawiyah	1.80±0.14	189.8	0.9107	1.4679±0.02
5	Gharian	1.55±0.05	186.0	0.9100	1.4678±0.01
6	Tajoora	1.60±0.08	186.0	0.9114	1.4680±0.04
7	Alnowahi-4-	1.29±0.03	193.8	0.9107	1.4680±0.02
8	Tarhoona	1.06±0.10	190.6	0.9112	1.4679±0.00
9	Tarhoona (Spanish)	0.78±0.03	199.9	0.9101	1.4681±0.02
10	Benwaleed	1.39±0.01	184.1	0.9107	1.4679±0.01
11	Misalata	1.06±0.12	202.9	0.9112	1.4677±0.01
12	Ghamata	1.10±0.04	203.5	0.9100	1.4679±0.04
13	Wadi Ekaam	1.54±0.06	185.4	0.9102	1.4681±0.03
14	Alkhoms	1.20±0.01	187.6	0.9111	1.4679±0.03
15	Zleetin	1.02±0.07	185.6	0.9105	1.4680±0.04
16	Alwashka	1.90±0.15	194.6	0.9112	1.4680±0.01
17	Gharife	1.10±0.02	197.7	0.9108	1.4678±0.03
18	Benghazi	0.80±0.01	188.2	0.9125	1.4680±0.02

σ : standard deviation

Refractive indices of these samples at temperature of 25 ^{O}C, lies between (1.4676-1.4680). Kenan T. et al. (2007) reported the refractive indices of five Turkish olive oils in range from 1.467-1.469 [52]. Mahmoud N. et al,(2010) reported the refractive index of two varieties of olive oil (Roghani & Zard) in Iran, for Roghani olive oil were 1.4680. and for Zard olive oil were 1.4672 [46] . Borchani1 C. et al, (2010), reported the refractive index of a commercial olive oil were 1.471 ± 0.001 [53].

The density of olive oil samples at temperature of 25 ^{O}C found to be in the range between (0.9100-0.9125) g/ml . Kenan T. et al. (2007) reported the density of five Turkish olive oils in range from 0.910 - 0.912 g/ml [51]. Mahmoud N. et al, (2010) reported the density of two varieties of olive oil (Roghani & Zard) in Iran, for Roghani olive oil were 0.9147 g/ml, and for Zard olive oil were 0.9127 g/ml [46].

The maximum free acidity level was recorded for 'Alwashka (1.90±0.15%) while 'Tarhoona (Spanish)' sample had the lowest free acidity value (0.78±0.03%) The acidity value for all samples grade as a virgin olive oil, (less than 2.2%). Kenan T. et al. (2007) reported the acidity (as oleic acid %) of five Turkish olive oils in range from 0.5-1.7% [52]. Mahmoud N. et al,(2010) reported the acidity (as oleic acid %) of two varieties of olive oil (Roghani & Zard) in Iran, for both olive oil were 1.43±0.04% [46]. Borchani1 C. et al, (2010), reported the acidity of a commercial olive oil were 0.56 ± 0.04% [53].

The maximum level of saponification was recorded for Ghamata sample (203.5 mg/g), while the lowest value was in Ajjmayl sample (182.3 mg/g). Kenan et al. (2007) reported the saponification of five Turkish olive oils in range from 183.7-190.1 mg KOH/g [52]. Mahmoud N. et al, (2010) reported the saponification of two varieties of olive oil (Roghani & Zard) in Iran, for Roghani olive oil were 177.88±0.62 mg KOH/g , and for Zard olive oil were 178.94±0.24 mg KOH/g [46].

3.2 UV Absorbance analysis of samples

The UV analysis of the 18 Libyan olive oil were performed and the results are shown in Table 3.2.

Table 3.2: The mean values of the absorbance measurements at 232 and 270 nm of Libyan olive oil

No.	Sample site	Abs232	Abs270	Delta K
1	Nalute	2.125	0.100	<0.01
2	Ajjmayl	2.190	0.105	<0.01
3	Beer-ghanam	2.299	0.180	<0.01
4	Azzawiyah	2.920	0.220	<0.01
5	Gharian	2.280	0.120	<0.01
6	Tajoora	2.840	0.204	<0.01
7	Alnowahi-4-	2.200	0.097	<0.01
8	Tarhoona	2.860	0.214	<0.01
9	Tarhoona (Spanish)	2.075	0.117	<0.01
10	Benwaleed	2.800	0.100	<0.01
11	Misalata	2.957	0.198	<0.01
12	Ghamata	2.220	0.156	<0.01
13	Wadi Ekaam	2.365	0.156	<0.01
14	Alkhoms	1.951	0.111	<0.01
15	Zleetin	2.900	0.134	<0.01
16	Alwashka	2.880	0.209	<0.01
17	Gharife	2.280	0.115	<0.01
18	Benghazi	2.325	0.216	<0.01

The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems. These absorptions are conventionally indicated by K value, which are characterized by the K232 and K270 values (for extra virgin olive

oil the values must be < 2.5 and 0.22 respectively). The delta K values for extra virgin olive oil should be < 0.01.

All results obtained for UV analysis shown that all studied samples were considered as extra virgin olive oil. (see appendix I). Manel Issaoui et al (2010), reported that the K232 values of virgin olive oils from four Tunisian cultivars (north and south Tunisia) range from 1.4- 2.2. and for K270 values were around 0.2 [42]. Abouzar H. et al (2010). reported that K232 values of five olive oil cultivars (*Olea europea* L.) in Iran were in the ranges from 0.60 -1.22 , and K270 values range between 0.082-0.093 [43].

3.3. Fatty acid composition

Total fatty acids composition is an essential aspect of the qualitative evaluation of olive oil. It is also used as a means allowing to make sure of the authenticity of olive oils and to discover frauds with other vegetable oils . However, the metabolism and the lipid levels in the olive fruit could be affected by environmental factors, such as light, temperature and water stress [54]**.**

The fatty acid composition has previously been used by a number of authors as a parameter for oil classification [55]. In this study, the composition of fatty acids in Libyan olive oil that obtained by gas chromatography analysis were listed in Table 3.3 The highest value of oleic acid content was in Nalute sample (76.2%) and the lowest oleic acid content was recorded for Wadi Ekaam sample (53.7%). Palmitic acid content was the highest in Beerl-ghanam sample (20.1%).The highest value of linoleic acid content was in Misalata sample (25.2%), While Wadi Ekaam sample was the highest in stearic acid value (4.3%).

Table 3.3: Fatty acid composition of Libyan olive oil samples

No.	Sample site	Palmitic Acid (C16:0) %	Oleic Acid (C18:1)%	Seatric Acid (C18:0) %	Linoleic Acid (C18:2) %
1	Nalute	8.7	76.2	1.9	11.4
2	Ajjmayl	16.8	72.6	0.6	7.6
3	Beer-ghanam	20.1	61.8	0.4	14.4
4	Azzawiyah	14.8	63.7	1.1	18.5
5	Gharian	12.5	62.7	1.6	19.9
6	Tajoora	17.9	61.6	3.4	20.8
7	Alnowahi-4-	9.4	73.1	0.8	14.4
8	Tarhoona	10.4	71.3	2.7	11.9
9	Tarhoona (Spanish)	13.1	67.2	1.2	17.1
10	Benwaleed	19.5	55.6	1.1	20.8
11	Misalata	12.8	57.3	2.1	25.2
12	Ghamata	11.2	73.4	0.8	12.7
13	Wadi Ekaam	18.1	53.7	4.3	20.8
14	Alkhoms	14.8	63.0	2.1	18.1
15	Zleetin	7.7	70.6	1.3	17.1
16	Alwashka	17.6	63.7	0.9	15.8
17	Gharife	13.7	59.2	1.3	23.4
18	Benghazi	16.9	70.2	1.3	9.5

Hatice U. A. and Gülcan Ö.(2011), reported that fatty acid composition of olive oil extracted from Memecik olive cultivar in Turkey were palmitic (C16:0) 11.38, stearic (C18:0) 1.25, oleic (C18:1) 76.33, and linoleic (C18:2) 10.34 [56].

Samia Dabbou et al. (2011), reported the fatty acids content of Tunisian olive oils. palimtic acid was within the range of 9.45-11.25%, stearic acid from 2.6-2.95%, oleic acid from 66.21-72.81%, and linoleic acid from 10.92-14.92% [57].

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Tawalbeh (2005), reported that oleic acid content in olive oil obtained from Nabali Baladi and Romi cultivars were 75.7% and 76.5%, respectively [44]. Al-Rousan (2004), reported that oleic acid content in olive oil extracted from Nabali Muhasan cultivar grown in north of Jordan contains 69.9% oleic acid, 14.1% palmitic acid, 8.7% Lenoleic acid, and 2.8% stearic acid [45].

3.3.1 The statistical analysis of fatty acids results

The resulting dendrogram

The dendrogram is fairly simple to interpret. Remember that our main interest is in similarity and clustering. The vertical axis of the dendrogram represents the distance or dissimilarity between clusters. The horizontal axis represents the objects and clusters. At the bottom of the dendrogram, each observation is considered its own cluster. Vertical lines extend up for each observation, and at various (dis)similarity values, these lines are connected to the lines from other observations with a horizontal line. The observations continue to combine until, at the top of the dendrogram, all observations are grouped together. The height of the vertical lines and the range of the (dis)similarity axis give visual clues about the strength of the clustering. Long vertical lines indicate more distinct separation between the groups. Long vertical lines at the top of the dendrogram indicate that the groups represented by those lines are well separated from one another. Shorter lines indicate groups that are not as distinct.

The resulting dendrogram of fatty acid composition is shown in Figure 3.1, revealed three major groups. The first group includes seven varieties: Azzawiyah, Alwashka, Tarhoona (Spanish), Garife, Tajoora, Beerl-ghanam, and Alkhms shows medium level of oleic acid (60%-68%). The second group includes seven another varieties: Ajjmayl, Ghamata, Zleetin, Tarhoona, Alnowahi-4-, Benghazi, and Nalute have a high levels of oleic acid (70%-85%). The third group includes four varieties: Wadi Ekaam,

Benwaleed, Misalata, and Gharian have low level of oleic acid (53%-59%), and high level of linoleic acid.

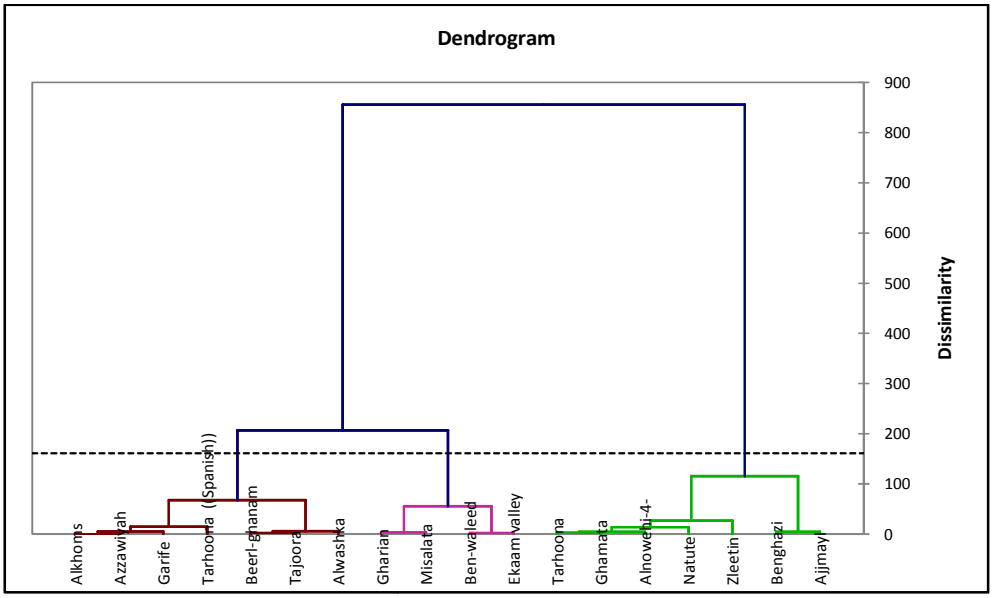

Figure 3.1: Dendrogram of the normalized data of fatty acid composition of Libyan olive oil samples

Figure 3.2: Illustrated the profile plot of the normalized data of fatty acid composition of Libyan olive oil samples.

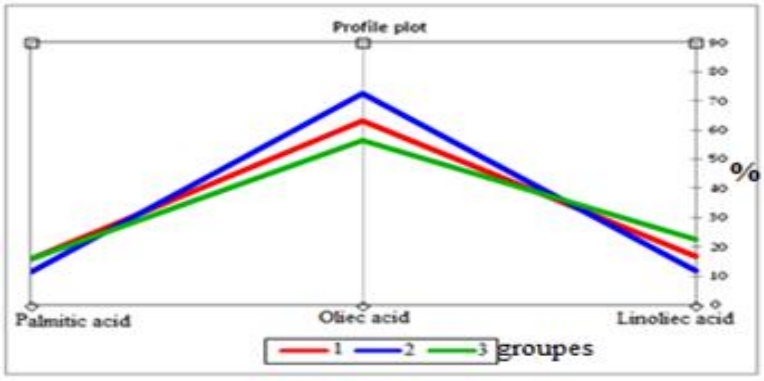

Figure 3.2: Profile plot of the normalized data of fatty acid composition of Libyan olive oil samples

Table 3.4 shows the percentage of saturated and unsaturated fatty acid of Libyan olive oil cultivars.(see appendix II)

Table 3.4: Percentage of saturated and unsaturated fatty acid in %

No.	Sample site	SFA %	USFA %	MUFA %	PUFA %	$\frac{\text{MUFA}}{\text{PUFA}}$
1	Nalute	11.5	88.5	71.2	17.3	4.12
2	Ajjmayl	19.1	80.9	72,9	8.1	9.00
3	Beer-ghanam	22.9	77.1	62.3	14.8	4.21
4	Azzawiyah	16.7	83.3	64.6	18.7	3.45
5	Gharian	15.8	84.2	63.9	20.4	3.13
6	Tajoora	23.6	76.4	62.3	14.1	4.40
7	Alnowahi-4-	11.7	88.3	73.8	14.7	5.02
8	Tarhoona	15.4	84.6	72.2	12.4	5.80
9	Tarhoona (Spanish)	15.2	84.8	67.6	17.2	3.93
10	Benwaleed	23	77	56.1	20.9	2.68
11	Misalata	16.2	83.8	58.2	25.6	2.27
12	Ghamata	13.3	86.8	73.9	12.9	5.73
13	Wadi Ekaam	24.2	75.8	54.5	21.3	2.55
14	Alkhoms	17.8	82.2	63.9	18.3	3.50
15	Zleetin	11.5	88.5	76.9	11.7	6.60
16	Alwashka	20.1	79.9	63.9	16.0	4.00
17	Gharife	16.3	83.7	60.0	23.7	2.53
18	Benghazi	19.5	80.5	70.9	9.6	7.40

USFA: unsaturated fatty acids.
MUFA: monounsaturated fatty acids.
PUFA: polyunsaturated fatty acids.

The percentage of saturated fatty acid in Libyan olive oil was within the range (11.5-24.2%), and the percentage of unsaturated fatty acid also was within the range (75.8-88.5%). The ratio of percentage of monounsaturated to percentage of polyunsaturated fatty acids (MUFA/PUFA), was lies between (2.27-9.0%). The highest ratio was

recorded in Ajjmayl sample, and the lowest ratio was in Misalata sample. Analysis of the mono-unsaturated and poly-unsaturated fatty acid speaks to the stability of the product. This ratio depending on the altitudes of the locations [58].

The highest percentage of saturated fatty acids value was recorded in Wadi Ekaam cultivar (24.2), and the lowest contents were in Nalute and Zleetin cultivars (11.5).

Manel I. et al. (2010), reported that the percentage of saturated fatty acid for Tunisian cultivars were within the range of (15.8-21.0), and the percentage of unsaturated fatty acid within the range (78.4-83.4). The ratio of monounsaturated to polyunsaturated fatty acid (MUFA/PUFA) was in the range (2.8-4.1) [42]. Abouzar H.et al. (2010), reported that the percentage of saturated fatty acid for Iran cultivars within the range (13.7-18.3), and the percentage unsaturated fatty acid lies in the range (80.9-84.2) [5].

Fadil T. et al. (2010), reported that the percentage saturated fatty acid for Albania cultivars were within the range (12.92- 14.56), and for unsaturated fatty acid percentage were lies between (85.4-87.2). The ratio of monounsaturated to polyunsaturated fatty acids (MUFA/PUFA) was in the range (7-10.4) [58].

3.4 Phenolic acids results

Phenolic acid is the minor content of olive oil components. Varies depending on the cultivar, climate, ripeness of the olives at harvesting, and the processing system [59]. The amount of phenolic compounds in olive oil is an important factor when evaluating its quality because natural phenols contribute to its resistance to oxidation especially during storage period and improve its taste. Several phenolic acids such as gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p- and o-coumaric, ferulic and cinnamic acid have been identified and quantified in virgin olive oil (in quantities lower than 1 mg of analyte per Kg of olive oil) [38].

The common individual phenolic compounds, which were identified in this study, were caffeic acid, vanillic acid, p-coumaric acid, ferulic acid, catechol, p-hydroxy benzoic acid, resorcinol, synergenic acid, and syringic acid. (see appendix III)

The antioxidant potential of the isolated phenolic compounds is shown in Table 3.5. It is concluded from the results in Table 3.5, that Libyan olive oil contains ten phenolic

acids compounds, that contribute significantly to give color, taste and distinctive flavor of oil and to be protected as antioxidants, and its vital role health. It is also noticed that the amount of these compounds in the oil samples depending on the type of olive , degree of maturity, and oil extraction method. This is useful to distinguish the quality of oils.

As can be noticed from Table 3.5, Misalata sample contain the highest phenolic acids, while Ben-waleed sample did not contain any of these acids, this is due to the weather or poor storage.

As reported by different authors, the amount of total phenols, which is 200 –500 ppm on the average [60].

Mohammed D. N (2007), recorded that the phenolic acids of Syrian olive oil were: caffeic acid ranged from1.1 to 5.2 ppm, ferulic acid ranged from 0.1-4.2 ppm, syringic acid ranged from 0.4 to 2.2 ppm, vanillic acid ranged from 0.7 to 4.5 ppm, and p-coumaric acid ranged from 10.4 to 70.9 ppm [46]. Shahat M., et al. (2013), recorded that the phenolic acids of some libyan olive oil varieties were: caffeic acid renged from 18.21 to 36.25 ppm, ferulic acid ranged from 6.18 to 15.49 ppm, syringic acid ranged from 8.51 to 17.54 ppm, vanillic acid ranged from 1.80 to 17.41 ppm, p-coumaric acid ranged from 5.82 to 10.11 ppm, and p-hydroxy benzoic acid ranged from 13.52 to 30.09 ppm [2].

Table 3.5: Phenolic Acids Components of Libyan Olive Oil Cultivars (ppm)

Sample area	P-Coumaric acid	Ferulic acid	Sinapic acid	Resorcinol	P-Hydroxy Benzoic	Vanillic acid	Syringic acid
Nalute	nd	nd	6.33	nd	nd	nd	nd
Ajjmayl	3.4	nd	4.00	nd	nd	nd	nd
Beer-ghanam	nd	5.2	nd	7.3	nd	nd	nd
Gharian	3.5	5.00	nd	nd	10.7	nd	7.18
Tajoora	nd	13.1	nd	nd	nd	nd	nd
Alnowahi-4-	nd	nd	6.70	5.90	nd	nd	nd
Tarhoona	2.8	nd	nd	nd	nd	nd	nd
Tarhoona (Spanish)	nd	nd	1.97	nd	nd	2.18	nd
Misalata	3.7	3.40	6.32	nd	nd	4.80	nd
Ghamata	nd	nd	1.1	nd	nd	nd	nd
Ekaam valley	4.3	nd	nd	nd	nd	nd	nd
Alkhoms	4.9	nd	2.73	nd	nd	nd	4.50
Zleetin	nd	nd	2.26	nd	1.22	nd	nd
Alwashka	1.5	nd	1.26	5.00	nd	nd	nd
Gharife	6.4	3.52	nd	7.00	nd	nd	nd
Benghazi	nd	nd	6.32	nd	nd	nd	nd

nd< 1 mg/kg

3.4.1 The statistical analysis of phenolic acids results

The resulting dendrogram of phenolic acids is shown in figure 3.3.

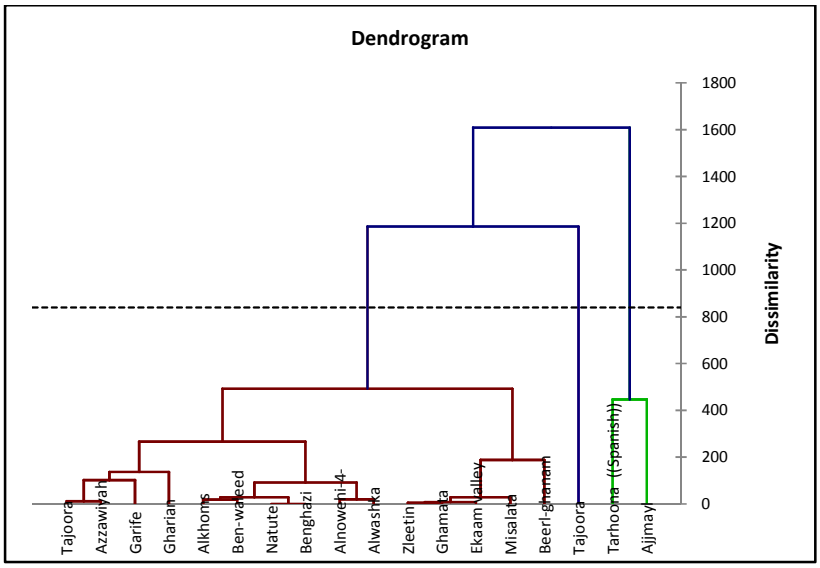

Figure 3.3:The resulting dendrogram of phenolic acids

From the figure 3.3 it was concluded that the Libyan olive oils can be divided into three major groups according to the phenolic acids concentrations. The first group includes the olive oil samples collected from, Azzawiyah, Alwashka, Benghazi, Ghamata, Zleetin, Garife, Wadi Ekaam, Tajoora, Ben-waleed, Alnowehi-4-, Misalata, Gharian, Beer-ghanam, Nalute, and Alkhoms. This group has the lowest values of phenolic acid . The second group include Tarhoona (Spanish) and Ajjmayl samples. The last group represented by Tarhoona samples only (Figure 3.4)

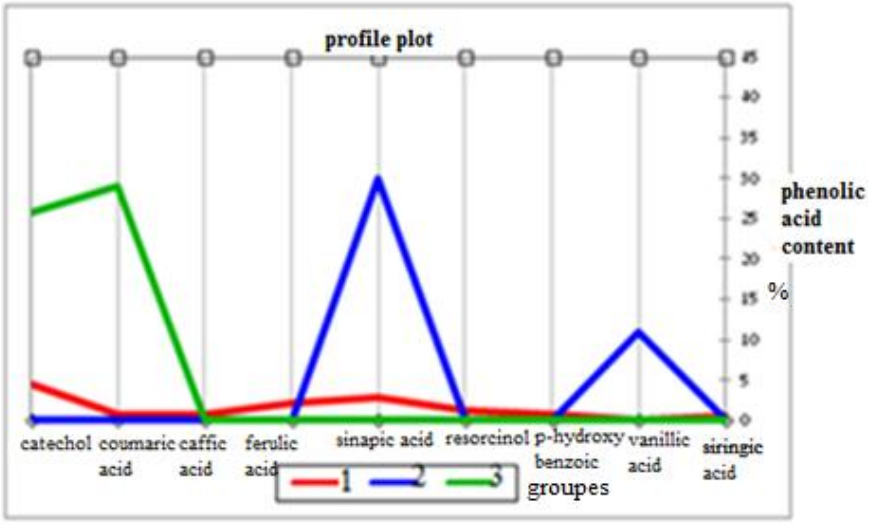

Figure 3.4: Profile plot of the normalized data of phenolic acids of libyan olive oil samples

3.5 Comparison of Libyan olive oil results with some other countries

From the Figrue 3.5 illustrates the comparison between the libyan olive oil, the Turkey and Tunisan olive oil by the content of oleic acid, FAO is also illustrated.

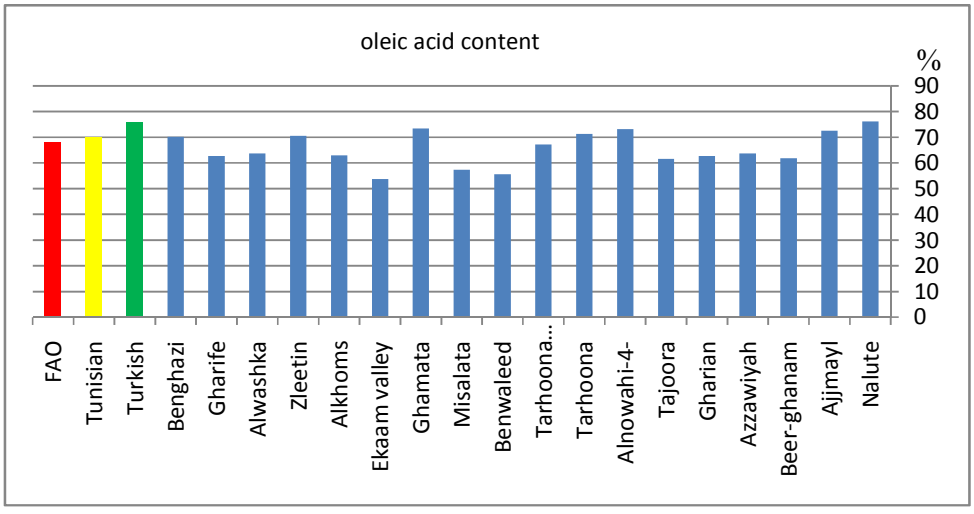

Figure 3.5: The comparison between the Libyan olive oil samples with Turkish olive oil and Tunisan olive oil by the content of oleic acid

Figure 3.6 illustrates the comparison between the Libyan olive oil samples with Turkish and Iranian olive oil in term of acidity

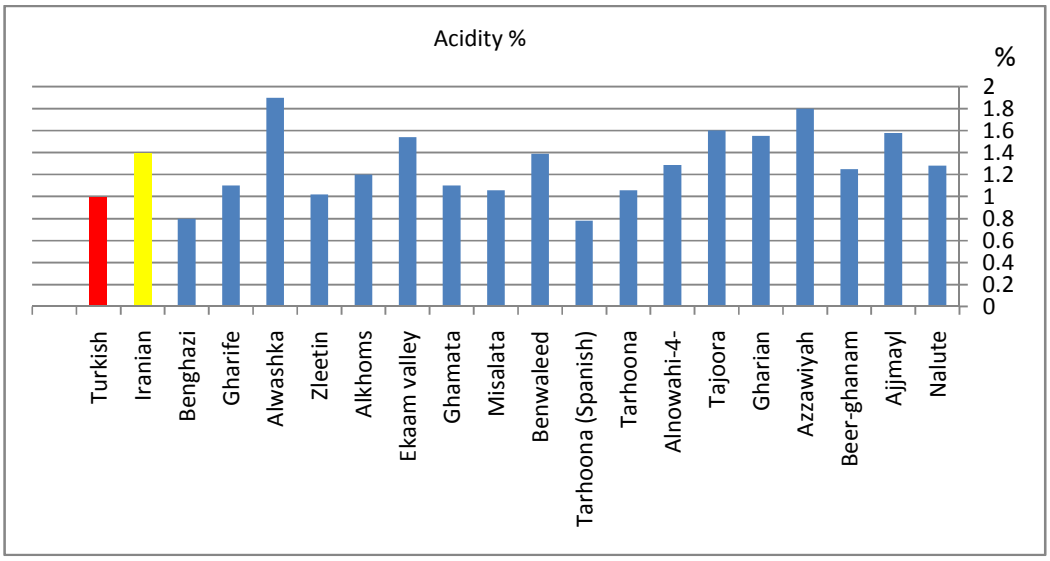

Figure 3.6: The comparison between the Libyan olive oil samples with Turkish olive oil and Iranian olive oil by acidity

CHAPTER IV

CONCLUSIONS and RECOMMENDATION

4.1 General conclusions

Several years ago, olive oil received a wide recognition throughout the world for its superior quality compared to other oils, and because of its impact on human health. Several chemical analyses and quality tests were applied to characterize its composition and to evaluate its quality, and to detect adulteration. This study can be considered as a preliminary characterization of Libyan olive oils in terms of phenolic compounds and fatty acid composition. In addition, the study includes the quality parameters (acidity, saponification value, density, refractive index, and UV absorbance values (delta K, K232 and K270). The results of physico-chemical Properties of this study demonstrated that the studied samples of Libyan olive oil can be graded as a virgin olive oil. The acidity lies between (0.78 and 1.90%), and the saponification value ranged from (182.3-203 mg/g KOH),the density was in the range of (0.9100-0.9125 g/ml) and refractive index was between (1.4677-1.4681). The absorbance measurements at 232 and 270 nm did not exceed the limit of 2.60 and 0.25. The values of K232 and K270 were significantly affected by ripening degree.

The qualitative and quantitative analysis of the fatty acids composition of Libyan olive oil were performed by gas chromatography. The results of this study demonstrated that the Libyan olive cultivars studied shows the best fatty acid composition (lowest palmitic acid, which is the major saturated fat and has high levels of mono- unsaturated oleic acid). All the samples were found to contain an oleic acid ($C_{18:1}$) ranging from 55.6% to76.2% , with the exception Wadi Ekaam sample that has 53.7%. and palmitic acid ($C_{16:0}$) ranging from7.7% to 20.1%. Distinctly, it was found that Libyan olive oil has saturated fatty acids values between (11.5 and 23.8), and unsaturated fatty acids values between (76.4 and 88.5). It has been demonstrated, that a good quality index is assured when the oleic acid value is greater than 57.7% [54] . The fatty acid composition of olive oil depends primarily on the cultivar, and environmental factors such as time of harvest, location, season or climatic conditions (in particular the temperature and rainfall during fruit growth and ripening)[10].

Phenolic acids concentrations of Libyan olive oil samples were determined by high performance liquid chromatography. It was noticed from the distribution of phenolic

acids in olive oils of different samples cultivars were markedly differ. Major phenolic compounds in Libyan olive oils are, vanillic acid, *p*-coumaric acid, cinnamic acid, caffeic acid, sinapic acid, p-hydroxy benzoic, resorcinol, and siringic acid. The concentrations of phenolic acids values that obtained were high and ranged between (1.22-39.4 ppm) .These concentrations depending on the cultivar, climate, ripeness of the olives at harvesting, and the processing. In summary, Libyan olive oil is less quality than Chetoui oil (the most abundant olive cultivar in Tunisia). This is due to the simple difference in the weather or because of the olive harvest at an inconvenient time or poor storage method.

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4.2 Recommendations

Olive oil has many benefits to protection from many diseases, such as heart disease, blood doll, and high blood cholesterol. Also because the Libya is one of the most important Mediterranean countries of olive oil producing. Therefore we must recommends the following:-

- Through the study of the quality of Libyan olive oil its noticed that some samples shows high acidity and its returned for several reasons, include the bad storage of oil or/and a early harvest of olive tree. Therefore we advises olive farmers and producers to take care of good storing of the olive fruits and olive oil after processing,in addition they must choose the right harvest time (optimal time to harvest of olive is between the last week of October and the first week of November).
- Monounsaturated fatty acids/polyunsaturated fatty acids (MUFAs/PUFAs) ratios, is present in a wide range of (2.27–9.00%). The Ajjmayl sample is distinguishable from the others due to its considerably higher MUFAs/PUFAs ratio. This ratios depending on storage of olive oil, transfer and picking of olive fruit. So storage, picking and transfer must be taking with care.
- The Libyan olive oil according to this study is good for the human consumption Because of it has low acidity in the range of (0.8-1.9%), and so it classified as Virgin olive oil.
- Although this study covers large part of Libyan Agricultural zone, the Green Mountain (Ajabal –Alakhdur) was not included in this study, so we recommends the researchers to investigate and study this area.
- The researchers finally recommends the government and the Ministry of Agriculture to encourage and support the farmers of the olive oil trees and helps them by accepting their harvest.

References

1. Apostolos Kiritsakisa, A.K., Konstantinos Kiritsakisc., Chemical analysis, quality control and packaging issues of olive oil. European Journal of Lipid Science and Technology, 2002. 104: p. 628–638. .
2. S. Movahed, B.K., H.Ahmadi Chenarbon, Evaluation of acidity and fatty acid compositions in Iranian and imported olive oils. . International Research Journal of Applied and Basic Sciences. , 2012. 3(6): p. 1140-1142.
3. Alegria Carrasco-Pancorbo, L.C., Alessandra Bendini, Antonio Segura-Carretero, Tullia Gallina-Toschi, and Alberto Fernandez-Gutiarrez1, Analytical determination of polyphenols in olive oils. Journal of Separation Science, 2005. 28: p. 837–858.
4. M. Del Carlo, E.R., G. Procida, F. Murmura, A. Cichelli, Characterization of extra virgin olive oils obtained from different cultivars. . Pomologia Croatica 2006. 12: p. 29-41.
5. Abouzar Hashempour, R.F.G., Davood Bakhshi, Samaneh Asadi Sanam, Fatty acids composition and pigments changing of virgin olive oil (Olea europea L.) in five cultivars grown in Iran. . Australian Journal of Crop Science 4 2010. 4(4): p. 258-263.
6. Baccouri, B., Temime, S.B., Campeol, E., Cioni, P.L., Daoud, D., Zarrouk, M. , Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. . Food Chemistry 2006. 102: p. 850-856.
7. Olfa Baccouri, A.B., Lorenzo Cerretani, Mokhtar Guerfel, Béchir Baccouri, Giovanni Lercker, Mokhtar Zarrouk, Douja Daoud Ben Mile. , Comparative study on volatile compounds from Tunisian and Sicilian monovarietal virgin olive oils. Food Chemistry, , 2008. 111: p. 322-328.
8. Luchetti, F., Importance and future of olive oil in the world market – an introduction to olive oil. . European Journal of Lipid Science and Technology 2002. 104: p. 559-563.

9. Visioli, F.a.G., C. , Biological Properties of Olive Oil Phytochemicals. Critical Reviews in Food Science and Nutrition 2002. 42(3): p. 209-221.
10. Grigg, D., Olive oil, the Mediterranean and the world. Geo Journal, 2001. 53: p. 163-172.
11. Carrasco A., L.C.L., Bendini A., Segura-Carretero A., Gallina-Toschi T., and Fernandez-Gutierrez A, Analytical determination of polyphenols in olive oils journal of Separation Science 2005. 28: p. 837-858.
12. M.M.Chakrabary, Chemistry And Technology Of Oils And Fats. New Delhi. Sriniway Puri, Printo Graphic Systems, 2003: p. Pp. 155.157.
13. International Olive Oil Council (IOOC), Doc. IOOC/T.15/NC no. 1/Rev. 6, June 10, . 1993.
14. NATIONS, F.A.A.O.O.T.U., "FAOSTAT Crops processed 2010 data for olive oil" 2010.
15. International Olive Oil Council. 2007. http:/.internationaloliveoil.org (accessed April 10.
16. Hashim, Y.Z.H.Y., Gill, C.I.R., McGlynn, H., Rowland, I.R. , Components of olive oil and chemoprevention of colorectal cancer. . Nutrition Reviews 2005. 63: p. 374-386.
17. Servili, M.a.M., G.F, Contribution of phenolic compounds to virgin olive oil quality. European Journal of Lipid Science and Technology 2002. 104: p. 602-613.
18. Organizzatione nazionale assaggiatori olio di olive (ONAOO). 2003. Chemical-physical characteristics of olive oils, 2003.
19. Zarrouk W., B.B., Taamalli W., Trigui A., Daoud D.,and Zarrouk M. , Oil fatty acid composition of eighteen Mediterranean olive varieties cultivated under the arid conditions of Boughrara (southern Tunisia). . Grasas Y Aceites,, 2009. 60(5): p. 498-506.
20. Manzi, P., Panfili, G., Esti, M., Pizzoferrato, L., Natural antioxidants in the unsaponifiable fraction of virgin olive oils from different cultivars. Journal of the Science of Food and Agriculture 1998. 77: p. 115-120.

21. A. Cert, W.M.a.M.C.p.-C., Methods of preparation of fatty acid methyl ester (FAME). Grasas y Aceites 2000. 51: p. 447-456.
22. Emily Waterman, M.P., and Brian Lockwood, B. Pharm, Ph D, M. R. Pharm. S, Active Components and Clinical Applications of Olive Oil. Alternative Medicine Review 2007. 12: p. 331-334.
23. Velasco, J., & Dobarganes, C. , Oxidative stability of virgin olive oil. . European Journal of Lipid Science and Technology, 2002. 104(9-19): p. 661-676.
24. Youssef Ameni, T., Mokhtar Guerfel, Chedly Abdelly, Mokhtar Zarrouk and Guido Flamin, The phenolic compounds and compositional quality of Chétoui virgin olive oil: Effect of altitude. . African Journal of Biotechnology 2013. 11(55): p. 11842-11850.
25. M.Tsimidou, polyphenols and quality of virgin olive oil in retrospect. italian journal of food science, 1998. 10: p. 99-116.
26. Aleksander Siger1, M.N.-K.A. and E. Lampart-Szczapa, the content and antioxidant activity of phenolic compounds in cold-pressed plant oils. Journal of Food Lipids, 2007. 15: p. 137-149.
27. Maurizio Servili, R.S., Sonia Esposto, Agnese Taticchi, GianFrancesco Montedoro, Guido Morozzi, Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. Journal of Chromatography A, 2004. 1054: p. 113-127.
28. Nahit Gençer, S.S., Oktay Arslan., Antioxidant Properties of Various Olive Cultivars. Journal of Applied Biological Sciences 2009. 3(1): p. 23-27.
29. Robbins, R.J., Phenolic Acids in Foods: An Overview of Analytical Methodology. Journal of Agricultural and Food Chemistry, 2003. 51(10): p. 2866-2887.
30. Aluyor, E.O., Ozigagu, C. E., Oboh, O. I. And Aluyor,.(Chromatographic Analysis of Vegetable Oils: A Review. . A Review. Scientific Research And Essay 2009. 4(4): p. 191-197.
31. Cicerale S., L.L., and Keast R, Biological Activities of Phenolic Compounds Present in Virgin Olive Oil. International Journal of Molecular Sciences 2010. 11: p. 458-479.

32. Angerosa, F., Mostallino, R., Basti, C., & Vito, R. , Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. . Food Chemistry, , 2000. 68: p. 283–287.

33. Marco D.R. Gomes d.a Silva, A.M.C.F., Maria J. B. Cabrita and Raquel Garcia Olive Oil Composition: Volatile Compounds, Olive Oil - Constituents, Quality, Health Properties and Bioconversions. Edited by Dr. Dimitrios Boskou 2012.

34. Benicasa, C.D.N., A. Lombardo, N. Perri, E. ;Sindona, G. & Tagarelli, A., Assay of Aroma Active Components of Virgin Olive Oils from Southern Italian Regions by SPME-GC/Ion Trap Mass Spectrometry. Journal of Agricultural and Food Chemistry., 2003. 51: p. 733- 741.

35. Kiritsakis, A.K., Nanos, G.D., Polymenopoulos, Z., Thomai, T., Sfakiotakis, E.M., , Effect of fruit storage conditions on olive oil quality. . Journal of the American Oil Chemists' Society, 1998. 75: p. 721-724.

36. Caponio, F.G., T. Pasqualone, A, Phenolic compounds in virgin olive oils: influence of the degree of olive ripeness on organoleptic characteristics and shelf-life. . European Food Research and Technology 2001. 212: p. 329-333.

37. José L. Quiles, M.C.R.-T.a.P.Y., Olive Oil And Health.7th ed. London. CAB International 2006: p. pp.47-50.

38. Alessandra Bendini, L.C., Alegria Carrasco-Pancorbo, Ana Maria Gómez-Caravaca, Antonio Segura-Carretero, Alberto Fernández-Gutiérrez and Giovanni Lercker, Phenolic Molecules in Virgin Olive Oils: a Survey of Their Sensory Properties, Health Effects, Antioxidant Activity and Analytical Methods. An Overview of the Last Decade. Molecules, 2007. 12: p. 1679-1719.

39. Rosa M. Lamuela-Raventós, E.G., Montse Fitó, Ana-Isabel, M.C. Castellote, M. Carmen De La Torre-Boronat And M., And C. López-Sabater., Interaction of Olive Oil Phenol Antioxidant Components with Low-density Lipoprotein. Biological Research, 2004. 37: p. 247-252.

40. Najafzadeh M., R.F., and Ali sharif, Physicochemical properties of two varieties of oliveoil (Roghani & Zard) in Iran. International e-Conference on novel Food Processing, , 2013: p. 1-7.

41. Al-Ghamdy, F.B.S.M., Nawal Mostfa Abdul Aziz & Amani Alawi Al-Rasheedi., Evaluation of Virgin Olive Oil Quality Presented in Local Market in Jeddah. 2008. 149.
42. Manel Issaoui , G.F., Faten Brahmi , Samia Dabbou , Kaouther Ben Hassine , Amani Taamali ,Hechmi Chehab , Myriem Ellouz , Mokhtar Zarrouk , Mohamed Hammami, Effect of the growing area conditions on differentiation between Chemlali and Chétoui olive oils. . Food Chemistry 2010. 119: p. 220-225.
43. Abouzar Hashempour, R.F.G., Davood Bakhshi, Samaneh Asadi Sanam, Fatty acids composition and pigments changing of virgin olive oil (Olea europea L.) in five cultivars grown in Iran. American Journal of Cultural Sociology 2010. 4(4): p. 258-263.
44. Y, T., A study of selected sensory and chemical quality aspects of "Rumi" and "Nabali" olive oil. Ph. D. Thesis. . amautah Lil-Buhuth Wad- Dirasat, 2005. 19: p. 51-65.
45. W., A.-R., amautah Lil-Buhuth Wad- Dirasat, , 2004. 19: p. 51-65.
46. N., M.D., Determine the types and proportions of natural phenolic compounds in Syrian virgin olive oil. . Arab Federation for Food Industries, 2007.
47. Nieisen, o.S.S., Food Analysis.2nded. Aspen. Ruth Bloom. 'Aspen Publishers, , 1998: p. Pp 99.104
48. Ali Muhammad, M.A., Alam Zeb, Said Wahab, Saleem Khan and Ihsanullah.. Physicochemical Analysis and Fatty Acid Composition of Oil Extracted From Olive Fruit Grown in Khyber Pakhtunkhwa, Pakistan. International Journal of Basic & Applied Sciences 2013. 13: p. 36-39.
49. Douglas A. Skoog, D.M.W., F.Games Holler And Stanley R. Crouch. , Analytical Chemistry: An Introduction. 7th Ed. United States Of America.Emily Barrosse. Thomoson Learing, Lnc, 1999: p. Pp71-73.
50. Shaker, M.A.a.A., A. A., Relationship between volatile compounds of olive oil and sensory attributes. . International Food Research Journal 20, 2013. 20: p. 197-204.

51.Tsimidou, P.M.P.a.M., Solid Phase Extraction: Applications To The Chromatographic Analysis Of Vegetable Oils And Fats. . Grasas Y Aceites 2002. 53(1): p. 84-95.

52. Kenan Tanılgana, M.M.o.a.A.u., Physical and chemical characteristics of five Turkish olive (Olea europea L.) varieties and their oils. . Grasas Y Aceites, , 2007. 58(2): p. 142-147.

53. C. Borchani1, S.B., Ch. Blecker, and H. Attia. , Chemical Characteristics and Oxidative Stability of Sesame Seed, Sesame Paste, and Olive Oils. Journal of Agricultural Science and Technology 2010. 12: p. 585- 596.

54. D. Krichene, A.A., B. Baccouri, G.Q. Fregapane, M.D. Salvador and M. Zarrouk, Territorial Investigation Based on the Chemical Composition of Chemlali Virgin Olive Oils. Asian Journal of Biochemistry, 2009. 4: p. 1-12.

55. Wissem Zarrouk, B.B., Wael Taamalli, Ahmed Trigui, Douja Daoud and Mokhtar Zarrouk., Oil fatty acid composition of eighteen Mediterranean olive varieties cultivated under the arid conditions of Boughrara (southern Tunisia). . Grasas Y Aceites, , 2009. 60(5): p. 498-506.

56. Hatice Uğurlu Asık, G.Ö., Physical, Chemical and Antioxidant Properties of Olive Oil Extracted from Memecik Akademik Gıda 2011. 9(2): p. 13-18.

57. Samia Dabbou, F.B., Sihem Dabbou, Manel Issaoui, Samira Sifi and Mohamed Hammami, Antioxidant capacity of Tunisian virgin olive oils from different olive cultivars African Journal of Food Science and Technology 2011. 2(4): p. 92-97.

58. Dritan Topi, F.T., Eltion Halimi, Virgin Olive Oil Production From The Major Olive Varieties In Albania. . Agriculture & Forestry, , 2012. 58(2): p. 87-95.

59. Montserrat Fitó, R.d.l.T., Magí Farré-Albaladejo,Olha Khymenetz, Jaime Marrugat and María-Isabel Covas, Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. Ann Ist Super Sanità 2007. 43(4): p. 375-381.

60. Sonia Ben Temime1, T.W., Baccouri Bechir, Abaza Leila, Daoud Douja And Zarrouk Mokhtar, Changes In Olive Oil Quality Of Chétoui Variety According To Origin Of Plantation. . Journal of Food Lipids 2006. 13: p. 88-99.

Appendix I

Table I.1. The mean values of the absorbance measurements at 266 and 274 nm of Libyan olive oil

No.	**Sample site**	Abs266	Abs274
1	Nalute	0.110	0.100
2	Ajjmayl	0.108	0.103
3	Beer-ghanam	0.202	0.170
4	Azzawiyah	0.221	0.218
5	Gharian	0.130	0.118
6	Tajoora	0.210	0.196
7	Alnowahi-4-	0.100	0.087
8	Tarhoona	0.220	0.200
9	Tarhoona (Spanish)	0.120	0.111
10	Benwaleed	0.110	0.095
11	Misalata	0.200	0.190
12	Ghamata	0.160	0.148
13	Wadi Ekaam	0.165	0.144
14	Alkhoms	0.123	0.100
15	Zleetin	0.140	0.129
16	Alwashka	0.210	0.207
17	Gharife	0.220	0.185
18	Benghazi	0.225	0.190

Appendix II

GC Estimation of fatty acid profile

Table II.1. Fatty acid profiles of (% total fatty acids) of Nalute samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.2
5	Tridecanoic acid	C13	0.1
6	Myristic acid	C14	0.3
7	Pentadecanoic acid	C15	0
8	Palmitolic acid	C16:1	0.6
9	Palmitic acid	C16	8.7
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	76.2
12	Stearic acid	C18	1.9
13	Linoleic acid	C18:2	11.4
14	Linolenic acid	C18:3	0.3
15	Arachidic acid	C20	0.1
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0.1
18	Saturated fatty acids		11.5
19	Unsaturated fatty acids		88.5

Table II.2. Fatty acid profiles of (% total fatty acids) of Ajjmayl samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.9
7	Pentadecanoic acid	C15	0.2
8	Palmitolic acid	C16:1	0.2
9	Palmitic acid	C16	16.8
10	Hexadecanoic acid	C17	0.3
11	Oleic acid	C18:1	72.6
12	Stearic acid	C18	0.6
13	Linoleic acid	C18:2	7.6
14	Linolenic acid	C18:3	0.5
15	Arachidic acid	C20	0.2
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0.1
18	Saturated fatty acids		19.1
19	Unsaturated fatty acids		80.9

Table II.3. Fatty acid profiles of (% total fatty acids) of Beer- ghanam samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0.1
4	Lauric acid	C12	0.1
5	Tridecanoic acid	C13	0.2
6	Myristic acid	C14	1.1
7	Pentadecanoic acid	C15	0.1
8	Palmitolic acid	C16:1	0.5
9	Palmitic acid	C16	20.1
10	Hexadecanoic acid	C17	0.6
11	Oleic acid	C18:1	61.8
12	Stearic acid	C18	0.4
13	Linoleic acid	C18:2	14.4
14	Linolenic acid	C18:3	0.4
15	Arachidic acid	C20	0.2
16	Behenic acid	C22	0.1
17	Erucic acid	C22:1	0
18	Saturated fatty acids		22.9
19	Unsaturated fatty acids		77.1

Table II.4.Fatty acid profiles of (% total fatty acids) of Azzawiyah samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.3
7	Pentadecanoic acid	C15	0.1
8	Palmitolic acid	C16:1	0.9
9	Palmitic acid	C16	14.8
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	63.7
12	Stearic acid	C18	1.1
13	Linoleic acid	C18:2	18.5
14	Linolenic acid	C18:3	0.2
15	Arachidic acid	C20	0.3
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0
18	Saturated fatty acids		16.7
19	Unsaturated fatty acids		83.3

Table II.5.Fatty acid profiles of (% total fatty acids) of Gharian samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.1
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.9
7	Pentadecanoic acid	C15	0.1
8	Palmitolic acid	C16:1	1.1
9	Palmitic acid	C16	12.5
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	62.7
12	Stearic acid	C18	1.6
13	Linoleic acid	C18:2	19.9
14	Linolenic acid	C18:3	0.5
15	Arachidic acid	C20	0.4
16	Behenic acid	C22	0.1
17	Erucic acid	C22:1	0.1
18	Saturated fatty acids		15.8
19	Unsaturated fatty acids		84.2

Table II.6. Fatty acid profiles of (% total fatty acids) of Tajoora samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.3
5	Tridecanoic acid	C13	0.1
6	Myristic acid	C14	1.2
7	Pentadecanoic acid	C15	0.2
8	Palmitolic acid	C16:1	0.7
9	Palmitic acid	C16	17.9
10	Hexadecanoic acid	C17	0.3
11	Oleic acid	C18:1	61.6
12	Stearic acid	C18	3.4
13	Linoleic acid	C18:2	13.8
14	Linolenic acid	C18:3	0.3
15	Arachidic acid	C20	0.2
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0
18	Saturated fatty acids		23.6
19	Unsaturated fatty acids		76.4

Table II.7.Fatty acid profiles of (% total fatty acids) of Alnowahi-4- samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.2
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.4
7	Pentadecanoic acid	C15	0
8	Palmitolic acid	C16:1	0.5
9	Palmitic acid	C16	9.4
10	Hexadecanoic acid	C17	0.6
11	Oleic acid	C18:1	73.1
12	Stearic acid	C18	0.8
13	Linoleic acid	C18:2	14.4
14	Linolenic acid	C18:3	0.3
15	Arachidic acid	C20	0.1
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0.2
18	Saturated fatty acids		11.7
19	Unsaturated fatty acids		88.3

Table II.8. Fatty acid profiles of (% total fatty acids) of Tarhoona samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	.71
7	Pentadecanoic acid	C15	0.3
8	Palmitolic acid	C16:1	0.9
9	Palmitic acid	C16	10.4
10	Hexadecanoic acid	C17	0.2
11	Oleic acid	C18:1	71.3
12	Stearic acid	C18	2.7
13	Linoleic acid	C18:2	11.9
14	Linolenic acid	C18:3	0.5
15	Arachidic acid	C20	0.1
16	Behenic acid	C22	0.1
17	Erucic acid	C22:1	0
18	Saturated fatty acids		15.4
19	Unsaturated fatty acids		84.6

Table II.9. Fatty acid profiles of (% total fatty acids) of Tarhoona (Spanish) samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.1
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.4
7	Pentadecanoic acid	C15	0.1
8	Palmitolic acid	C16:1	0.4
9	Palmitic acid	C16	13.1
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	67.2
12	Stearic acid	C18	1.2
13	Linoleic acid	C18:2	17.1
14	Linolenic acid	C18:3	0.1
15	Arachidic acid	C20	0.2
16	Behenic acid	C22	0.1
17	Erucic acid	C22:1	0
18	Saturated fatty acids		15.2
19	Unsaturated fatty acids		84.8

Table II.10. Fatty acid profiles of (% total fatty acids) of Benwaleed samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.1
5	Tridecanoic acid	C13	0.1
6	Myristic acid	C14	1.5
7	Pentadecanoic acid	C15	0.2
8	Palmitolic acid	C16:1	0.5
9	Palmitic acid	C16	19.5
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	55.6
12	Stearic acid	C18	1.1
13	Linoleic acid	C18:2	20.8
14	Linolenic acid	C18:3	0.1
15	Arachidic acid	C20	0.4
16	Behenic acid	C22	0.1
17	Erucic acid	C22:1	0
18	Saturated fatty acids		23
19	Unsaturated fatty acids		77

Table II.11. Fatty acid profiles of (% total fatty acids) of Misalata samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.2
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.4
7	Pentadecanoic acid	C15	0
8	Palmitolic acid	C16:1	0.9
9	Palmitic acid	C16	12.8
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	57.3
12	Stearic acid	C18	2.1
13	Linoleic acid	C18:2	25.2
14	Linolenic acid	C18:3	0.4
15	Arachidic acid	C20	0.6
16	Behenic acid	C22	0.1
17	Erucic acid	C22:1	0
18	Saturated fatty acids		16.2
19	Unsaturated fatty acids		83.8

Table II.12. Fatty acid profiles of (% total fatty acids) of Ghamata samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0.1
6	Myristic acid	C14	0.7
7	Pentadecanoic acid	C15	0
8	Palmitolic acid	C16:1	0.4
9	Palmitic acid	C16	11.2
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	73.4
12	Stearic acid	C18	0.8
13	Linoleic acid	C18:2	12.7
14	Linolenic acid	C18:3	0.2
15	Arachidic acid	C20	0.3
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0.1
18	Saturated fatty acids		13.3
19	Unsaturated fatty acids		86.7

Table II.13. Fatty acid profiles of (% total fatty acids) of Wadi Ekaam samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0.1
2	Caprylic acid	C8	0.1
3	Capric acid	C10	0
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	1.2
7	Pentadecanoic acid	C15	0.1
8	Palmitolic acid	C16:1	0.8
9	Palmitic acid	C16	18.1
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	53.7
12	Stearic acid	C18	4.3
13	Linoleic acid	C18:2	20.8
14	Linolenic acid	C18:3	0.5
15	Arachidic acid	C20	0.2
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0
18	Saturated fatty acids		24.2
19	Unsaturated fatty acids		75.8

Table II.14. Fatty acid profiles of (% total fatty acids) of Alkhoms samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.1
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.3
7	Pentadecanoic acid	C15	0.3
8	Palmitolic acid	C16:1	0.9
9	Palmitic acid	C16	14.8
10	Hexadecanoic acid	C17	0.1
11	Oleic acid	C18:1	63
12	Stearic acid	C18	2.1
13	Linoleic acid	C18:2	18.1
14	Linolenic acid	C18:3	0.2
15	Arachidic acid	C20	0.1
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0
18	Saturated fatty acids		17.8
19	Unsaturated fatty acids		82.2

Table II.15. Fatty acid profiles of (% total fatty acids) of Zleetin samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0.1
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0.1
6	Myristic acid	C14	1.4
7	Pentadecanoic acid	C15	0.3
8	Palmitolic acid	C16:1	0.6
9	Palmitic acid	C16	7.7
10	Hexadecanoic acid	C17	0.3
11	Oleic acid	C18:1	70.6
12	Stearic acid	C18	1.3
13	Linoleic acid	C18:2	17.1
14	Linolenic acid	C18:3	0.2
15	Arachidic acid	C20	0.3
16	Behenic acid	C22	0.1
17	Erucic acid	C22:1	0
18	Saturated fatty acids		11.5
19	Unsaturated fatty acids		88.5

Table II.16. Fatty acid profiles of (% total fatty acids) of Alwashka samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0.1
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0.2
5	Tridecanoic acid	C13	0.2
6	Myristic acid	C14	0.6
7	Pentadecanoic acid	C15	0
8	Palmitolic acid	C16:1	0.2
9	Palmitic acid	C16	17.6
10	Hexadecanoic acid	C17	0.3
11	Oleic acid	C18:1	63.7
12	Stearic acid	C18	0.9
13	Linoleic acid	C18:2	15.8
14	Linolenic acid	C18:3	0.2
15	Arachidic acid	C20	0.2
16	Behenic acid	C22	0.2
17	Erucic acid	C22:1	0
18	Saturated fatty acids		20.1
19	Unsaturated fatty acids		79.9

Table II.17. Fatty acid profiles of (% total fatty acids) of Gharife samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0
6	Myristic acid	C14	0.6
7	Pentadecanoic acid	C15	0.1
8	Palmitolic acid	C16:1	0.7
9	Palmitic acid	C16	13.7
10	Hexadecanoic acid	C17	0.2
11	Oleic acid	C18:1	59.2
12	Stearic acid	C18	1.3
13	Linoleic acid	C18:2	23.4
14	Linolenic acid	C18:3	0.3
15	Arachidic acid	C20	0.4
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0.1
18	Saturated fatty acids		16.3
19	Unsaturated fatty acids		83.7

Table II.18.Fatty acid profiles of (% total fatty acids) of Benghazi samples

No.	Fatty Acid		Fatty Acid %
1	Butyric acid	C4	0
2	Caprylic acid	C8	0
3	Capric acid	C10	0
4	Lauric acid	C12	0
5	Tridecanoic acid	C13	0.1
6	Myristic acid	C14	0.7
7	Pentadecanoic acid	C15	0.2
8	Palmitolic acid	C16:1	0.7
9	Palmitic acid	C16	16.9
10	Hexadecanoic acid	C17	0.2
11	Oleic acid	C18:1	70.2
12	Stearic acid	C18	1.3
13	Linoleic acid	C18:2	9.5
14	Linolenic acid	C18:3	0.1
15	Arachidic acid	C20	0.1
16	Behenic acid	C22	0
17	Erucic acid	C22:1	0
18	Saturated fatty acids		19.5
19	Unsaturated fatty acids		80.5

Appendix III

Some of HPLC chromatograms of the phenolic acids extracted from Libyan olive oil samples

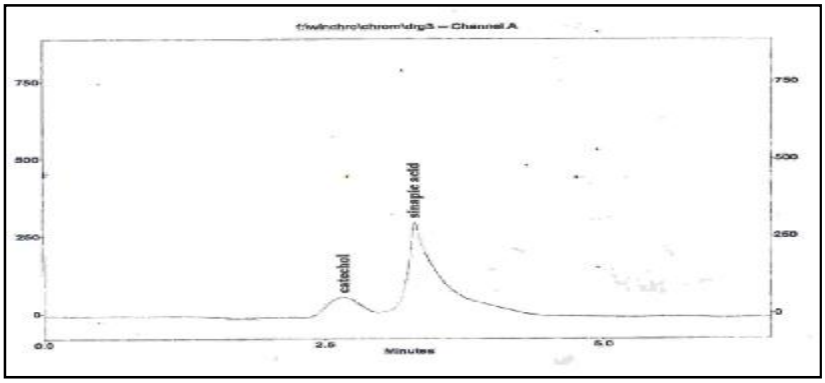

Figure III.1: HPLC chromatograms of the phenolic acids extracted from Nalute

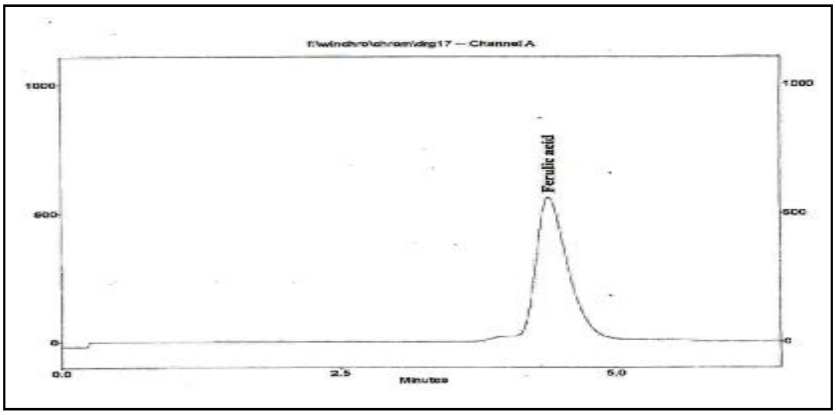

Figure III.2: HPLC chromatograms of the phenolic acids extracted from Tajoora

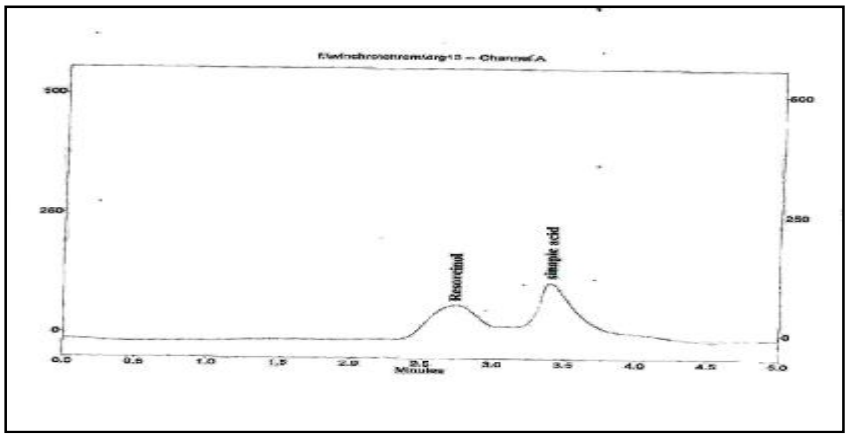

Figure III.3: HPLC chromatograms of the phenolic acids extracted from Alnowahi-4-

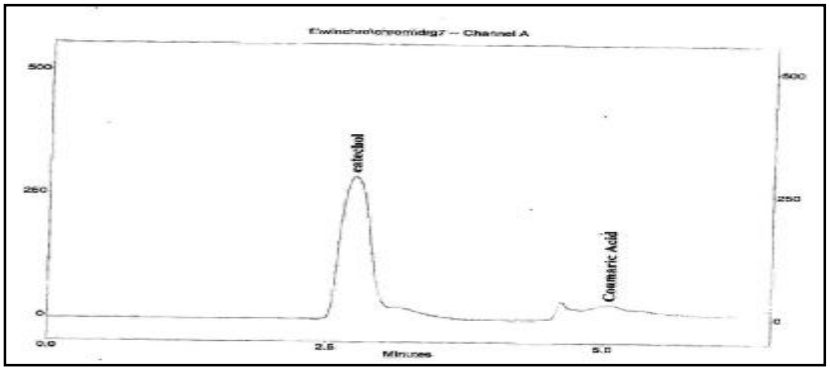

Figure III.4: HPLC chromatograms of the phenolic acids extracted from Wadi Ekaam

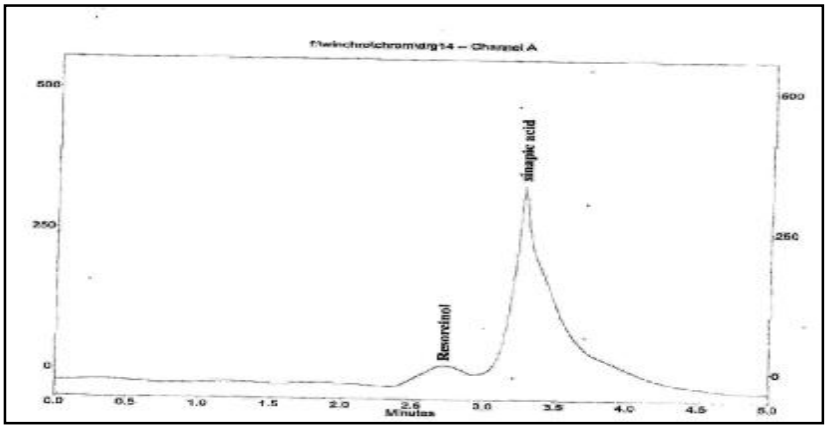

Figure III.5: HPLC chromatograms of the phenolic acids extracted from Alwashka

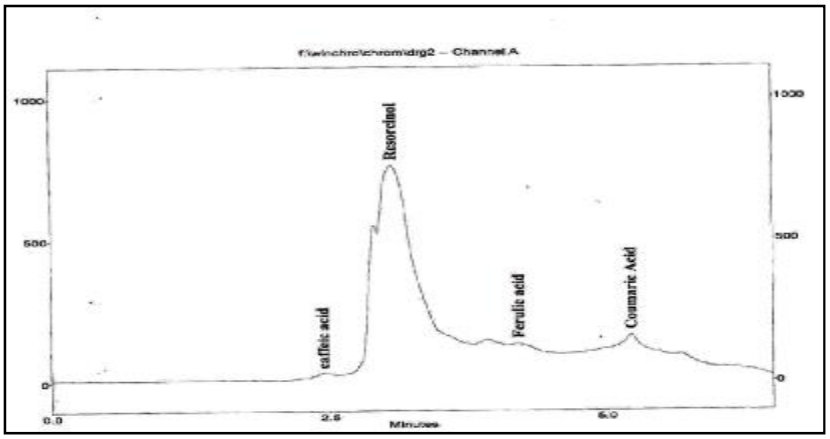

Figure III.6: HPLC chromatograms of the phenolic acids extracted from Gharife

ملخص

زيت الزيتون يختلف عن باقي الزيوت النباتية الأخرى في احتواه على عالية من المركبات الفينولية و محتويات الأحماض الدهنية غير المشبعة . لأنه يحتوي على مستوى عال في الأحماض الدهنية غير المشبعة (حامض الأوليك) ؛ وقد تم ربط المقاومة للأكسدة زيت الزيتون و الحماية ضد بعض الأمراض لهذه المكونات من زيت الزيتون.

في هذا العمل ، لتحديد التراكيب الأحماض الدهنية و محتويات المركبات الفينولية (الأحماض الفينول) من زيت الزيتون لليبي ، وقد تم جمع ثمانية عشر عينة زيت الزيتون لليبي من مختلف المناطق الزراعية في ليبيا ، من مدنية بنغازي في شرق الي مدنية نالوت في غرب البلاد ، حوالى 1150 كم على طول ساحل البحر الأبيض المتوسط .

تم فصل مكونات الأحماض الدهنية في زيت الزيتون الليبي عن طريق تقنية فصل سائل- سائل قبل القياس بواسطة تقنية كرماتجرافيا الغاز(GC)، و تم فصل الأحماض الفينولية عن طريق استخراج المرحلة الصلبة قبل القياس بواسطة كرماتجرافيا السائل (HPLC).

حيث كانت قيم حمض الأوليك في عينات الزيت لتكون ضمن المستويات المثلى (55-83 ٪) باستثناء عينة وادي كعام. وكانت انواع الأحماض الفينولية الموجودة في العينات هي : حامض كافيك ، الكاتيكول ، وحمض فرولك ، حامض كيماريك ، وحامض هيدروكسي البنزويك ، وحمض فانيليتش ، الريسورسنول ، حمض سيرجينيك ، حمض سينابيك. تم العثور على مجموعة من الأحماض الفينولية لجميع العينات لتكون بين (1.22 حتى 4.39 جزء في المليون) . تم قياس ألحموضة و رقم التصبن ، الامتصاص في البنفسجي فائقة في K232 K270 ،و الكثافة و معامل الانكسار أيضا قيست للمعرفة جودة زيت الزيتون . هذه الطرق المستخدمة لتحديد جودة زيت الزيتون و مقاومته للتأكسد.

نستنتج من هذه الدراسة أن معظم عينات زيت الزيتون الليبي ذات نوعية جيده و كانت معظم العينات صنفت على اساس زيت زيتون بكر .