Deanship of Graduate Studies

Al-Quds University

Correlation Between Chemical and Sensorial Characteristics of Virgin Olive Oil

Prepared By:

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Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledges, and that this thesis (or any part of the same) has not been submitted for the higher degree to any other university or institute.

Signed: *Safaa'*

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Date: 10 / 8 / 2024

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ABSTRACT

Palestine is an emerging nation in virgin olive oil production, yet little research has been conducted on its olive oils to date. Notably, there is a gap in the literature regarding the oils' chemical and organoleptic properties and the Pearson correlations between chemical and sensory parameters. The set of characteristics that determine an olive oil's appeal to consumers is known as its quality, which can also be described from commercial, nutritional, or organoleptic perspectives. Extra virgin olive oil (EVOO) derives its nutritional benefits from minor components concentration of antioxidants and high oleic acid content. Therefore, these quality factors support olive oil's pricing and consumption demands compared to other edible oils.

In this study, the olive oil used was provided directly by farmers at the olive oil presses. Eight samples were collected from each farmer, sealed in airtight containers, and stored in a refrigerator until analysis. The samples were gathered under various conditions in late October 2022. The aim of this work was to evaluate the chemical parameters, including Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant Activity (AA), Acidity, and Peroxide Value (PV), as well as the organoleptic quality of eight olive oil samples from different farms in Palestine: Qarawat Bani Zaid farm (S1), Masha farm (S2), Anzeh (S3), Farkh (S4), East Bani Zaid 2 (S5), Fruit Fall Trees 1 (S6), Al-Yamoon (S7), and Kofer Qadom (S8). Sensorial evaluation was conducted by a panel accredited by the Jordan Standards and Metrology Organization as International Olive Council (IOC). The correlation between chemical and sensorial evaluations was also analyzed.

For an olive oil to be classified as extra virgin, it must have a median defect score of zero or a median score of one of the five olive oil defects (Fusty, Musty, Rancid, Winey vinegar and Others perceived with the greatest intensity as zero, and a median fruitiness score above zero. Antioxidant activity and polyphenol concentration, which both have a strong positive correlation with fruitiness, are highly indicative of olive oil quality. Furthermore, the absorption coefficients K270 and K232 have been identified as the most sensitive chemical

tests for determining the quality of fresh olive oil. Fruity attributes negatively correlate with both K270 and K232 values. Also Fruity attributes negatively correlate with Defects. Chemical indices like absorption coefficients K232 and K270, coupled with sensory evaluations, determine olive oil quality. Total flavonoid concentration emerges as a critical chemical marker, impacting quality, while acidity and sensory defects play lesser roles. Total phenolic content significantly correlates with antioxidant activity, underscoring its importance in enhancing olive oil quality. K270 and K232 absorption coefficients prove particularly sensitive in determining olive oil quality, while peroxide value shows no significant association with EVOO. Strong correlations between antioxidant activity, total phenolic content, and sensory defects highlight their pivotal roles in defining olive oil quality. Sensory evaluation alone is insufficient for accurate quality assessment, necessitating complementary chemical analysis. Extra virgin olive oil include a median score of zero for faults and a positive median score for fruitiness. Antioxidant activity and polyphenol concentration exhibit strong positive correlations with fruitiness, underscoring their significance in olive oil quality assessment. Moreover, the absorption coefficients K270 and K232 emerge as sensitive chemical indicators for determining fresh olive oil quality, with fruitiness showing a negative correlation with both. Extra virgin olive oil (EVOO) stands as the pinnacle of olive oil quality, subject to stringent chemical and sensory regulations. Renowned for its superior taste and health benefits, EVOO represents the highest grade among olive oil varieties. Despite being the most challenging to produce, the effort is well justified by its exceptional quality.

The criteria for defining extra virgin olive oil include a median score of zero for faults and a positive median score for fruitiness. Antioxidant activity and polyphenol concentration exhibit strong positive correlations with fruitiness, underscoring their significance in olive oil quality assessment. Moreover, Total flavonoid concentration emerges as a critical chemical marker, impacting quality, while acidity and sensory defects play lesser roles.
the absorption coefficients K270 and K232 emerge as sensitive chemical indicators for determining fresh olive oil quality, with fruitiness showing a negative correlation with both, while peroxide value shows no significant association with EVOO.

This study highlights the insufficiency of sensory evaluation alone, emphasizing the importance of complementing it with chemical analysis. However, there remains a lack of comprehensive research on Palestinian olive oils, particularly regarding their minor

components profiles and organoleptic properties, as well as their correlations with chemical factors.

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LIST OF ABBREVIATIONS

AA : Antioxidant Activity

ALA : Alpha-Linolenic Acid

CUPRAC : CUPRAC ion reducing antioxidant capacity

DAG : Diacylglycerols

DPPH: 2,2-Diphenylpicrylhydrazyl

EVOO : Extra Virgin Olive Oil

FFA : Free Fatty Acids

IOOC : International Olive Oil Council

FRAP : Ferric Ion Reducing Antioxidant Power Assay

GA : Gallic Acid

IOOC : International Olive Oil Council

L linoleic acid

Ln linolinic acid

L˙ :fatty acid radical

LOO˙: fatty acid peroxyl radical

LOOH : lipid hydroperoxides

LOX : Lipoxygenase Pathway

MAG : Monoacylglycerols

MUFA : Monounsaturated Fatty Acid

OO: Olive Oil

O oleic acid

P Palmitic acid

PV Peroxide value

RSA radical scavenging activity

RI Refractive index

S stearic acid

TAA total antioxidant activity

TPTZ Tripyridyltriazine

TAG : Triacylglycerols

TFC : Total Flavonoid content

TPC : Total phenol content

LOX : Lipoxygenase Pathway

OO: Olive Oil

PCA : Principle Component Analysis

P : Probability distribution

PT : Panel test

PUFA : Polyunsaturated Fatty Acid

PV: Peroxide Value

SD : Standard Deviation

SIMCA : Soft Independent Modelling Class Analogy

TAG : Triacylglycerols

TFC : Total Flavonoid content

TPC : Total phenol content

UV : Ultraviolet

VOO : Virgin Olive Oil

CHAPTER 1

INTRODUCTION

In the Palestinian territories, olive trees are a significant crop that are mostly farmed for their oil. With 7.8 million fruit-bearing olive trees, olive farming is reported to have made up 57% of the cultivated area in the Palestinian territory in 2011 (IOC, 2011). 24,700 tonnes of olive oil were produced in 2014 from an estimated 108,000 tons of olives that were crushed (IOC, 2014). Approximately 100,000 households make their principal income from olives. Among the most significant trees in Palestine are olive trees, which represent the Palestinian people's ties to the land and their origins. The region's soil and climate are responsible for producing some of the best olive oil in the world. The United States (United nations, 2008) Ninety-five percent of the world's 750 million olive trees are grown in the Mediterranean region. According to the International Olive Oil Organization 2 664 000 tons of olive fruits were produced worldwide in 2008 (International Olive Oil Organization, 2008).

About 45% of Palestine's agricultural output is made up of olives and the fruits that are used to make extra virgin and virgin olive oil. About 100,000 hectares, or 45% of the agricultural area in Palestine, is planted with olive trees (Basheer- Salimia et al., 2009). 32–35 thousand metric tons of olive oil are anticipated to be extracted annually with the use of 270 oil press plants located in Gaza and the West Bank (Salimia et al, 2009) . In Palestine, almost 93% of the olive harvest is used to make oil, which accounts for 18% of the country's whole agricultural sector. The Palestinian olive oil farmer receives approximately 2.3 US dollars per litter, with sales ranging from 6 to 7 US dollars (United Nations, 2008).

Before the season.. Here is Palestine’s olive oil production for the last 10 years(Agriculture and Statistics, 2023). According to the Economic Survey of official data issued by Agriculture and Statistics, Palestine's oil production in 2022 reached 36 thousand tons, while

in 2021 season it reached 17 thousands and 500 tons. In 2020 season , Palestine's oil productions reached 14,500 tons of oil , which is one of the worst numbers recorded at the level of oil production in about 11 years. In 2019 , it was a diamond year, as Palestine recorded the best oil production in its history, with 40 thousand tons .

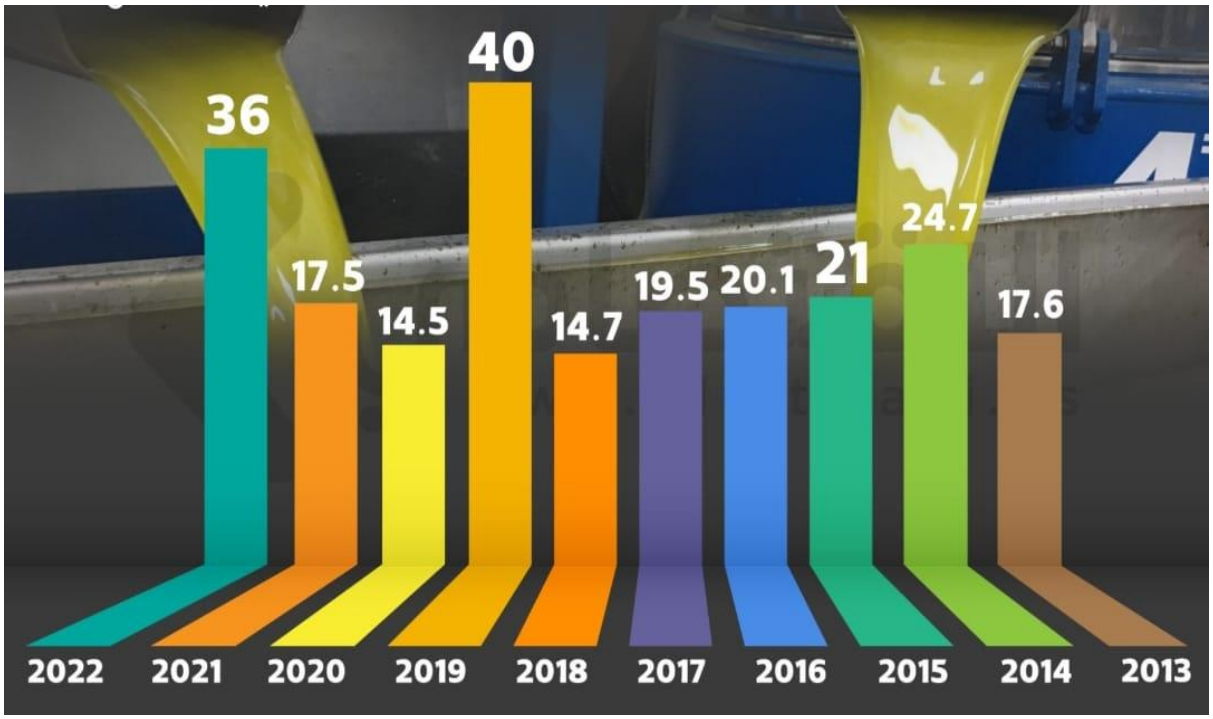

Figure 1 Palestine's olive oil production for the last 10 years According to the Economic Survey of official data issued by Agriculture and Statistics, the value in thousand tons (Aliqtisadi) (Agriculture and Statistics, 2023)

The area planted with olive trees amounted to about 575.2 thousand dunums, representing 85% of the total area planted with trees in Palestine. There are about 12.5 million trees in Palestine, of which less than 10 million are fruit bearing olive trees (Agriculture and Statistics, 2023).

Virgin Olive oil can be distinguished from other edible vegetable oils by its distinct aroma, taste, color, and nutritional qualities. The olive business is therefore very concerned about preserving its product without losing these beneficial qualities. One of olive oil's many health benefits is an increase in blood lipid profile, which occurs when good HDL (high density lipoprotein) cholesterol levels are dramatically raised in the bloodstream and harmful LDL (low density lipoprotein) cholesterol levels are lowered. Consuming olive oil lowers the chance of developing coronary heart disease, diabetes, some malignant tumors (such as

endometrial, digestive system, and skin tumors), some cancers, including breast, prostate, and colon cancers, and a few other chronic illnesses (Perez-Jimenez, et al. 2007).

Oxidation is one of the main reasons olive oil loses its quality. The extraction process and storage conditions are two important technological aspects that affect the composition and oxidative stability of virgin olive oils (Aparicio, et al. 1999).

Either light (photo oxidation) or dark (autoxidation) conditions can cause oxidation, as can the action of enzymes (enzymatic oxidation). Because it contains more natural antioxidants and less polyunsaturated fatty acids than other vegetable oils, olive oil is thought to be more resistant to oxidation. It is the abundance of oleic acid, which makes up 56–84% of all fatty acids, that distinguishes olive oil from other vegetable oils(Perez-Jimenez, et al. 2007). Natural antioxidants can be found in large quantities in olive oil. These include phenolic compound, tocopherols, and carotenoids, which may work in a variety of ways to provide a strong defense against attacks by free radicals. While phenolic compounds are mainly studied for their antioxidant activity, they also exhibit significant biological activity in vivo and may help prevent diseases associated with excessive formation of oxygen radicals that exceed the body's ability to defend against them (Aparicio, et al. 1999). The set of characteristics that determine an olive oil's attractiveness to consumers is known as its quality; it can also be characterized from a commercial, nutritional, or organoleptic standpoint. Extra virgin olive oil (EVOO) is rich in oleic acid, which contributes to its nutritional value. It also contains minor components, including phenolic compounds, which give the oil its scent. As a result, these quality factors support olive oil's price and consumption needs, placing it higher among vegetable oils when compared to other edible oils [Vacca, V. et al., 2006].

The development of trustworthy analytical techniques is required to guarantee that olive oil quality matches labeling and to establish the product's authenticity by looking for any flaws in processing, storage, and adulteration circumstances(Perez-Jimenez, et al. 2007). In order to improve product quality, increase international trade, and increase consumption, the International Olive Council (IOC) and European Communities Legislation (EC) define the identity characteristics of olive oil by defining analytical methods and standard limit values of the quality parameters such as peroxide value (PV), acidity, Ultra violet (UV) absorbance values (K232 and K270), and organoleptic characteristics (odor, taste, and color) for olive oils [Jehad- Abbadi et al, 2014]. Olive oil is classified as extra virgin, virgin, and lampante oil based on chemical testing and organoleptic qualities, which indicate the edible quality and commercial values of the oil. Extra virgin olive oil is the best grade; it must have a free

acidity of less than 0.8%, a peroxide value of no more than 20 milliequivalent O2 kg-1 oil, and a distinct flavor that is representative of the fruit it is made from. It must also contain zero defects and more than zero positive attributes, as determined by a certified taste panel. [M. Cosio and others, 2011].

In addition to adding to the body of knowledge regarding the chemical and sensory qualities of virgin olive oil, my research highlights the significance of studying Palestinian olive oil in order to identify the best agricultural and technological practices that can be applied to subtropical climates and produce virgin olive oil that is of superior quality from both a sensory and chemical standpoint. Palestine is a developing nation that produces virgin olive oil, but little research has been done on its oils up to this point. In particular, there is a lack of information in the literature regarding the chemical and organoleptic properties of its oils as well as the relationships between chemical and sensory parameters(Vacca et al, 2006).As per the official protocol of the International Olive Council (IOC), since there is no scientific information available regarding the sensory attributes of Palestine virgin olive oils assessed by skilled tasters (IOC, 2016). This study examines the mean chemical characteristics of virgin olive oil produced in Palestine, namely total phenolic compound, total flavonoid content, and antioxidant activity. The association between these parameters and sensory evaluation is then established.

An "analysis panel" consisting of eight to twelve tasters who have been qualified and trained by regulatory agencies should conduct this analysis. A panel leader, who oversees the group, compiles the ratings assigned to the favorable (fruity, bitter, and pungent) and unfavorable (sensory defects) Fusty, Musty, Rancid, Winey vinegar and Others sensory qualities. The virgin olive oil is classified as extra virgin, virgin, or lampante based on the median values of fruity and sensory flaws (Amelio, 2019; Bertoncini & Testa, 2014; International Olive Council, 2015a). Correlations between chemical and sensory parameters are subsequently determined.

OLIVE OIL AND QUALITY

1.1. Olive Oil

In Mediterranean regions, olives (Olea europea L.) are traditionally grown as the major crop in subtropical climates. It is thought to have originated in Mesopotamia and has been

grown for many years in North Africa and southern European nations that border the Mediterranean (Murkovic et al. 2004). One of the earliest known vegetable oils is olive oil, which is mostly grown in the nations that border the Mediterranean. It is an organic fruit juice with a distinct composition and quality that is made from the fruit of the Olea europea tree. In addition, olive oil is one of the rare oils that may be eaten unrefined, maintaining all of its natural components.

An extended lifespan in excellent health is said to be linked to the traditional Mediterranean diet, which includes olive oil as a main ingredient. Due to customer preference for less processed goods and growing interest in the Mediterranean diet, the consumption of olive oil has also expanded outside of the Mediterranean region. There is a growing consumer desire for food quality to be maintained at a high level between purchase and consumption. These expectations stem from the necessity to prevent undesired changes in sensory quality in addition to the essential requirement that the meal remain safe (Morello, et al. 2003).

The cultivation of olive trees, as well as the procedures and duration of olive processing, harvesting, and storing, all affect the quality of virgin olive oil. The olive cultivar, the pedo climatic conditions of agriculture, and the irrigation, fertilization, and pruning of olive trees are all particularly significant factors in determining the quality of olive oil. The timing of harvest can have a big impact on output, oil stability, sensory qualities, and oil quality. It is essential to carefully extract olive oil from undamaged fruits at the peak of ripeness in order to produce a distinctively fragrant and delicately flavored olive oil. This demonstrates how, in order to select an ideal harvest, the quality of olive oil from a variety of harvest seasons and cultivars must be determined (Baccouri, et al. 2006).

Numerous qualitative elements of the oil produced inside specific olive orchards have been demonstrated to be influenced by the maturity of the olives (Olea europaea L.) and the processing procedure. The most significant degradation occurs when olives are stacked into big piles during harvest season and kept at room temperature for a few weeks or more before being processed for oil extraction. The fruit may secrete fluid under pressure in the olive pile during storage, which might create the ideal environment for the growth of bacteria and fungus. In these circumstances, the inner portion of the pile may experience anaerobiosis while the outer portion experiences aerobic losses. Additionally, the generation of heat from breathing may hasten the decline of the fruit and eventually cause the breakdown of cell structure. Oil extracted from these damaged olives can be high in acidity and low in stability and can develop a high content of volatile acids (acetic or butyric) that causes a characteristic musty smell (Agar, et al. 1998).

The steps involved in processing olives are milling, mixing, pressure (or centrifugation, depending on whether one uses a traditional or centrifugal method), and oil phase separation. To extract the oil from the olives, high-speed rotating machinery creates centrifugal force that increases the difference between the specific weight of the immiscible liquid and the solid matter. In addition to preserving the oil's exquisite flavor, proper storage methods for olive oil also prevent it from going bad and becoming rancid, which would lower the oil's nutritional value More than any other edible oil, olive oil may be stored for years before going rancid if handled correctly. Olive oil should be stored properly and used within a few months to ensure its healthy phytonutrients remain intact and available. This is because olive oil's monounsaturated fatty acid 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 flaxseed oil, which should always be refrigerated and never heated (Koutsaftakis et al. 1999).

Because virgin olive oil contains a high concentration of carotenoids, monounsaturated fatty acids, tocopherol, and phenolic components, it is more stable than other edible oils. Conversely, premium olive oils are abundant in polyphenols, which not only provide health advantages but also extend the shelf life of the oil significantly. After processing and during storage, temperature, light exposure, and oxygen interaction are the main variables influencing the quality of olive oil. Light is the catalyst for the chemical reactions that eventually cause the oil to deteriorate. Chlorophyll and other sensitizers may contribute to the process of photooxidation (Koutsaftakis et al. 1999).

The shelf life of the oils is significantly impacted by the type of packaging. Carefully chosen storage container can cause damage to oil that has been treated to maximize palatability. For as long as possible, it is ideal to keep the product quality at its peak. Published papers on the behavior of different packing materials include those by (Koutsaftakis et al. 1999) and (Mendez and Falque ,2006). Studies by (Kanavouras et al. 2004), Gutierrez and (Fernandez 2002), and (Cecchi et al. 2006) examined specific distinctive flavor components that developed in extra virgin olive oil when it was stored under varied circumstances and packaged with different materials. The stability of extra virgin olive oil under various commercial conditions and bottle types was researched by Pagliarini et al. (2000). They found that diverse controlled bottling, transit, and storage conditions in supermarkets did not significantly affect the oil's stability. Vekiari et al. (2007) refuted the claim that glass functions as a barrier to oxygen, preventing the passage of some components that degrade in its presence. However, it permits direct light exposure to the olive oil, which may exacerbate

oxidative rancidity due to the oil's susceptibility to photooxidation. As a result, it is not recommended that extra virgin olive oil be stored in PVC bottles as this is not the best way to preserve the oil's quality, because it involves the release of toxic chemicals such as dioxins and phthalates, which are harmal to both human health and environment.

Glass, especially tinted glass, ceramics, porcelain, and non-reactive metals like stainless steel provide the greatest storage containers. Reactive metal containers, like those made of copper or iron, shouldn't be used to keep olive oil since the chemical reaction between the metal and the oil may deteriorate the oil and perhaps release pollutants. Because light accelerates the oxidation process, olive oil should be stored in a cool, dark location(Vekiari et al. 2007)

(Caponio, et al. 2005) mentioned how light affects the quality of EVOO while it's being stored. They came to the conclusion that oils exposed to light had a shorter shelf life than oils stored in darkness and that oils exposed to light could not be classified as extra virgin after just two months. It is possible to refrigerate olive oil without noticeably sacrificing its flavor or quality. If olive oil is maintained in a dark, somewhat constant-temperature environment, it should be stored beyond that at regular room temperature (21–25°C). If stored properly, olive oil can last anywhere from nine to over eighteen months (Gomez-Alonso, et al. 2007). Olive oil matures and loses quality over time, becoming more acidic. The three main indicators that olive oil is no longer fit for human consumption are rancidity, a taste or smell similar to wine, and a metallic flavor.

There is a need to develop reliable analytical methods to ensure compliance of olive oil quality with labeling, and to determine the genuineness of the product by the detection of eventual defects during adulterations, processing and storage conditions. Therefore, the International Olive Oil Council (IOOC) and European Communities Legislation (EC) define the identity characteristics of olive oil by specifying analytical methods and standard limit values of the quality parameters such as peroxide value (PV), Acidity%, Ultra violet (UV) absorbance values ($K_{23}2$ and K_{270}) and organoleptic characteristics (odor, taste and color) for olive oils in order to improve product quality, exp and international trade, and raise its consumption (Abbadi et al, 2014).

Numerous factors such as olive variety, environmental, climatic, soil and cultivation conditions, age of the tree, olive ripeness, olive health, etc. are involved in the composition differences in virgin olive oil during its formation in the fruit (Velasco& Dobarganes, 2002).Various factors from intrinsic to agronomic, affect phenolic compounds. Olive cultivars, together with geographical origin, are responsible for the typicality of olives,

Tableolives, and olive oils. Attention has to be paid to agricultural practices and cultivation systems (Malheiro et al, 2015).

1.2. Designations and Definitions of Olive Oil

The following labels and definitions are used in its marketing (International Olive Council 2007).

Virgin olive oil : is the oil that is extracted from olive tree fruit using only mechanical or other physical processes, particularly heat ones, that do not change the oil's composition and that only involve washing, decantation, centrifugation, and filtration. The free fatty acid content of virgin olive oils is categorized based on grams of oleic acid per 100 grams of olive oil. % Acidity < _2.0, peroxide value< _20, K232 < _2.60, K270 <_0.25.

Extra virgin olive oil: A virgin olive oil's free fatty acid acidity, measured in grams per 100, should not be greater than 0.8 grams (represented as oleic acid). % Acidity <_ 0.8, peroxide value<_ 20, K232 <_ 2.50, K270 <_0.22

Virgin olive oil: Virgin olive oil with no more than 2 grams of free fatty acid (represented as oleic acid) per 100 grams. % Acidity < _2.0, peroxide value<_ 20, K232 < _2.60, K270 <_0.25

Regular virgin olive oil: The amount of free fatty acid acidity, or oleic acid, in an ordinary virgin olive oil should not be more than 3.3 grams per 100 grams.

Lampante virgin olive oil is virgin olive oil that is not suitable for eating as is: Virgin olive oil with more than 3.3 grams of free acidity (also known as oleic acid) per 100 grams. It is meant to be used in technology or for refining.

Refined olive oil is made from virgin olive oils using refining techniques that preserve the original glyceridic structure. It has a maximum of 0.3 grams of free fatty acids per 100 grams, which is represented as oleic acid.

Olive oil: A food-grade oil made from a mixture of virgin and refined olive oils. It possesses a free fatty acidity of no more than 1 gram per 100 grams, which is represented as oleic acid.

Olive-pomace oil is the oil that is produced by subjecting olive pomace to physical or solvent

treatments; it does not include the oils that are produced through reesterification procedures or any mixtures with other types of oils. The following terms and meanings are used in its marketing:

Crude olive-pomace oil: Olive-pomace oil is meant to be refined and used either for technical or human consumption purposes.

Olive pomace oil that has been refined: oil that has been extracted from crude olive pomace oil using processes that preserve the original glyceridic structure. It has a maximum of 0.3 grams of free fatty acids per 100 grams, which is represented as oleic acid.

Olive pomace oil: An oil made from a combination of virgin olive oils and refined olive pomace oil that is suitable for human consumption. This oil's free fatty acidity shouldn't be more than one gram per 100 grams (IOC, 2015).

Riviera olive oil: An oil that may be eaten straight out of the bottle after it has been purified and combined with natural olive oil. According to the Turkish Food Codex 2000, the free fatty acidity should not exceed 1.5 grams per 100 grams. The combination of refined and virgin olive oil is referred to as olive oil with free fatty acid acidity not more than 1 gram per 100 grams, under EU (1991) and IOOC (2007). As figure (1.2)

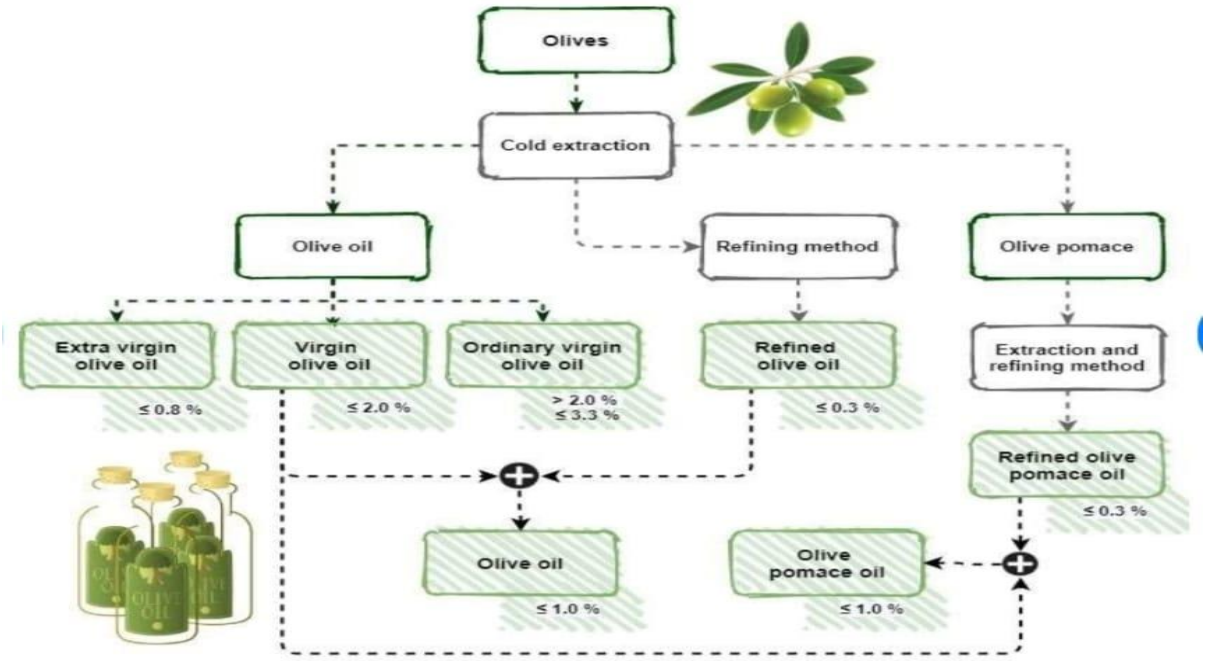

Figure 1.2 Obtention ways and olive oil classification(International Olive Council 2007)

1.3. Olive Oil Chemical Composition

As shown in figure (2.2), the chemical makeup of olive oil can be divided into two categories: major and minor components. Triacylglycerols (TAG) and the family of glyceridic compounds composed of mono-(MAG) and diacylglycerols (DAG) comprise over 98% of the structure of olive oil. Phospholipids, waxes, and esters of sterols, aliphatic and triterpenic alcohols, carotenoids, chlorophylls, hydrocarbons, antioxidants, volatile chemicals, etc. are minor ingredients that make up around 2% of its composition. In the nine quality and purity analyses, in the research on authenticity and genuineness, and, more recently, in the studies on olive oil trace abilities and health, minor constituents are significant.

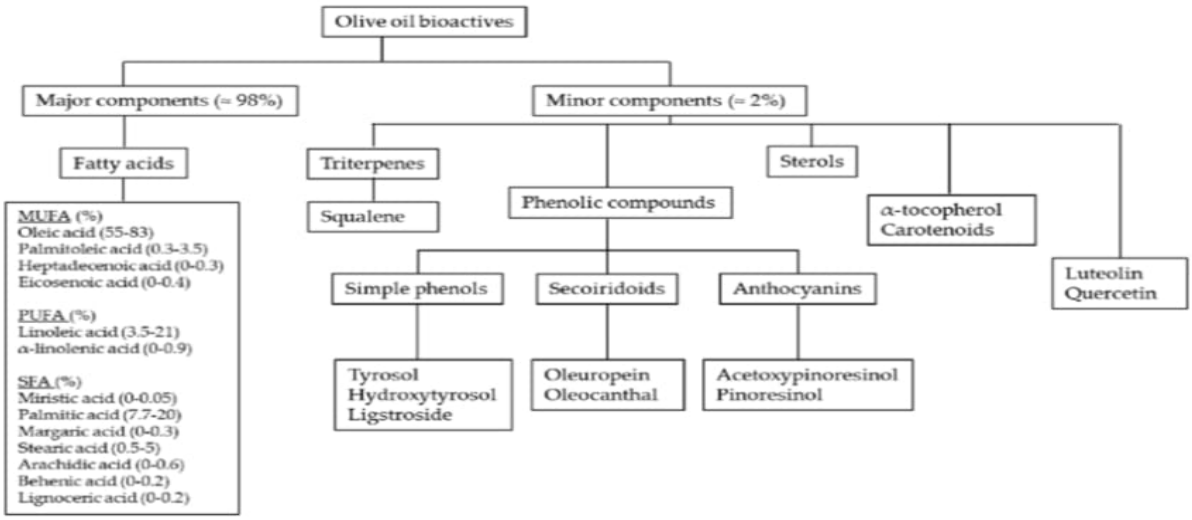

Figure 1.4 Main bioactive compounds in extra-virgin olive oil(EVOO) –(Marika Massaro, 2020).

1.3.1. Major Components

Triacylglycerols are one of the main constituents of olive oil, commonly referred to as saponifiable compounds (TAGs). Fatty acids are bonded in groups of three with a unit of glycerol in an olive oil molecule. TAGs, or triacylglycerol molecules, are the units in question. They are only regarded as high-quality oil when the fatty acids are bonded in these

tiny units. A unit of triacylglycerol can change from being a diacylglycerol to a monoacylglycerol by losing one or two fatty acids(Yildirim, 2009).

Free fatty acid is the term used to describe the fatty acid that is extracted from triacylglycerol. To produce TAGs, any three or more fatty acids can be linked to the glycerol unit. The carbon chains might be saturated, monounsaturated, or polyunsaturated, and they can also have varying lengths(Yildirim, 2009). What distinguishes one oil from another is the proportional amount of them. Fatty acids are the main ingredients of olive oil (Figure 1.4).

Fatty acids are simple structures made up of long chains of various numbers of carbon atoms. There are only a few types of fatty acids in olive oil, but the proportions of each strongly influence the characteristics and nutritive value of the oil.

Myristic (14 carbon atoms) and lignoceric (24 carbon atoms) are the two primary fatty acids found in olive oil. The most notable ones include the polyunsaturated linoleic and linolenic acids, as well as the monounsaturated oleic and palmitoleic acids. Table 2.1 shows the fatty acid compositions of extra virgin olive oil, pomace oil, and olive oil. The predominant monounsaturated fatty acid found in olive oil, oleic acid, makes for 55-83% of the total fatty acids. In terms of health, lower incidence of coronary artery disease have been attributed to the Mediterranean region's high oleic acid intake. Additionally, by lowering the LDL/HDL ratio, olive oil enhances the lipid profile associated with cardiovascular risk (Martinez-Gonzalez and Sanchez-Villegas 2004).

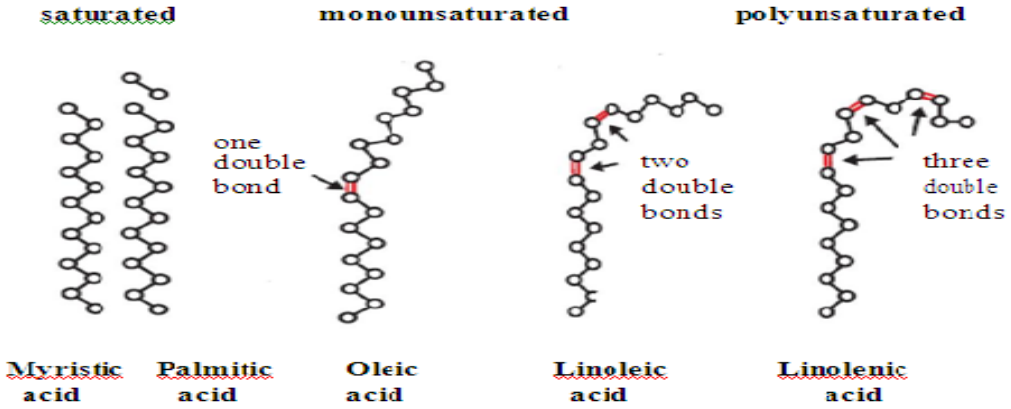

Figure 1.5: Forms of some fatty acids in olive oil (Yildirim, 2009)

Table 2.1Olive Oil Fatty Acid Composition (%) (Dottorato& Alimenti, 2009).

Oleic	C18:1 n-9	55 to 83
Palmitic	C16:0	7.5 to 20
Linoleic	18:2 n-6	3.5 to 21
Stearic	C18:0	0.5 to 5
Linolenic	18:3 n-3	<0.9
Palmitoleic	16:1 n-7	0.3 to 3.5
Arachidic	C20:0	<0.6
Margaric	C17:0	<0.3
Margaroleic	C17:1 n-8	<0.3
Lignoceric	C24:0	<0.3
Gadoleic	C20:1 n-11	0.1 to 0.4
Behenic	C22:0	<0.2
Myristic	C14:0	<0.1

1.3.2. Minor Components

Sterols: Sterols are lipids with significant nutritional value that should be regularly measured in meals. The main sterols found in olive oils are 4-desmethylsterols and β-Sitosterol. Δ5-avenasterol, stigmasterol, sitostanol, and cholesterol are examples of minor sterols. Olive oil also contains the triterpene dialcohols erythrodiol and uvaol, in amounts varying from 10 to 200 mg/kg oil. The most common sterol was βsitosterol, and the overall sterol concentration varied from 687 to 2.479 mg/kg depending on the kind of oil. To differentiate between olive oil and seed oil, one can use stigmasterol and the quantity of erythrodiol plus uvaol (Martinez-Vidal, et al. 2007). Olive oil's compositional analysis of its sterol fraction can be used to determine how pure the oil is and whether it contains any other plant oils. This conclusion also makes it possible to characterize the kind of olive oil (Dobarganes & Velasco 2002).

Squalene: accounting for over 90% of the hydrocarbon fraction in olive oil, squalene is the main hydrocarbon and ranges from 200 to 7500 mg/kg oil or even higher (800–12000 mg/kg oil). It is thought that squalene has a role in olive oil's ability to prevent some types of cancer. In a recent investigation into the minor components of Italian olive oils made from six distinct varieties of olives at varying stages of ripeness, it was discovered that squalene loss

increased more than α-tocopherol loss when oil samples were stored in the dark (Manzi, et al. 1998). This was linked to the potential regeneration of α-tocopherol from squalene, suggesting that this highly unsaturated hydrocarbon has antioxidant properties.

Pigments: The solubilization of the lipophilic carotenoid and chlorophyll pigments found in the source fruit gives virgin olive oil its color. 0.9 to 2.3 ppm of lutein and 1.0 to 2.7 ppm of β-carotene can be found in virgin olive oil (Psomiadou and Tsimidou 2002). By means of light filtering, singlet oxygen quenching, sensitizer inactivation, and free radical scavenging, carotenoids, and particularly β-carotene, can reduce the rate of oil oxidation. Carotenoids and the products of their oxidation may function as prooxidants in vegetable oils when there is no light (Velasco and Dobarganes 2002). These elements have the ability to convert light energy into chemical compounds. They thus function as prooxidants when kept in a bright environment. Chlorophylls function as strong prooxidants in the presence of light, sensitizing the production of O2, but they also function as antioxidants in the absence of light, potentially via giving free radicals hydrogen (Endo et al. 1985, Francisca and Isabel 1992). Because they function as singlet oxygen quenchers or photo-sensitizers, respectively, carotenoids and chlorophylls are thought to have a significant role in maintaining the quality of edible oils (Cert et al. 2000).

Tocopherols: Olive oil contains the naturally occurring forms of tocopherols α, β, γ, and δ. Olive oil contains α-tocopherol, also known as vitamin E, in amounts between 150 and 300 parts per milliliter. These substances are very significant for human health since they have antioxidant qualities and function as vitamins (particularly vitamin E). The concentration and existence of other antioxidants in olive oil are the primary determinants of their antioxidant activity. When stored in light and in the presence of chlorophyll, tocopherols function as singlet oxygen quenchers and improve the oxidative stability of vegetable oils (Cert, et al. 2000).

Phospholipids: The range of phospholipid concentrations in olive oils is 40–135 mg/kg. The primary phospholipids found in olive oils include phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, and phosphatidic acid. The oxidative stability of the olive oils or the physicochemical state of hazy (veiled) olive oil may be impacted by their presence. Phospholipids' antioxidant properties stem from an amino group that can chelate metals and maintain their active form. (Velasco and Dobarganes,2002), they have the ability to work in concert with phenolic compounds and tocopherols to increase their antioxidant activity. Hydrophilic and hydrophobic groups coexist in phospholipid molecules. The edible oil contains hydrophobic

groups, while the hydrophilic phospholipid groups are found on the oil's surface. In addition to lowering edible oil's surface tension, phospholipids may speed up the rate at which oxygen diffuses into the oil from the headspace, accelerating the oxidation of the oil (Choe and Min 2006).

Phenolic Compounds: At least thirty phenolic compounds are present in VOO. The main phenolic components are hydroxytyrosol-based derivatives of oleuropein, which are potent radical scavengers and antioxidants. A hydroxyl group on a benzene ring is a component of the complex class of molecules known as phenolic compounds. These hydrophilic chemicals are also present in olive oil, although they are concentrated in the pulp of the olive. Numerous classes fall under the umbrella of the class of phenols, including simple phenolic acids and their derivatives, such as tyrosol and hydroxytyrosol, vanillic, coumaric, and caffeic acids, as well as more complex substances like lignans (1–13 acetoxypinoresinol and pinoresinol), flavones (apigenin, luteolin), and secoiridoids of oleuropein and ligstroside. The main phenolic components found in virgin olive oil are listed in Table 2.2. There are two distinct constitutive carbon frameworks in phenolic acids, which are the hydroxycinnamic and hydroxybenzoic structures. They present in olives with the chemical structure of C6–C1 (benzoic acids) and C6–C3 (cinnamic acid) (Garrido Fernandez, et al. 1997).

well as their antioxidant and health-promoting capabilities, have all been linked to phenolic acids (Nergis and Unal 1991). The potential protective effect of phenolic acids from oxidative damage—such asFoods' color and sensory attributes, as cancer, heart disease, and stroke—when ingested through fruits and vegetables has sparked renewed study in these compounds (Masaki, et al. 1997). In particular, a number of phenolic acids have been discovered and quantified in VOO (in levels lower than 1 mg of analyte kg-1 of olive oil), including gallic, protocatechuic, phydroxybenzoic, vanillic, caffeic, syringic, p- and o-coumaric, ferulic, and cinnamic acid. Phenolic acids can form conjugations with other phenolics, organic acids, carbohydrates, lipids, amino compounds, and terpenoids. examined the existence of hydroxy-isochromans(Bianco , colleagues 2002)

In actuality, hydrolytic activities mediated by glycosidases and esterases enhance the amount of hydroxytyrosol and carbonylic compounds during the malaxation step of VOO extraction, favoring the presence of all components required for the production of isochroman derivatives. By using the HPLC-MS/MS approach, two hydroxy-isochromans—which are produced when hydroxytyrosol reacts with either benzaldehyde or vanillin—have been identified and measured in commercial VOOs.

The secoiridoids oleuropein, demethyloleuropein, and ligstroside are the main complex phenols in virgin olive oil. Secoiridoids are characterised by the presence of elenolic acid in its glucosidic or aglyconic form (Bianco and Uccella 2000). The secoiridoids, which are glycosidated compounds, are produced from the secondary metabolism of terpenes as precursors of several indole alkaloids (Soler-Rivas, et al. 2000) and are characterised by the presence of elenolic acid in its glucosidic or aglyconic form. Especially, they are formed from a phenyl ethyl alcohol (hydroxytyrosol and tyrosol), elenolic acid and, eventually, a glucosidic residue. Oleuropein is the ester between 2-(3,4-dihydroxyphenyl) ethanol (hydroxytyrosol) and the oleosidic skeleton common to the glycosidic secoiridoids of the Oleaceae. Oleuropein and demethyloleuropein are hydrolyzed by endogenous β -glycosidases to 14 the dialdehydic form of elenolic acid linked with 3,4-dihydroxyphenylethanol (3,4-DHPEA-EDA) and 3,4-dihydroxyphenylethanol-elenolic acid (3,4-DHPEA-EA) during crushing and malaxation (Bendini, et al. 2007).

The primary phenolic alcohol is hydroxytyrosol, which can exist as a simple or esterified phenol with elenoic acid, forming oleuropein and its aglycone, or as a component of the verbascoside molecule (Servili et al. 1999). Depending on the hydroxyl group to which the glucoside is bound, hydroxytyrosol can also exist in several glycosidic forms (Bianco et al. 1998, Ryan et al. 2001).

According to Owen et al. (2000), lignans, (+)-1-acetoxypinoresinol, and (+)-pinoresinol are additional compounds found in the phenolic fraction. The lignan fraction of various plants, including Forsythia species and Sesamum indicum seeds, commonly contains the compound (+)-pinoresinol. On the other hand, the bark of Olea europeae L. (olive) contains (+)-1 acetoxypinoresinol, (+)-1-hydroxypinoresinol, and their glycosides. (olive) (Kato, et al. 1998).

By scavenging free radicals and chelating metals, the phenolic compounds in olive oil worked as antioxidants mostly during the early stages of autoxidation (Deiana, et al. 2002). There are additional reports of phenolic component changes in virgin olive oils with storage. Cinquanta et al. (1997) investigated the changes in simple phenols after storing them in the dark for eighteen months. Due to the hydrolysis of their complex derivatives in the first stage, they discovered a significant increase in the levels of tyrosol and hydroxytyrosol. At the conclusion of the storage period, they also observed a quick loss of hydroxytyrosol relative to that of tyrosol. The most potent antioxidant in the oxidation of olive oil was hydroxytyrosol. Among phenolic compounds, o-diphenols such as caffeic acid are oxidized to quinones by ferric ions and become ineffective in inhibiting iron-dependent free radical chain reactions in

oil (Keçeli and Gordon 2002). However, hydroxytyrosol, tyrosol, vanilic acid, p-coumaric acid were not oxidized by the ferric ions.

Because flavonoids have positive health effects on cancer and heart disease, they are also a major issue. Depending on the presence of an OH group at C-3, a saturated single bond between C-2 and C-3, a combination of no carbonyl at C-4 and an OH group at C-3, or none at all, flavones, flavonols, flavanones, and flavanols are the several subtypes of flavonoid aglycones. The flavonoid group of phenolic chemicals in VOO contained luteolin and apigenin, as reported by Rovellini et al. (1997) and Morello et al. (2005). Both luteolin and apigenin can come from lutein-7-glucoside or rutin, respectively.

Table (A.1) Major classes of phenolic compounds in VOO(Servili, et al. 2004)

Major classes of phenolic compounds in VOO
Phenolic acids and derivatives
Vanillic acid
Syringic acid
p-coumaric acid
o-coumaric acid
Gallic acid
Caffeic acid
Protocatechuic acid
p-hydroxybenzoic acid
Ferulic acid
Cinnamic acid
4-(Acetoxyethyl)-1, 2-dihydroxybenzene
Benzoic acid
Hydroxy-isochromans
Phenolic alcohols
Hydroxytyrosol
Tyrosol
(3,4-Dihdroxyphenyl)ethanol-glucoside
Secoiridoids
3, 4-DHPEA (3, 4-DHPEA-EDA)
(p-HPEA-EDA)
(3, 4-DHPEA-EA)
Ligstroside aglycon
Oleuropein
p-HPEA-derivative
Dialdehydic form of oleuropein aglycon
Dialdehydic form of ligstroside aglycon
Lignans
(+)-1-Acetoxypinoresinol
(+)-Pinoresinol

Table (B.1) Major classes of phenolic compounds in VOO(Servili, et al. 2004)

Flavones
Apigenin
Luteolin

The sensory and antioxidant qualities of premium olive oils are mostly determined by the quantities of phenolic compounds, which are also highly significant. The quantity of oleuropein and its hydrolytic derivatives influences the quality of the oil and olives (Limiroli, et al. 1995). According to Cinquanta et al. (1997), Visioli and Galli (1998), Brenes et al. (1999), the location, variety, and stage of maturation of the olives at harvest determine the olive oil's absolute phenolic component content.

Volatile Compounds: Low molecular weight chemicals that easily evaporate at room temperature are known as volatile compounds. Olive oil's distinctive scent, and especially its green and fruity characteristics, come from a variety of volatile compounds that are produced when polyunsaturated fatty acids are broken down during the oil extraction process by a series of enzymatic reactions called the lipoxygenase (LOX) pathway (Angerosa, et al. 2000, Angerosa, et al. 2004).

Table 2. Defined major volatile compounds in VOO(Ryan et al, 2002).

Aldehydes	**alcohols**	**esters**	**hydrocarbons**	**ketone**	**furans**
3-Methylbutanal	Methanol	Methyl acetate	Octane	2-Butanone	Ethylfuran
(E)-2-Pentenal	Ethanol	Ethyl acetate	2-Methylbutan	3-Pentanone	
(Z)-2-Pentenal	1-Hexanol	Butyl acetate	Nonane	1-Penten-3-one	
Hexanal	1-Penten-3-ol	Hexyl acetate	Hexane	2-Octanone	
(E)-2-Hexenal	(E)-3-Hexen-1-ol	(Z)-3-Hexenyl-acetate			
(Z)-3-Hexenal	(Z)-3-Hexen-1-ol	Ethyl propanoate			
Heptanal	(E)-2-Hexen-1-ol				
2-4-Heptadienal	1-Octen-3-ol				
Octanal	Terpineol				
Nonanal	3-Methylbutan-1-ol				
2,4-Nonadienal					
2,4-Decadienal					
(E)-2-Undecenal					

Some of the volatiles in virgin olive oil are created during the manufacturing of virgin olive oil when enzymatic reactions in the presence of oxygen damage cell structure. Other volatiles are found in the intact fruit tissue. According to Morales and Tsimidou (2000), the primary

17 precursors of volatile chemicals are amino acids (leucine, isoleucine, and valine) and fatty acids (especially linoleic and alpha-linolenic). Although extrinsic factors (such as temperature, soil, harvesting and extraction circumstances) may change the intrinsic sensory profile of olive oil, it has been shown that the quantities of volatile chemicals rely on the enzymatic activity (Salas, et al. 2005). (Morales and Aparicio 1999).

Aldehydes (hexanal, trans-2-hexenal, acetaldehyde), alcohols (methanol, hexan-1-ol, 3-methylbutan-1-ol), esters (methyl acetate, ethyl acetate, hexyl acetate), hydrocarbons (2-methylbutane, hexane, nonane), ketones (2-butanone, 3-methyl-2-butanone, 3-pentanone), furans, and other unidentified volatile substances are all thought to be responsible for the scent of olive oil. C6 and C5 volatile compounds are the main volatiles found in virgin olive oils (Angerosa et al. 2004, Cimato et al. 2006). The main volatile substances found in VOO are also shown in Table 2

Major or minor volatile compounds have a critical role in olive oil quality and serve as helpful quality indicators. In addition to volatile substances, non-volatile substances like phenolic compounds also amplify the sensation of bitterness by their pungency, astringency, and metallic qualities (Dobarganes & Velasco 2002).

1.4. Quality of Olive Oil

A product's quality can be described as the set of features or aspects that are important in determining how much the user will accept the product. One can define the quality of olive oil from a commercial, nutritional, or organoleptic standpoint. While volatile molecules have a significant influence on the aroma of extra virgin olive oil (EVOO), its high levels of oleic acid content and lesser components, including phenolic compounds, are the source of its nutritional value. Olive oil's distinct flavor and nutritional benefits make it a more desirable product to purchase when compared to other edible oils. Good quality control of olive oil should be ensured throughout the production and storage line in order to meet customer expectations. Analytical parameters are measured and particular limit values are defined in order to maintain the quality of olive oils (El Riachy et al, 2011).

The International Olive Oil Council,(IOC, 2015) established by the nations that produce olive oil in an effort to increase output and enhance the quality of the oil, has suggested a traditional index that quantifies the oil's quality and implicitly guarantees its authenticity. Olive oil quality has been classified by the International Olive Oil Council and the European Commission using factors such as the amount of free fatty acid (FFA), peroxide value (PV),

UV specific extinction coefficients (K232 and K270), and sensory score. A crucial component in dividing olive oil into commercial grades is its FFA content. In addition to the quality standards established by the IOOC and EEC, variations in the major and minor constituents found in olive oils, as well as their concentrations, offer suggestions for enhancing olive oil quality, particularly with regard to packaging, marketing, and storage concerns. The European Community legislation developed the Protected Designation of Origin (PDO) mark to protect producers of premium olive oils and guarantee customer knowledge of product quality. The PDO mark permits the labeling of virgin olive oils with the names of the places where they are produced (IOC,2016)

The value of the oil rises with this accreditation. The subject of regional identification takes on greater significance when it comes to tiny manufacturing locations, which are frequently the source of high-quality products (Vissers et al, 2004).

Lastly, changes in the minor ingredients are also caused by hydrolysis, esterification, and oxidation while the oil is being stored. Determining the minor elements is therefore crucial for the analytical evaluation of the olive oils' quality, origin, extraction technique, refining process, and adulteration. The oxidative quality and color of olive oil are determined by the quality parameters that are discussed in the following sections (Servili et al, 2009).

1.4. 1 Factors Affecting The Quality of Olive Oil

Many factors, such as genetics (tree variety), agronomic factors (ripening stage, fertilization, irrigation, and harvesting practices), health of the drupe, environmental factors (temperature, day length, and sunlight duration), geographical factors, and postharvest processing (packaging materials and storage conditions) can all have an impact on the quality of olive oil [Abbadi et al, 2014]. Additionally, some EU EVOOs may be labeled with the Protected Denomination of Origin (PDO) designation, which ensures that the product's quality and its place of origin are closely related. This is made possible by a significant European rule. Just 50% of the olive oil produced worldwide is categorized as extra virgin grade due to the intricate interplay of these elements [Cosio, M. et al, 2011].

These variables all have a major impact on the extracted oil, particularly on its organolyptic qualities (Méndez and Falqué, 2005).Extra virgin olive oil's chemical makeup varies depending on the method used to extract it; notable differences were observed between oils extracted under pressure and those extracted using centrifuge methods (Gutfinger ,1981). The

oil obtained through centrifugation had a larger phenol content than the oil obtained by pressure, which was characterized by higher values of acidity, pigments, and oleic acid.

It can be challenging to define average concentration of polyphenolic chemicals (Tsimidou, 1998). However, their quantity may vary from 40 to 900 mg kg-1 if they are assessed colorimetrically as total phenols in the oil's methanolic extract. However, some oils were also reported to have larger amounts (up to 1000 mg kg-1) (Blekas et al, 2004).90% of the total tocopherol content in polyphenol tocopherols is composed of the α-homologue, which is the lipolytic (hydrophobic) portion of the eight recognized "E-vitamins." There is α-Tocopherol in its free form; reported amounts range from 55-370 mg kg-1 oil, depending on cultivar potential and technological considerations. Aliphatic and triterpenic alcohols as additional minor compounds(Boskou ,2006)

After extraction, oxygen availability, high temperature, light action, and metals can all have an impact on the olive oil's commercial life. These elements may contribute to the triglyceride in olive oil breaking down. The first mono-hydro peroxide that was produced underwent a number of decomposition processes, resulting in an off flavor and odor, which in turn caused oxidative and hydrolytic degradations that degraded the oil's quality (Garca et al., 2003).

1.4.1.1. Acidity Determination (Free Fatty Acid Content) (FFA)

The percentage (in weight) of free fatty acids in the oil under investigation is expressed by the free acidity percent. Free fatty acids are typically found in oils as well. When triglycerides are generated, the action of enzymes (lipase) found naturally in olive fruit gradually increases the acidity by assisting the fatty acids in separating from the triglyceride molecule (lipolysis). The oil's acidity is caused by the lipolytic activity of lipase, which releases 19 free fatty acids. The fruit-growing microorganisms' enzymes have the potential to have the same lipolytic activity. Thus, in order to obtain a product which is organoleptically better and has lower acidity, it is necessary to preserve the olives well. Consequently, FFA reflects the stability of oil and its susceptibility to rancidity(Kiritsakis et al. 1998).

The primary method for determining acidity is titration with potassium hydroxide. The process calculates the percentage of oleic acid, or free fatty acids (FFA) that are contained in the oil. The free fatty acidity, which gauges the oil's quality, is a reflection of the care that

went into its production and storage. Additionally, the basic criterion for dividing olive oil into its many categories is its acidity value.

However, Kiritsakis et al. (1998) state that acidity is not the ideal criterion for determining the quality of olive oil because an oil with a low acidity may not have as excellent of a flavor or aroma as one with a relatively high acidity. The maximum acidity for extra virgin olive oil is 0.8% (European Union Commission's 1991).

1.4.1.2. Peroxide Value (PV)

The peroxide value (PV), which is an important indicator of quality, is a measurement of the total peroxides in olive oil represented as miliequivalent of O2 kg−1 oil (meq O2/kg oil). The official EU method is based on titrating iodine that has been freed from potassium iodide using oil-based peroxides life(Ruíz, et al,.2001). . Put another way, one of the most basic indicators of the extent of lipid peroxidation is the peroxide value, which is a measurement of the active oxygen bound by the oil and represents the hydroxyperoxide value. The more oxidation-related deterioration, the higher the number. After pressing, the peroxide value often rises progressively over time.

Twenty meq O2/kg of oil is the maximum standard value for peroxide. Table 3 shows the higher standard peroxide indices for other types of olive oil as determined by European Regulation. Peroxide levels above 20 often indicate that the oil is less stable and has a shorter shelf life (Nouros, et al. 1999).

1.4.1.3. Specific Absorption Coefficients (UV Absorbance Values) (K232 and K270)

To estimate the oxidation stage of olive oil, the specific absorption coefficients (specific extinction) in the ultraviolet band must be determined. The production of conjugated diene and triene in the olive oil system as a result of oxidation or refining operations is linked to the absorption at specific wavelengths at 232 and 270 nm in the ultra violet range. According to Kiritsakis et al. (2002), chemicals of secondary oxidation (aldehydes, ketones, etc.) contribute to K270, whereas molecules of conjugated diene oxidation contribute to K232. Table 3 displays the upper standard UV absorption values.

Table 3. European Regulation Standard limit values for olive oil quality parameters

Category	Acidity (%)	Peroxide index	K_{232}	K_{270}	ΔK	Sensory evaluation of defects	Sensory evaluation of fruity
Extra virgin olive oil	≤ 0.8	≤ 20	≤ 2.50	≤ 0.22	≤ 0.01	$Md = 0$	$Mf > 0$
Virgin olive oil	≤ 2.0	≤ 20	≤ 2.60	≤ 0.25	≤ 0.01	$Md \leq 3.5$	$Mf > 0$
Lampante olive oil	> 2.0	–	–	–	–	$Md > 3.5$	–

Quality parameters for olive oil depending on its category (modified from European Regulation 1348/2013)

Table 4 Summary of quality index of olive oil as indicated in codex regulations

Test	**Type of alteration**	**Alteration Factors**	**What does it measure**	**Extra virgin**	**Virgin**	**Ordinary virgin**	**Lam--pante**
Acidity	Hydrolysis	Mold, Fermentation ,Maturity too much advanced, Olive fly, bad storage condition such as temperature and humidity	The percentage of free fatty acids	Maxi 0.8 %	Max 2 %	Codex ≤ 3.3 %	Codex > 3.3 %
Peroxide index	Oxidation	Maturity too much advanced, Frost, Ageing, Ventilation, Light, Heat.	The hydro peroxides (-OOH)	Maxi 20.0 milliequivalents O_2 kg^{-1} oil			--- --
K 232	Oxidation	Ageing, Ventilation, Light, Heat, Olive fly	The hydroperoxides of C18 :2 and conjugated diene decomposition	Maxi 2.5	Max 2.6	-----	-----
K 270	Oxidation	Ageing, Ventilation, Light, Heat,	Secondary products of oxidation (triènes= combined refining)	Maxi 0.22	Maxi 0.25		Max 0.11
Panel test	Various	Various	Sensorial intensity	MD*= 0 MF* > 0	0 <MD≤2 .5 MF* >0	Codex 2.5 <MD≤ 6	Codex MD > 6

MD: median of main defect MF: median of fruity

Extract from training AFIDOL France - "Monitoring the alteration of olive oil"(2008), (Baldassari, 2008).

1.5 Antioxidant Activity determination(AA)

1.5.1 Antioxidants

1.5.2 Definition

Antioxidants are defined as compounds that have the ability to considerably delay or prevent the oxidization of oxidizable substrates when present at lower concentrations than those substrates (Antolovich et al, 2002).

1.5.2. Olive Oil's Antioxidants

Alpha-tocopherol, or vitamin E, carotenoids, and phenolic compounds—which include both complex and simple phenols like oleuropein and hydroxytyrosol—are all examples of antioxidants whose efficacy has been shown in vitro and more recently in vivo (Lobo et al, 2010).

1.5.3. Mechanism of action of antioxidants

Demonstrating additional benefits in the prevention of aging and some illnesses. Virgin olive oil is very high in these compounds and has a potent antioxidant action that guards against the development of cancer and damage from free radicals (scavenger activity) (Hamid et al, 2010).

1.5.4. Classification of antioxidants

Primary, or natural antioxidants, and secondary, or manufactured antioxidants, are the two categories of antioxidants. Ascorbic acid and its derivatives, tocopherols, gallic acid esters, sodium salt of erythorbic acid, BHA, BHT, and other chemicals like THBP and TBHQ are examples of primary antioxidants (antioxidants proper). Secondary antioxidants are compounds that have antioxidant properties but also have other purposes. Secondary antioxidants include lecithin, sulphites, and sulphur dioxide (Butnariu & Grozea, 2009)

Free radicals target every major class of biomolecule, with a particular focus on cell membrane polyunsaturated fatty acids (PUFA). Because lipid peroxidation, a type of oxidative damage to polyunsaturated fatty acids, is a self-reinforcing chain reaction, it can be very harmful (Lobo et al., 2010).A fatty acid radical ($L^{\cdot}$) is produced by oxidation of the

PUFA (eqn (1)), and this radical quickly takes on oxygen to create a fatty acid peroxyl radical (LOO\, eqn (2)). The chain reactions are carried by peroxyl radicals. Further oxidation of PUFA molecules by the peroxyl radicals can start additional chain reactions that result in the production of lipid hydroperoxides (LOOH) (eqn (3) and (4)), which can decompose into even more radical species (Esterbauer et al, 1990).

$LH + R^{\cdot} \rightarrow L^{\cdot} + RH$……..…..……..(1)
$L^{\cdot} + O2 \rightarrow LOO^{\cdot}$ …….…..…..……..(2)
$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$ ……..…….…….(3)
$LOOH \rightarrow LO^{\cdot} + LOO^{\cdot}$ + aldehydes ……..(4)

There are different methods to evaluate the antioxidant capacity of foodstuffs including olive oil among which are: DPPH, FRAP, CUPRAC and ABTS.

1.5.5. DPPH:

The standard 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (MacDonald-Wicks et al., 2006) measures compounds that are radical scavengers and is based on the idea that a hydrogen donor is an antioxidant. Figure 1.6 below illustrates the process by which DPPH• accepts hydrogen from an antioxidant. DPPH• is one of the few stable and commercially available organic nitrogen radicals (Wicks et al., 2006). The antioxidant effect is proportionate to the disappearance of DPPH• in test samples.

The most widely used technique for measuring DPPH• is now using a UV spectrometer due to its accuracy and ease of use. At 517 nm (purple), DPPH• exhibits a significant absorption maximum. When hydrogen from an antioxidant is absorbed, the color changes from purple to yellow and DPPH is formed. It is a stoichiometric process in terms of the quantity of absorbed hydrogen atoms. Consequently, it is simple to assess the antioxidant action by monitoring the decline in UV absorption at 517 nm. (Moon and others, 2009).

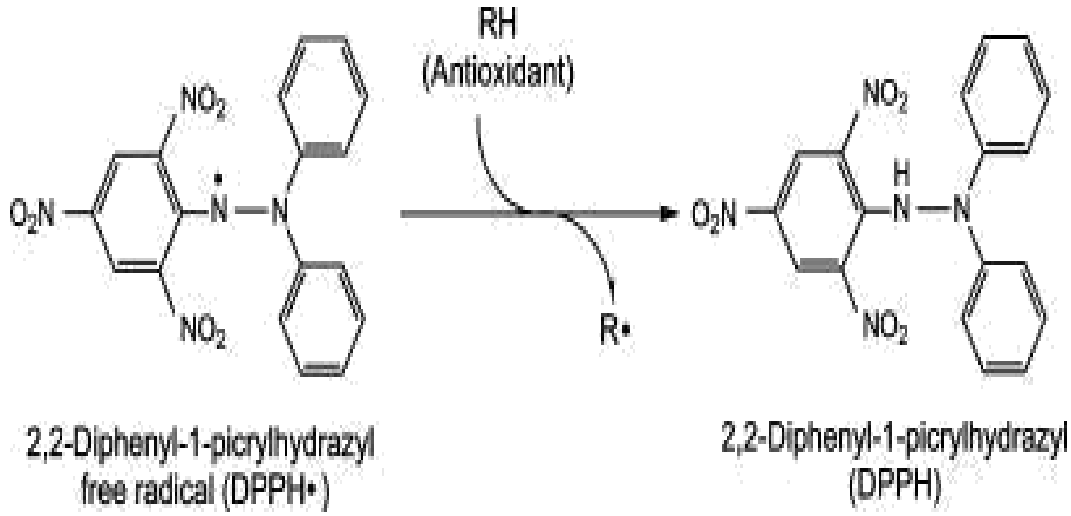

Figure 1.6 Mechanism of DPPH• free radical(Moon et al, 2009).

1.5.6 FRAP assay:

The Ferric Ion Reducing Antioxidant Power Assay (FRAP) is a straightforward, quick, affordable, and reliable approach that doesn't require any specialist tools. Under acidic circumstances, electron-donating chemicals (such as phenolic compounds) convert the yellow Fe3+TPTZ complex (2,4,6-tri (2-pyridyl)-1,3,5-triazine) to the blue Fe2+TPTZ complex in the FRAP method (Benzie et al., 1996; see Figure 1.8). The reaction and the creation of the blue complex will be accelerated by any electron-donating material whose half-reaction has a lower redox potential than Fe3+/Fe2+ TPTZ. Compounds with redox potentials less than 0.7 V, or the redox potential of Fe3+-TPTZ, are detected by the reaction (Prior et al, 2005).

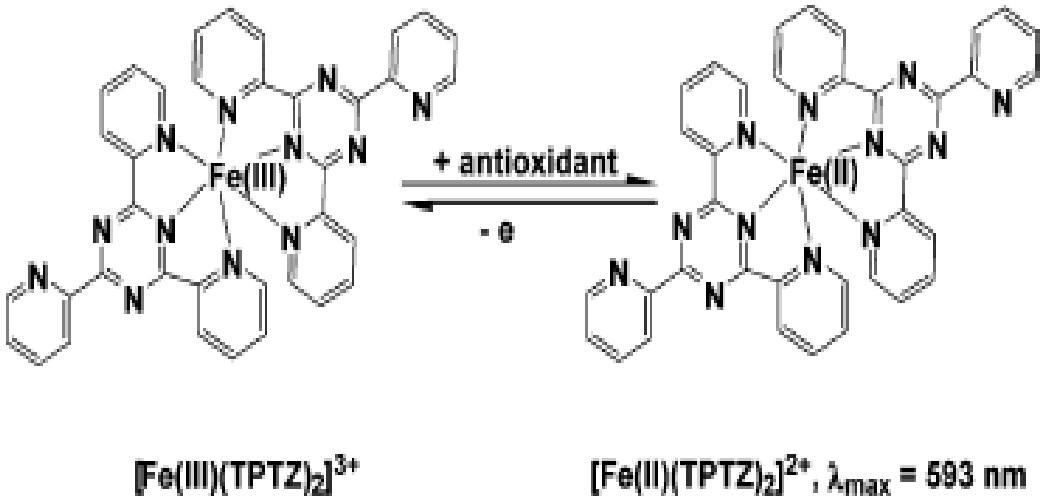

Figure (1.8) Reduction of yellow Fe^{3+} TPTZ complex (2,4,6-tri (2-pyridyl)-1,3,5-triazine) with antioxidants to the blue Fe^{2+} TPTZ complex by FRAP reagent (Prior et al, 2005)

1.5.7 CUPR A Cassay

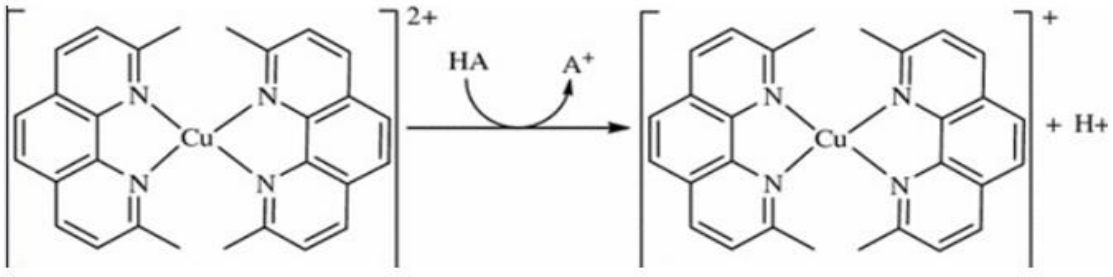

Figure 1.9 CUPRAC method (Apaket al,2006)

The authors of the putative CUPRAC approach are (Apaket al,2006). These experiments are based on the reduction of Cu+2 to Cu+ by all antioxidants working together or by polyphenols reducing in an aqueous-ethanolic medium (pH 7.0) with neocuproine (2,9-dimethyl-1,10-phenanthroline). This results in Cu+ complexes with a maximum absorption peak at 450 nm (Figure 1.9) (Lee et al, 2011). This technique uses the Cu+2-neocuproine (Cu+2-Nc) reagent as the chromogenic oxidizing agent to determine the antioxidant capacity of food constituents. A Cu+ combination with a maximal absorption peak at 450 nm is produced when reducing agents reduce Cu+2 in the presence of neocuproine (Tutemet al, 1991).

Figure1.9: CUPRAC reaction by an antioxidant molecule (HA: an antioxidant molecule, A+: an oxidized antioxidant molecule). Protons liberated in the reaction are neutralized by the ammonium acetate buffer (Tutemet al, 1991).

1.5.8 ABTS method

The formation of the bluish-green cation radical (ABTS•+) (Marc et al., 2004) is attributed to the nitrogen atom of ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) losing an electron. The nitrogen atom quenches the hydrogen atom in the presence of Trolox (or another antioxidant that donates hydrogen), resulting in the decolorization of the solution. According to Pellegrini et al. (2003) and Thaipong et al. (2006), as shown in Figure 1.10, potassium persulphate can oxidize ABTS. This produces the ABTS cation radical (ABTS•+), whose absorbance decrease at 743 nm was observed in the presence of Trolox, a standard antioxidant (Pisoschi & Negulescu 2012).

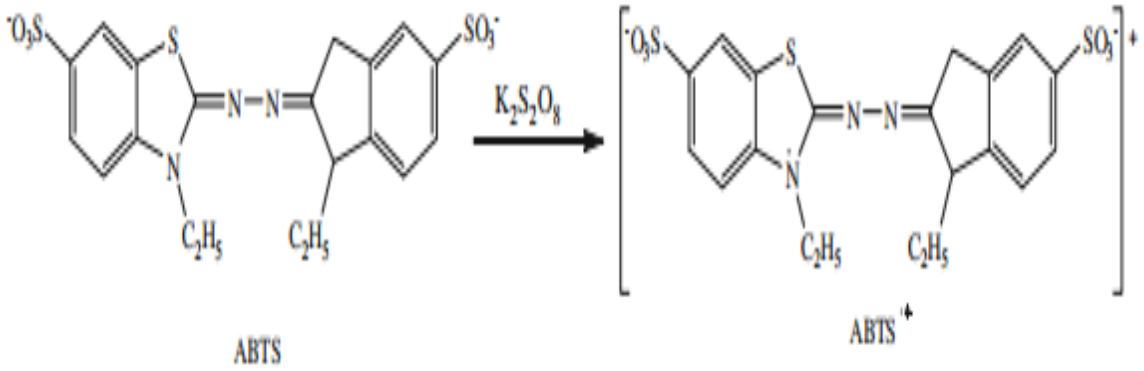

Figure 1.10 ABTS Method (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)(Pisoschi & Negulescu 2012).

The primary antioxidants are those that neutralize free radicals by either donating a hydrogen atom (hydrogen atom transfer or HAT) or by a single electron transfer (ET) mechanism. Meanwhile, secondary antioxidants are those that neutralize prooxidant catalysts. Three of the major antioxidant vitamins are beta-carotene, vitamin C, and vitamin E. DPPH· free radical reacts with an antioxidant (AH) or a radical species (R·) according to Eqs. (1), (2).DPPH· + AH → DPPH − H + A·DPPH· + R· → DPPH − R

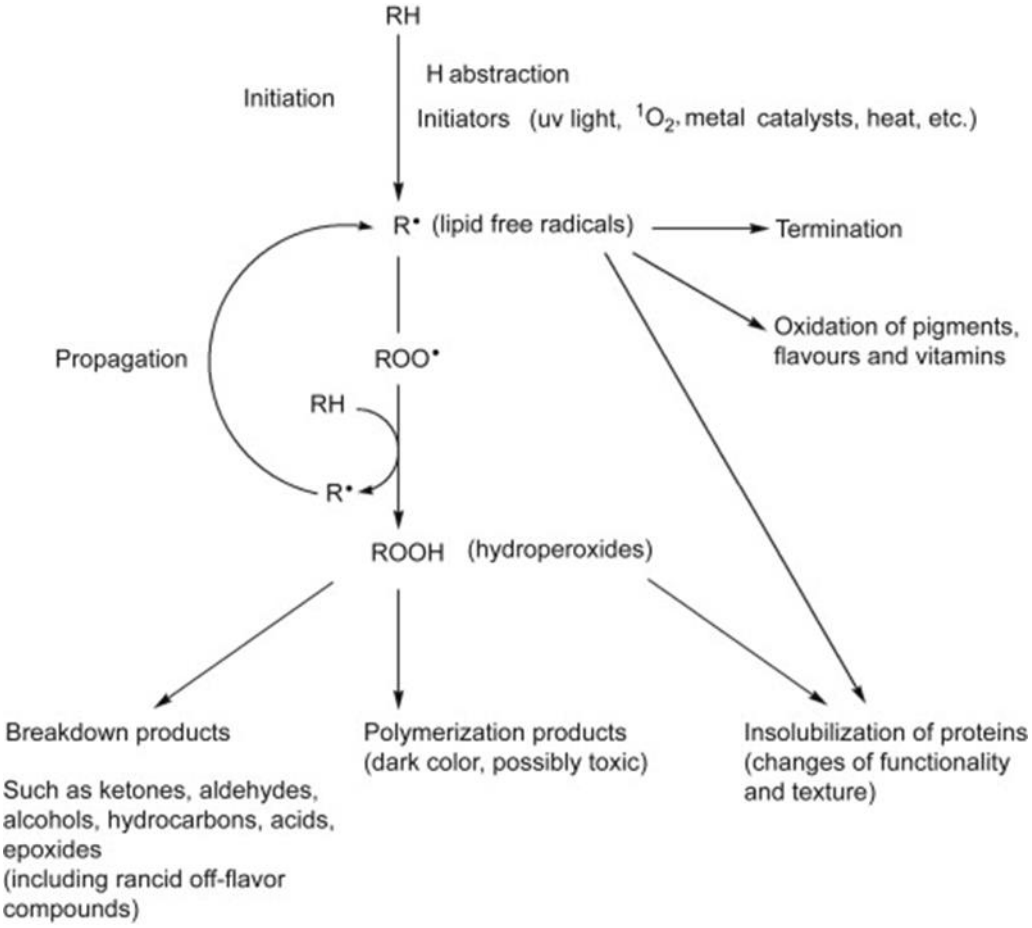

Figure 1.11 Mechanism of Antioxidant Activity (Pisoschi & Negulescu 2012).

1.6 Phenolic compounds

1.6.1 Definition

The most prevalent secondary metabolites found in plants are phenolic chemicals. These compounds belong to a complicated class that has a hydroxyl group on a benzene ring. Plant phenols are secondary metabolites with an aromatic ring that contain one or more hydroxyl molecules. They comprise a basic range of chemicals. According to their metabolic origin, plant phenols are classified as compounds that originate from the phenylpropanoid metabolism and the shikimate route (Ryan et al, 2002).

The literature now in publication uses a variety of terminology to describe these substances, depending on the matrix under investigation. These terms include phenols, phenolic contents, polyphenols, biophenols, and others. But when it came to Olea europaeae L. matrices, two of them—olive phenols and/or olive phenolic compounds—were chosen as the best (Uccella, 2000).

At least thirty phenolic compounds are present in virgin olive oil. Olive oil's total phenolic content and quantity can range from 100 to 1000 mg/kg.

In actuality, polyphenols are an intricate collection of substances with various chemical structures that are extracted from the oil using a methanol-water mixture. Phenolic molecules have an association with both the oil's biological characteristics and stability. In nature, the majority of phenolic chemicals exist in conjugated form. Houshia and Qutit (2014)

1.6.2. Functions in olive oil

a. Nutritional quality: The presence of phenols with high antioxidant activities increases the nutritional value of olive oils. (El Riachy et al, 2011).
b. Olive oil phenols' health benefits: Numerous studies have examined the advantages of olive oil phenols. Apart from the well-established antioxidant properties, they also appear to have antithrombotic and antihypertensive properties. In addition to their anti-aging properties, they were also linked to preventive effects against cardiovascular, neurological, and certain types of cancer Vissers et al. (2004)
c. Sensory quality: Phenols and volatiles together are primarily responsible for the sensory qualities of olive oils, giving them a subtle and distinct flavor that consumers find quite appealing. Servili et al. (2009)

d. Oxidative stability: Phenols are essential for both the oxidative stability and shelf life of virgin olive oils. They use strategies including metal-chelating properties, hydrogen atom transfer, and/or radical scavenging to prevent lipid oxidation even in the early stages. Jerman (2014).

1.6.3 Factors Affecting Their levels

Olives' amounts of polyphenols are influenced by their variety, harvesting ripeness, climate, and farming methods. During the growing season, irrigation has an impact on the fruit's polyphenol levels; sparingly watering raises the phenol content. Harvest timing influences the amount of polyphenols in the oil since they naturally decline as the olive fruit ripens; oils with earlier harvests have higher polyphenol values (Houshia & Qutit, 2014). While being milled and stored, the amount of polyphenols decreases. Since many polyphenols dissolve in water, they are lost along with the water from the vegetation when processed. In addition, polyphenol levels will slowly decrease during storage, as they dampen oxidation in the oil given these unavoidable losses, an initial high polyphenol level is essential for ensuring longer shelf life and greater health properties.
(Manach, 2004)(Figure1.4) shows the factors that affect olives and olive products phenolic composition and levels (Malheiro et al, 2015).

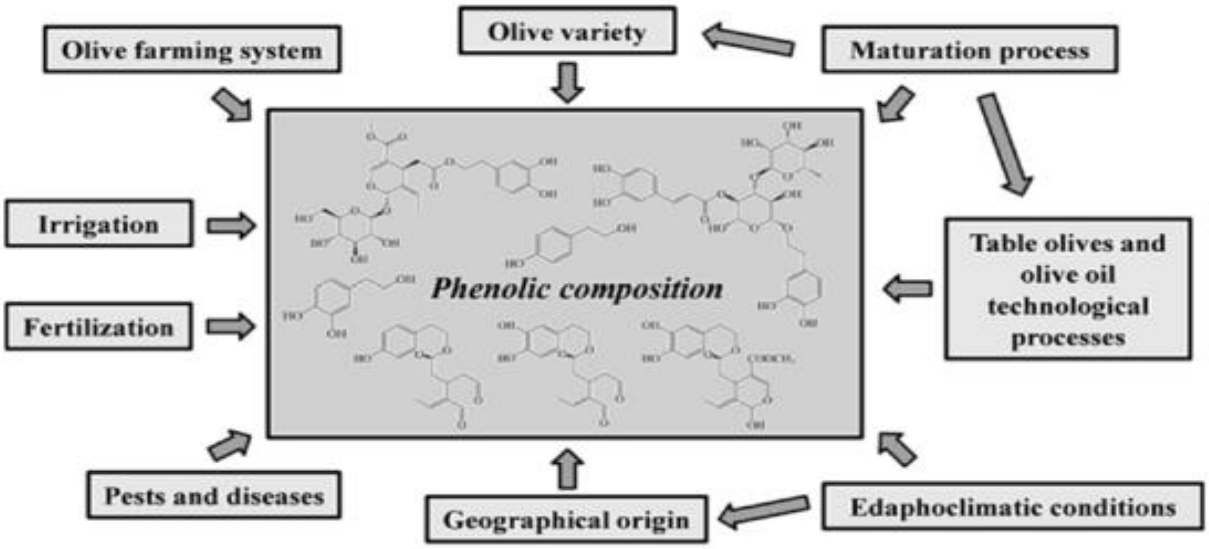

Figure 1.12 factors that affect olives and olive products phenolic composition (Malheiro et al, 2015)

1.6.4 Phenolic compounds

polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes

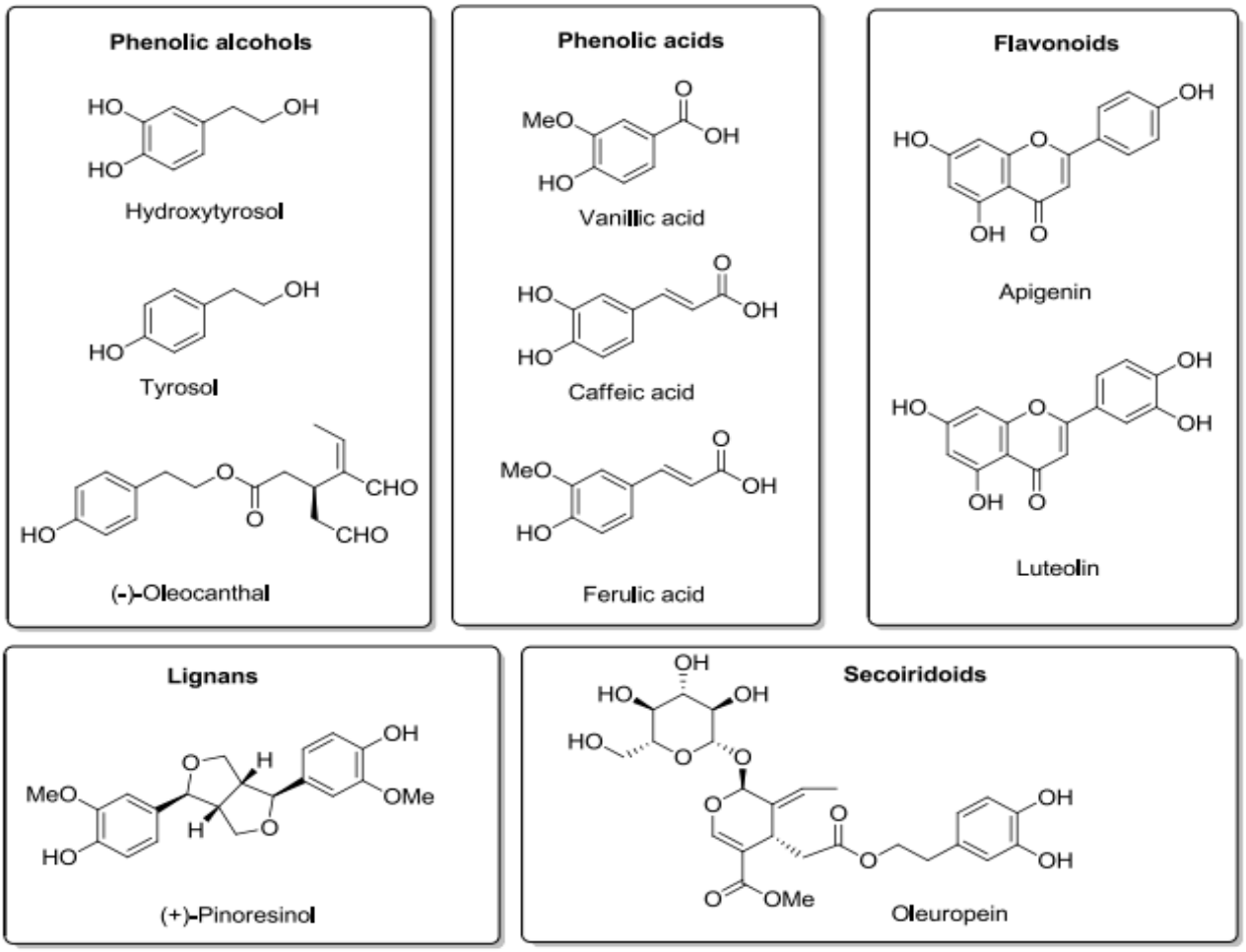

Figure 2.2 polyphenols may be classified into different groups as a function of the number(Rodríguez et al., 2015).

of phenol rings that they contain as well as the structural components that connect these rings. Phenolic acids, flavonoids, stilbenes, and lignans are the primary classes. The molecular structures of exemplary examples of the various types of polar phenolic compounds found in olive oil are depicted in (Figure 2.2) by Rodríguez et al. (2015). The molecular structures of exemplary examples of the several classes of polar phenolic compounds found in olive oil are shown in Figure 2.2 (Rodríguez et al., 2015).

1.7 Total flavonoid content

Important naturally occurring bioactive substances are flavonoids. Total flavonoid content (TFC) is commonly measured using the aluminum chloride colorimetric technique, which assumes that all flavonoids would respond equally when compared to a flavonoid standard. Plant secondary metabolites fall into three main categories: phenols, terpenoids, and alkaloids (Das & Gezici, 2018). Plant aromatic amino acids are the source of alkaloids, while aspartate, glutamate, or glycine within the cell is the source of phenols, including polyphenols. Conversely, glycolysis intermediates are used to make terpenoids.

Following solvent extraction, the total flavonoid content (TFC) is often measured colorimetrically. Using Al(III) as a complexing agent, the aluminum chloride colorimetric assay is a commonly used technique for measuring TFC in plant extracts (Das & Gezici, 2018).

1.7.1 Mechanism of action of Total Flavnoid content

Following solvent extraction, the total flavonoid content (TFC) of plants is often measured colorimetrically. Using Al(III) as a complexing agent, the aluminum chloride colorimetric assay is one of the commonly used techniques for measuring TFC in plant extracts. In order to identify flavonol compounds in medications, Christ and Muller first devised the test in 1960 (Christ & Müller, 1960). The process is predicated on Al(III)-flavonoid chelates forming. Because flavonoids include a lot of oxo and hydroxyl groups (Scheme 1), they can bind metal ions like Al(III) very well, usually at a 1:1 ratio, depending on the pH level and other experimental factors (Kasprzak, Erxleben, & Ochocki, 2015; Pyrzynska & Pękal, 2011). Over time, the first procedure underwent many modifications, including the inclusion of sodium nitrite (NaNO2) prior to the addition of AlCl3. Flavoniod-nitroxyl derivatives (Scheme 2) are generated by the selective nitrating action of sodium nitrite on aromatic vicinal diols (Barnum, 1977). These derivatives are identified by the emergence of a new absorption band at around 500 nm. An additional modification involves doing the Al(III)-flavonoid complexation with acetate salt present. In the current work, an experimental evaluation of the original assay and its modifications is conducted.

Its proponents state that the AlCl3 method of TFC detection can only be used if the metal chelates of each flavonoid contained in a sample exhibit quantitatively identical absorbance at a particular wavelength (Christ & Müller, 1960). Nevertheless, in spite of this reality, the

approach was widely and naively used to determine TFC under the false assumption that all flavonoids had the identical absorption spectra at the region of interest.

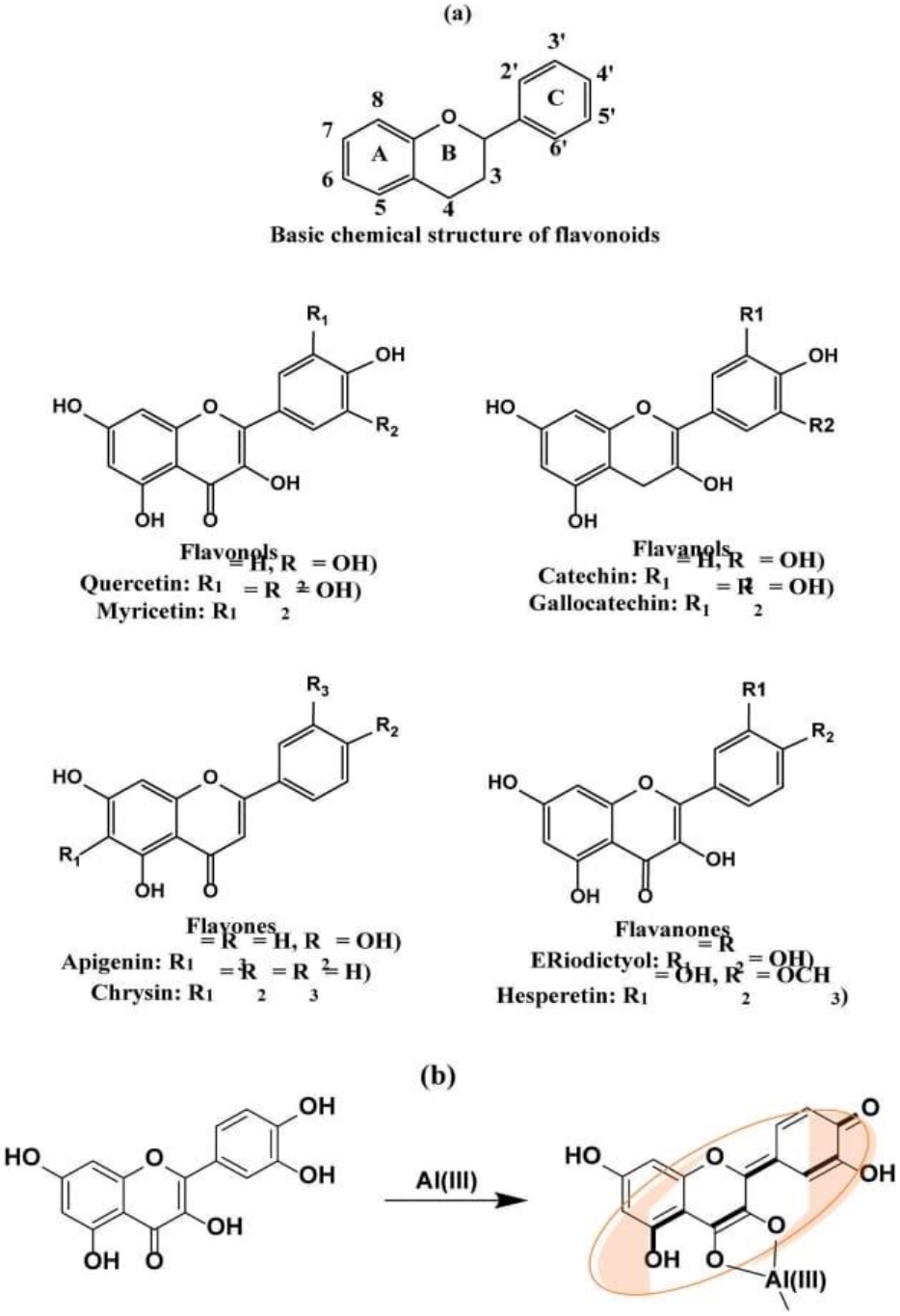

Figure 2.2.a Flavonoids: general chemical structure and examples(a) and illustration of Al(III)-quercetin chelate (b) (Christ & Müller, 1960).

In an experimental setting, yellow-colored Al(III)-flavonoid complexes are produced when AlCl3 is added without NaNO2, and their absorbance is then evaluated at a specific wavelength within the 410–440 nm range.

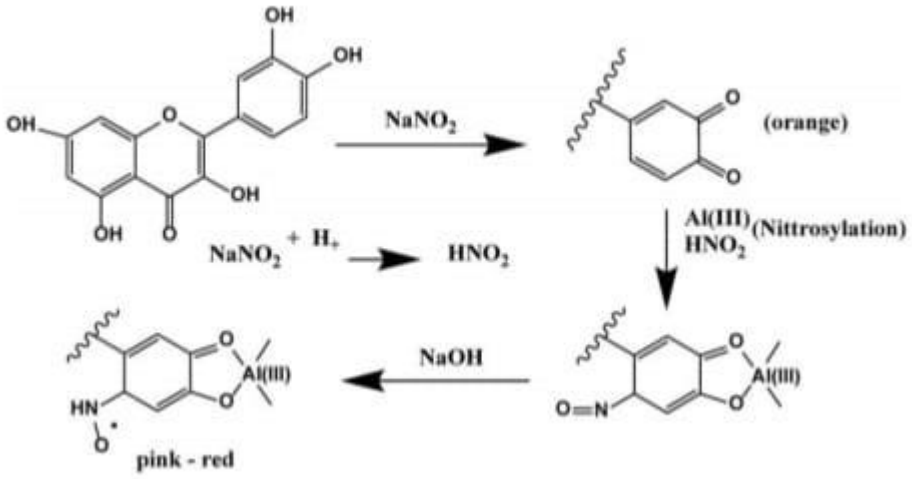

Figure 2.2.b Al(III)-flavonoids (quantifed is used as example) complexation in the presence of NaNO2 (Mekonnen & Desta,2021).

TFC is assessed using calibration curves that are based on a reference flavonoid standard that was measured at the same wavelength and under the same experimental conditions. The most often used flavonoid standards are rutin, quercetin, and catechin. Figure 2.2 c shows the structure of these compounds. The goal of the inquiry was to assess the aluminum chloride colorimetric assay critically in order to quantify the total flavonoid content. The following goals were established in order to accomplish this goal: (i) assessing the efficacy of the aluminum chloride colorimetric assay using real plant extracts and authentic flavonoid standard solutions; and (ii) determining the recoveries of authentic flavonoid standards that were spiked in both experimental solutions and real plant extracts in order to evaluate the validity and accuracy of the assay using the spike recovery approach (Mekonnen & Desta,2021).

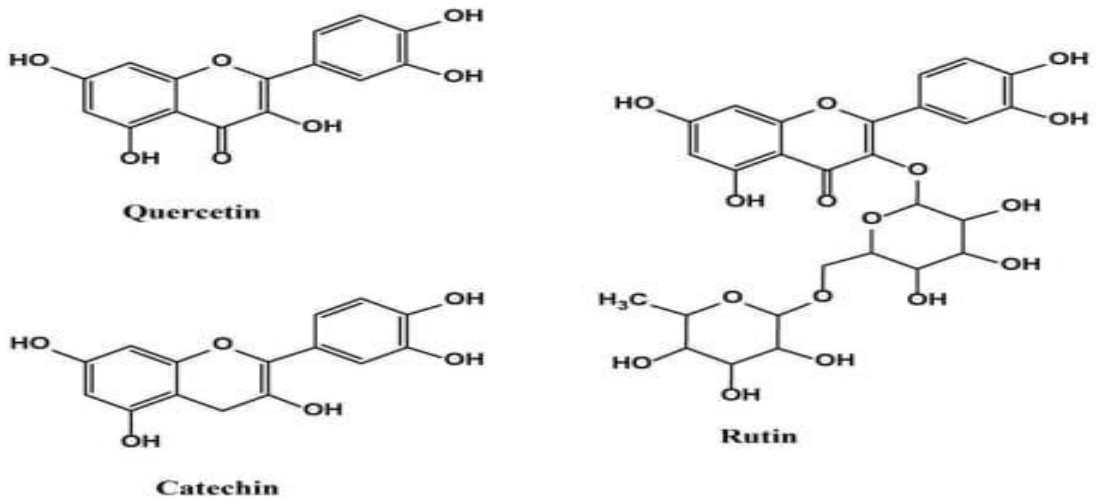

Figure 2.2.c Chemical structures of quercetin, catechin, and rutin(Mekonnen & Desta,2021).

1.8. Sensory evaluation

It is a crucial tool for evaluating quality and putting virgin olive oils in the commercial category. An "analysis panel" consisting of eight to twelve tasters who have been qualified and trained by regulatory agencies should conduct this analysis. A panel leader, who oversees the group, compiles the ratings assigned to the favorable (fruity, bitter, and pungent) and unfavorable (sensory defects) sensory qualities. The virgin olive oil is categorized based on the median values of fruity and sensory faults. Lampante, extra virgin, or virgin (International Olive Council, 2015), (Bertoncini & Testa, 2014), (Amelio, 2019)

Individuals choose their foods depending on a number of criteria, including price, taste, past eating experiences, and healthfulness. Even yet, and maybe even more importantly, they use data gathered from their senses: taste, smell, texture, and appearance. These sensory components can improve the allure of a food product, exhibit its quality and attractiveness, or satisfy the preferences and tastes of important demographics. Manufacturers can use information from food product sensory analysis for marketing, product development, and other purposes.

The scientific discipline of sensory assessment encompasses all methods used to measure, analyze, evaluate, and elicit human reactions to food attributes as experienced by the five senses: taste, smell, touch, sight, and hearing. Researchers are particularly interested in taste and scent in relation to invesgtive behavior

The sensory evaluation focuses on measuring a product's sensory qualities objectively, assessing consumers' subjective reactions to physical products, and interpreting their reactions by comprehending the product (Amelio, 2019).

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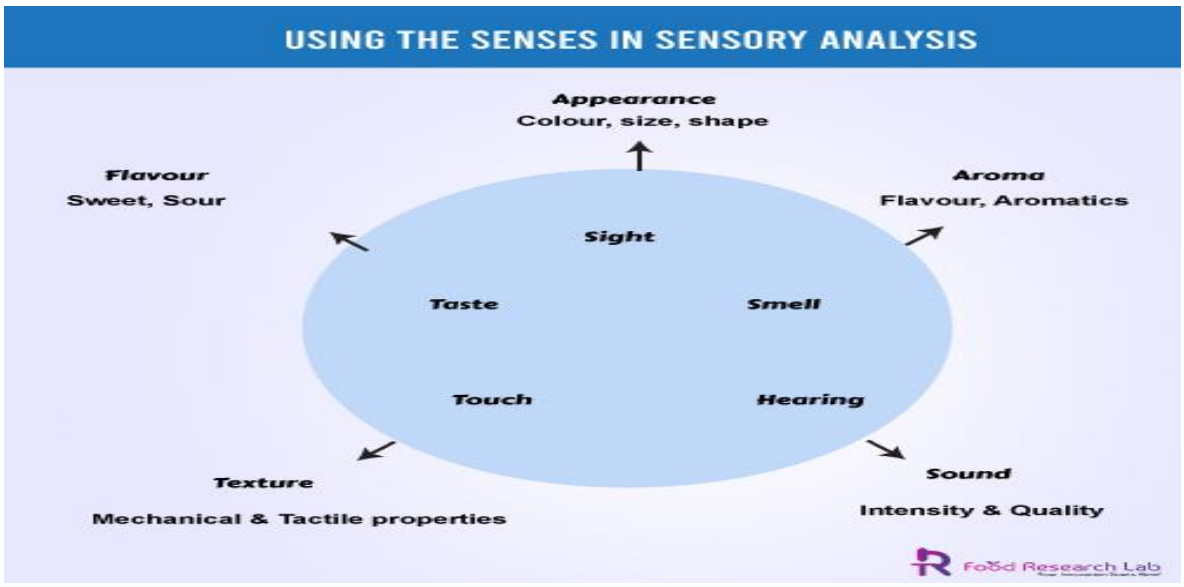

Figure 2.3 sensory evaluation of food using the senses in sensory analysis (R Food Research Lab) (Amelio, 2019)

Sensory analysis can be used for a variety of purposes, such as flavor profiling, shelf-life estimation, product success assessment, quality control, and identifying the variables that influence consumer preferences. It can assist you in making important choices about raw materials, parts, additives, packaging and storage settings, "best by" or expiration dates, and product optimization (Amelio, 2019).

1.8.1 .Difference Tests

Food products are compared using difference testing to check whether there are any differences. These qualities include the texture, flavor, and aroma, to name a few. The triangle test, the duo-trio test, and the paired comparison test are three distinct test types(IOC, 2015) .

They are as follows:

The Triangle Test: establishes whether two things have detectable sensory differences. It works particularly well when changes made to production may have affected the final product. After tasting all three samples, the panelists decide which is the most distinctive. A

triangle test might not be the ideal choice if there is significant flavor carryover is easy to understand between samples and panel lists find it confusing to evaluate three models instead of just two.

The Duo-Trio Test : can also be used to identify variations in the product brought about by adjustments to the suppliers of ingredients, storage, packaging, and other elements. The sample that is exact replica of a certain reference sample is indicated by the sensory eater. It. The purpose of Paired Comparison Tests is to identify the more prevalent attribute or preferred example between two samples. In the latter case, it is considered an acceptance test. One of the most popular attribute difference tests is easy for panelists to understand.

1.8.2. Descriptive Tests

A comprehensive profile of a food product's sensory characteristics and a qualitative evaluation of the strength of each feature are provided by a descriptive food sensory analysis. Test of Flavour Profile The five main components of the Flavor Profile technique are character features, attribute intensity, order of attribute appearance, aftertaste, and amplitude (the total impression of the analyzable and non-analyzable flavor components). Five points were initially assigned to the flavor profile: not present, threshold, minor, moderate, and vigorous. In order to support greater intensity.

Texture Profile Test: Five core qualities (hardness, cohesion, adhesiveness, viscosity, and elasticity) and three additional criteria (brittleness, chewiness, and gumminess) were offered for analyzing food texture by The Texturometer - A New Instrument for Objective Texture Measurement.

The Spectrum Descriptive Analysis applies statistical techniques, a more precise scale (often 150 points, depending on the product), a more extensive panel group (up to 15 people), and the rigorous training and organization of the Flavor and Texture Profile Methodologies to the descriptive data.

Qualitative Tests: A panel consisting of ten to twelve individuals is asked to rate the attributes of a product using a six-inch line scale with half-inch intensity indications.

Selective Choice Profiling Free Choice Profiling is different from the aforementioned approaches in two key aspects. First off, the members of each panel are "untrained" consumers. Even if they are provided instructions on the evaluation, they are not selected

based on their aptitude or experience in identifying minute variations in product attributes. Second, the participants mostly use "liking" or "acceptance" responses (among other qualifiers) as a means of measuring for each attribute rather than providing an empirical rating.

1.8.3. Affective Testing

The test method is clear and straightforward, so the panel of consumers will know how to reply. A technique for ascertaining the preferences of two individuals is the Paired Preference Test. After a discernible difference between the two wines has been established, a preference test can be conducted. This aids in selecting the wine blend or yeast fermentation technique to use.

Ranking Test: If more than two samples are examined, a preference ranking test may be finished. In most cases, a consumer can rank three to five pieces in a fair length of time. In this sensory evaluation method, the customer rates the samples according to their preferences, with a "1" denoting the sample that they would most like.

The Hedonic Tests: The degree to which one or more items are acceptable can be ascertained using the hedonic scale. This is a category scale that has five to nine odd categories, ranging from "like extremely" to "dislike highly." There's a midway ground that's neither like nor disliked. The customers rate the product on a scale based on their answers show as figure 2.4 classification of the main sensory testing procedures

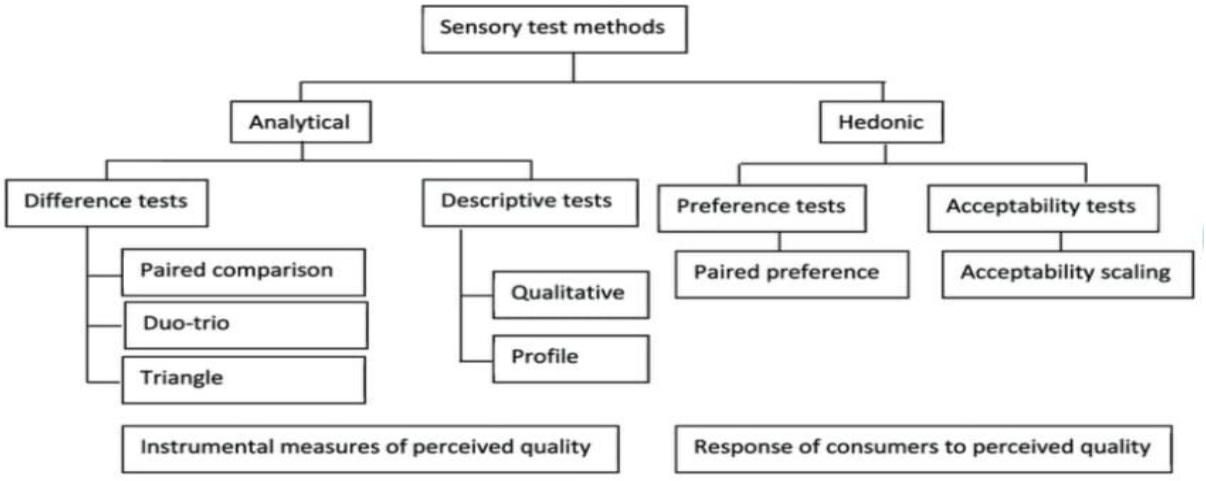

Figure 2.4 classification of the main sensory testing procedures (Amelio, 2019; Bertoncini & Testa, 2014; International Olive Council, 2015a)

A completely qualified analytical sensorial panel made up of five trained assessors also conducted the sensory evaluations for the olive oil sensorial analysis. After each taster

smelled the oil, they each judged it to be defective or not. The panelists then sampled the oils faultlessly and noted how strong the fruity, bitter, and pungent flavors were. attributes in line with the IOC's technique of organoleptic characterization of virgin olive oil.

1.9 Extra virgin olive oil

The highest grade of olive oil, or extra virgin olive oil (EVOO), is subject to the strictest chemical and organoleptic regulations. Because of this, extra virgin olive oil (EVOO) is the healthiest and tastes the best among the other grades(IOC, 2016) The most costly quality of olive oil to produce, extra virgin olive oil is also the hardest to make and necessitates both chemical and organoleptic evaluation. But as anyone who has ever tasted premium extra virgin olive oil will attest, it is well worth the effort. Extra virgin olive oil (EVOO) is defined as having superior flavor and aroma by the International Olive Council (IOC) and Codex Alimentarius, the two primary bodies that oversee the quality of olive oil. For an olive oil to be considered "extra virgin," it must have a median fruitiness score above zero and a median defect score of zero, or the median score of one of the 12 olive oil flaws that is judged as having the greatest intensity (more on that later). Additionally, extra virgin olive oil has the lowest free fatty acid level of any non-refined grade, as measured by oleic acid, which is less than 0.8 grams per 100 grams. (Refined olive oils have less free fatty acid since the refining process eliminates it.(IOC,2015)

Triglycerides, which bind three fatty acids to a glycerol backbone, have generally broken down, as shown by greater amounts of free fatty acids. This occurs when olive oil is produced using harmed or diseased fruit, when the grinding process is delayed, when the oil is exposed to high temperatures, or when other unfavorable storage circumstances exist(Uccella, 2000. The maximum permissible level of free fatty acids in extra virgin olive oil is 0.8 grams per 100 grams; nevertheless, many of the best EVOOs have free fatty acid contents that are closer to 0.3. The milliequivalent peroxide oxygen per kilogram of oil must be less than or equal to 20, in addition to the free fatty acid content(Hamid, F., & Hamid, F. H.2016).

t. The oil is less likely to be fresh over time the higher the peroxide value detected. This is because more oxidation has already occurred. Although the majority of governments adhere to the Codex Alimentarius and IOC criteria, California has a stricter definition for extra virgin olive oil, allowing a free fatty acid level represented as oleic acid of less than 0.5 grams per 100 grams. But the requirements for organoleptics are still the same. Extra virgin

olive oil is evaluated based on three main characteristics: the strength of these features, the absence of five typical flaws, and the chemical parameters(IOC, 2015)

Fruitiness, bitterness, and pungency—three of EVOO's favorable characteristics—are rated on a linear scale by a skilled panel of tasters.

A fruity oil is defined by its flavor and scent. Fresh, green, mature, and ripe are some common descriptions of it. However, bitterness is a taste that is felt on the tongue and is not as preferred in most dishes. Its existence, however, is a sign that an extra virgin olive oil rich in polyphenols was produced using fresh olives. Like some beers, chocolates, and coffees, bitterness is a flavor that one must develop. It takes some time to truly appreciate the flavor.

Also See: Some Food Proteins Lessen EVOO's Bitterness and Pungency

Pungency, a stinging sensation at the back of the throat linked to the polyphenol oleocanthal, is the third favorable EVOO property. Similar to the sense of chili peppers, pungency is likewise an acquired taste (IOC,2015).

To produce the most flavorful oil possible, producers of premium extra virgin olive oil must strike a balance between these advantageous characteristics.

Tasting panels note the five most frequently mentioned bad qualities as stated by the IOC, in addition to the good ones: frostbitten, fusty, musty, rancid, and winey. If an olive oil has any of these flaws, it cannot be classified as "extra virgin ."The olive oil sample tastes like damp wood because of the frostbitten olives(Antolovich et al, 2002; Ruíz et al,2001).. When frost damages the olive trees, a fault arises. When olives are incorrectly stored after harvesting but before grinding, they can ferment and cause spoiledness. Fustiness is identified by flavor as well as a muddy feeling that accumulates at the bottom of the container. The mustiness that lends olive oil its earthy or humid flavor results from yeast or fungi growing on the olives as a result of moist storage conditions or from not washing them. To be rancid is to be fat "gone bad." It happens when the oil oxidizes, which naturally happens over time with extended exposure to heat, light, or air. The taste of rancid oils is stale and waxy, leaving a greasy aftertaste (El Riachy et al, 2011).

Olive oil takes on a sour, vinegary, or acidic flavor when it gets winey. The flaw arises from improper cleaning of mill machinery, which allows olive waste to ferment and produce ethanol ethyl acetate, and acetic acid. EVOO is physically extracted without the need of chemical solvents or heat. In the olive groves is where the process starts. Farmers harvest their olives (either manually or mechanically) once the daily temperatures have dropped, and they promptly transport the fruits to the mill (Marc et al, 2004). Many growers in hotter

climates choose to harvest at night because the lower harvest temperatures help preserve the polyphenols in extra virgin olive oil. When the olives get to the mill, the leaves are taken off and a wash is performed.

The olives are taken to the grinder after being cleaned. The majority of contemporary mills grind the olives into a paste using a blade, disc, or hammer mill. Although they are less effective, stone mills are still used in traditional mills. The crushed olive paste is placed in the malaxer and swirled slowly, allowing the oil droplets to collect. At this point, olive oil begins to take on its distinctive smells and scents(Pisoschi& Negulescu, 2012).. To separate the oil from the water and pomace, or solid waste made up of stems and pits, the paste is taken from the malaxer and placed in the centrifuge. In the past, a hydraulic press was used for this; thus, the name "cold-pressed."

Many mills choose to centrifuge the surplus oil again after the initial centrifuging in order to eliminate any remaining water and pomace particles. From here, the oil is drained out and either transported for filtering or kept in stainless steel tanks filled with non-reactive inert gas.

The oil is classified as "extra virgin" if it satisfies the above described chemical and organoleptic requirements. Extra virgin olive oils are unique among other oils in that they have a multitude of health advantages that are related to monounsaturated fatty acids and bioactive substances like vitamin E and polyphenols. (Vissers et al, 2004).

The overwhelming majority of these health benefits come from the polyphenols in EVOO, which is why virgin olive oil and refined olive oil do not have the same health benefits. (Bertoncini & Testa, 2014).

CHAPTER TWO

Literature Review

Malika Douzane1 , Mohamed-Seghir Daas1, et al (2021) . This study set out to assess the physicochemical and organoleptic qualities of twenty olive oil samples that were gathered around the country and belonged to four different Algerian cultivars: Chemlal, Sigoise, Ronde de Miliana, and Rougette de Mitidja. According to physical-chemical and sensory analysis, 40% of the oils were categorized as "virgin olive oil," while 60% of the oils fall into the extra virgin category. Depending on the cultivar and place of origin, the principal component analysis (PCA) results showed significant variation in the fatty acid composition of the samples. The most prevalent acid, oleic acid, ranged from 64.84 to 80.14%. Extra virgin olive oils that meet quality standards might be given a label. Sigoise from Oran, Ronde de Miliana, and Rougette de Mitidja all had a lot of promise.

Zagoa, , Squeob , et al (2019) . performed Pearson's correlations between chemical and sensory indices, as well as the relationship between volatile profiles and organoleptic properties. Thus, providing information regarding these facets was the current study's goal. Although volatiles from anaerobic and aerobic fermentation were also occasionally discovered, the volatile profiles demonstrated the existence of the aldehydes responsible for the favorable property of volatile organic oils (VOOs), namely trans-2-Hexenal and hexanal.

El Riachy , et al (2018) . The current study aims to explore the effects of harvesting location, harvesting time, and processing method on the chemical composition and sensory qualities of olive oils made from the Lebanese olive variety known as "Baladi." Samples (n = 108) were taken from four processing systems, three distinct harvesting times, and North and South Lebanon. The findings demonstrated a significant relationship between oil quality, fatty acid composition, total phenols, and OSI and the origin, processing method, and harvest time.

Higher total phenol content (220.02 mg GAE/Kg) and higher OSI (9.19 h) were observed in the early harvest. Additionally, the samples from 3-phases and sinolea had the lowest free acidity (0.36% and 0.64%), and the highest OSI (9.87 and 9.84 h). Consumers were not unanimous regarding the studied factors, although samples recording high ranks were mostly from South using sinolea, 3-phases and press systems at early and intermediate harvest. The overall findings suggest that the selection of the harvesting time and of the processing system could have significant influence on the characteristics of the olive oil.

Abbadi, et al (2014) investigated the impact of storage containers on olive oil quality and confirmed that, at both storage temperatures, glass was the most effective container in preserving the quality of extra virgin olive oil (EVOO), whereas pottery performed the poorest. It was insufficient to grade the investigated olive oil that had been stored using sensory evaluation. Furthermore, it was evident that the most sensitive chemical test for determining the quality of stored olive oil was the absorption coefficient K270, which could also be employed as a quick indicator test.

Houshia, et al (2014): Measuring the overall concentration of polyphenol in several samples of Palestinian olive oil was the aim of the investigation. The Folin-Ciocalteau reagent was used to colorimetrically assess the methanol extracts' total polyphenol content. The total polyphenols were measured colorimetrically at 725 nm using a diluted extract or phenolic standard, Folin-Ciocalteau reagent, and aqueous Na2CO3. The procedure was calibrated using standard solutions containing gallic acid. In olive oil, the amount of polyphenols varies between 150 and 300 mg/kg.

M. El Riachy, et al (2012) investigated how the ripening index and genotype affected the phenolic profile of olive oils from advanced selections compared to their progenitors. Liquid-liquid extraction using a 60:40 (v/v) methanol-water mixture was used to characterize the phenolic profile. This was followed by chromatographic analysis using absorption and fluorescence detection in a sequential arrangement. The phenolic profile has been found to be influenced by both genotype and fruit ripening, with a stronger genetic influence on total phenols (34.73% and 20.45%, respectively) and individual phenols (16.99% to 49.25% and 1.58% to 23.77%, respectively). The acquired results also make it possible to identify the selections that have high levels of both total and individual phenol content.

These results suggest a strategy based on early harvesting of fruits (at the first three ripening indexes) for better comparison and selection of genotypes in further crosses in olive breeding programs aiming at improving the quality of virgin olive oil.

Dabbou, et al (2011): Discovered the total phenolic component in the virgin olive oil of the four different varieties and described several novel olive oil genotypes growing in Egypt. They reiterated the significance of virgin olive oil's tocopherol content in preventing lipids from autoxidating, extending its shelf life and nutritional value as a whole food. They discovered that the amount of α-tocopherol changed from 44.66 ppm in cultivar No. 1 during the first season to 290.82 ppm in cultivar 138.
examined the antioxidant potential of phenolic extracts from two mixed olive cultivars, Chaïbi, Oueslati, and four other Tunisian olive oils in connection to their α-tocopherol and lipid composition. With the exception of Oueslati oil (171.6 mg kg-1), all α-tocopherol concentrations were greater than 300 mg kg-1. The range of the total phenol concentration was 396–652 mg kg-1. According to the two methods of measuring virgin olive oil's antioxidant capacity, Mix2 had the greatest levels (0.9 mmol TE kg-1 and 72.3%, respectively) of total antioxidant activity by ABTS test and radical scavenging activity by DPPH assay. When tested with polar components, it demonstrated the connection between the lipid profile—which is critical to the virgin olive oils' shelf life—and their antioxidant capability. The study's findings suggest that Tunisian cultivars are a valuable source of bioactive substances with strong antioxidant capacities and beneficial synergistic effects.

Georgiou, et al (2010) this work aimed to map the total antioxidant capacity (TAC) of fifty Greek olive oil samples from the 2005–2006 season based on cultivar and production region. They also compared the Folin–Ciocalteu, 2, 2-diphenyl-1–picrylhydrazyl radical (DPPH), and 2, 2’-azino-bis (3-ethylbenzo–thiazoline-6–sulfonic acid (ABTS) tests for use with olive oil. Olive oil's total acidity content (TAC) as determined by the DPPH method ranges from 77 to 177 mM Trolox Kg-1, whereas antioxidant capabilities found in the hydrophilic fraction range between 5.42 - 22.5 mMgallic acid Kg-1 for the ABTS method and 1.29 - 9.95 mMTrolox Kg-1 for the DPPH method.
The total phenol content of olive oil ranges from 3.8 to 29.4 mMgallic acid Kg-1. The DPPH (r _ 0.89) and ABTS (r _ 0.69) assays used to measure the total antioxidant capacity in the hydrophilic fraction show a correlation with the total phenol concentration. There is a substantial correlation (r _ 0.81) between the ABTS readings and the hydrophilic fraction

DPPH values. The DPPH results for total olive oil, on the other hand, have weak correlations with the DPPH assay in hydrophilic fraction, the Folin-Ciocalteu method, and the ABTS assay. The use of a battery of assays helps to better characterize the antioxidant capacity of olive oil, even though total phenolic content exhibits high association with ABTS and DPPH values and may be a valuable indicator of olive oil antioxidant capacity.

Waterman, et al (2007) proved the effect of olive oil and the antioxidant activity especially main phenolicshydroxytyrosol, tyrosol, and oleuropein, which occur in highest levels in virgin olive oil on specific health conditions. Olive oil was seen in women who consumed ≥30.5 g/day.With respect to blood pressure reduction, an effect was seen with dietary supplementation of 40 g/day for men and 30 g/day for women, which equates to approximately 15 kg/year and 11 kg/year, respectively.

The study also shown how antioxidants are involved in several biological functions of olive oil. Monounsaturated fatty acids like oleic acid have been proven to be effective in preventing cancer, while squalene has also been linked to anticancer properties. Consuming olive oil can help prevent breast and colon cancer. Numerous studies have examined the oil's potential to lower blood pressure and low-density lipoprotein (LDL) cholesterol in relation to coronary heart disease (CHD). Oleuropein, hydroxytyrosol, and tyrosol have all been shown to have antimicrobial action against a variety of bacterial strains linked to respiratory and gastrointestinal illnesses. Eating entire olives may also be beneficial to health, even though most research has focused on the oil.

Cosio, M. S, et al (2006) verified the geographical origin and the uniqueness of specific extra virgin olive oils. Their dataset includes 36 Garda oils and 17 oils from other regions. Two classification models have been built by means of Counterpropagation Artificial Neural Networks in order to separate Garda and not-Garda oils, as follows: utilizing all of the chemical variables and sensor signals in the first place; utilizing electronic tongue sensors in the second place; and using four specific electronic nose sensors in the last place. Furthermore, 19 samples of commercial olive oil were used to test each model. The results from neural networks are rather good, and they suggest that the electronic nose is the best instrument for characterizing the oils under study. These findings have indicated that the use of electronic nose in conjunction with neural networks may offer a quick, affordable, and effective way to categorize and describe extra virgin olive oils from a certain region.

CHAPTER THREE

3.1 Materials and Method

3.1.1. Extraction Olive Oil Samples

Olive oil provided by Alreef for Investment and Agricultural Marketing. These samples were collected from various categories of olive oil across different fields in Palestine's West Bank. The olive oil for this study was sourced directly from the farmers at local presses. Each farmer provided eight samples, which were stored in glass containers and refrigerated until analysis. The collection occurred under diverse conditions in late October 2022. In total, eight samples representing different kinds of olive oil were gathered from various farms in Palestine, including Kufer Qadom farm, Masha farm, Anzeh, Farkh, East Bani Zaid, Fruit Fall Trees, and Al-Yamoon farm, as shown in Table 5. Subsequently, a correlation analysis between chemical and sensorial evaluations was performed.

Table 5 Abbreviations used for EVOO samples

Cultivators	Code / Sample
Qarawat bani zaid	S1
Fruit fall trees1	S2
Anzeh	S3
East bani zaid2	S4
Farkh	S5
Al-yamoon farm	S6
Masha farm	S7
Kufer Qadom farm	S8

3.1.2 Initial Quality tests

The tests conducted in this research include Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant Activity (AA), Acid Value (AV), Peroxide Value (PV), and Sensory evaluation (panel test).

The chemical reagents used for determining total phenolic compounds, as well as those used for measuring peroxide value (PV), acid value, total flavonoid content (TFC), and antioxidant activity (AA), are listed in Table 6

Table 6: Chemicals used in analysis

CHEMICALS	Peroxide value (PV)
(PV)	Glacial Acetic acide
	Chloroform
	Potassium iodide KI
	Sodium thiosulphate $Na_2O_3S_2.5H2O$
	Starch- water
(AV)	Phenolphthalien
	Ethyl alcohol
	Sodium hydroxide
	Ethanol
(TPC)	
	Folin- Ciocalteu reagent
	Deionized water
	Na_2CO_3
	Gallic acid
(TFC)	
	Distilled water
	$NaNo_2$
	$AlCl_3.6H_2O$
	NaOH
	Catechin
(AA)	ETHANOL
	DPPH

3.1.3 Extraction of virgin olive oil samples

After dissolving an aliquot of two grams of olive oil samples in twenty milliliters of n-hexane and transferring the liquid to a separatory funnel, three sections of ten milliliters of methanol-water mixture (80:20 v/v) were added. The extracts were then collected, and any leftover oil was washed away with twenty milliliters of n-hexane. After that, the extracts were kept cold until analysis.

3.2 Tests:

3.2.1 Determination of total phenolic content (TPC)

TPC was determined spectrophotometrically using Folin–Ciocalteu reagent. To 100µL of the sample extract , 2.9 ml of deionized water, 0.5 ml of Folin–Ciocalteu reagent and 2.0 ml of 20% Na_2CO_3 solution will be added. The mixture will be allowed to stand for 90 min and absorption will be measured at 760 nm against a reagent blank in UV–Vis spectrophotometer. Results will be expressed as gallic acid equivalent (mg GAE/100 g). (Singleton et al., 1999).

3.2.2. Determination of antioxidant activity (AA)by DPPH :

Materials used were: DPPH (2,2-diphenyl-1- (2,4,6-trinitrophenylhydrazyl**),** Methnol (95%), Torolox. AA was determined by UV-Vis Spectrophotometer.

A 3.9 mL aliquot of a 0.0634 mM of DPPH solution, in methanol (95%) was added to 0.1 mL of each extract (the extract mentioned before) and shaken vigorously. Change in the absorbance of the sample extract was measured at 515 nm for 30 min till the absorbance reached a steady state. The percentage inhibition of DPPH of the test sample and known solutions of Trolox were calculated by the following formula: %inhibition = (100× (A_0-A)/A_0 where A_0 was the beginning absorbance at 515 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the sample extract at 515 nm. Methanol (95%) was used as a blank. Results were expressed as µmol Trolox/g (Re et al, 1999).

(Different concentrations of Torolox for the calibration curve from 20-120ppm were used

3.2.3.Determination of Total flavonoid content (TFC)

TFC was analysed using the Aluminium chloride method (Zhishen et al., 1999). An aliquot (1 ml) of Olive oil extract in 10 ml of volumetric flask containing 4 ml of distilled water, 0.3 ml

portion of 5% $NaNO_2$ and 0.3 ml portion of 10% $AlCl_3.6H_2O$. The mixture is allowed to stand for 6 min at room temperature. Two millilitres of 1 N NaOH will be added and the solution will be diluted to 10 ml with distilled water. The absorbance of the solution versus a blank at 510 nm was determined by UV-Vis Spectrophotometer, will be measured immediately. Aqueous solutions of known Catechin concentrations in the range of 30– 200 ppm will be used for calibration. The results will be expressed as catechin equivalent (mg CE/100 g) (Zhishen et al, 1999).

3.2.4. Acidity Percentage Test

Acidity indicates the level of biochemical degradation of triglycerides. It corresponds to the amount (expressed in grams) of free fatty acids in 100 grams of olive oil. It is expressed as a percentage of free oleic acid; (the main fatty acid of triglycerides present in the olive oil (Boskou, 2006).

Materials used were: Ethanol (96%), NaOH, Phenolphthalein solution, Hot plate.

By using the AOAC method number 940.28 to determine AV in fats and oils, 7gm of oil sample was put into a dry and clean 250 ml Erlenmeyer flask, then 50 ml of 96% ethanol was neutralized with 0.1 N aqueous NaOH solution in presence of 2 ml phenolphthalein solution to produce faint permanent pink, then the neutralized ethanol was added to the oil in the flask, then the mixture was shaken vigorously and boiled on a hot plate for two minutes then titrated with 0.1 N aqueous NaOH solution until permanent faint pink colour appeared and persisted one minute.

Calculations

1. Results could be reported as acid value (number of milligrams of NaOH required to neutralize free fatty acids in 1 gram oil).

$$\text{Acid Value} = N \times V \times \frac{56.1}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where :

N = Normality of NaOH aqueous solution

V = Milliteres of NaOH aqueous solution required to neutralize fatty acids present in the used sample .

W = grams of the sample used in the test.

2. Results could be reported as acidity % - free fatty acids contents which mean the percent of F.F.A expressed as a certain fatty acid of specified molecular weight according to the type of oil under investigation. Normally oleic acid with a molecular weight of 282 is taken. In a number of cases an average molecular weight, more appropriate to the nature of the oil, is used. In each reported basis must be clearly stated:

$$\text{Acidity \%} = \frac{N \times V \times G \times 100}{1000 \times W} \qquad \text{.....(2)}$$

where N, V and W are the same symbols used in the calculation of acid value, G is the molecular weight of the appropriate fatty acid.

In normal cases acidity % is expressed as oleic acid , and G = 282.

If acidity is expressed as lauric acid, G = 200.

If acidity is expressed as palmitic acid, G = 256.

If acidity is expressed as ricinoleic acid, G = 298.

If acidity is expressed as erucic acid, G = 338.

The relation between acid value and acidity % :

$$\text{In normal cases, Acidity \%} = \frac{N \times V \times 282 \times 100}{1000 \times W}$$

$$\text{Acid value} = \frac{N \times V \times 56.1}{W}$$

$$\frac{\text{Acidity \%}}{\text{Acid value}} = \frac{N \times V \times 282 \times 100}{1000 \times W} \times \frac{W}{N \times V \times 56.1}$$

$$= 0.503$$

3.2.5 Peroxide value tests

Peroxide value is an indicator for evaluating the early stages of the degradation of triglycerides by oxidation which causes the appearance of odorous compounds (aldehydes, alcohols, ketones) that alter the flavour of oil and lead to rancidity (Boskou, 2006). Oxidation of oil is caused firstly by contact with atmospheric oxygen, then light (UV) and heat which act in the initiation stage of oxidation. Finally, metals such as iron and copper operate at extremely low levels in catalyzing the oxidation process (Baldassari, 2008). Peroxide value measures the oxygen content expressed as oxygen milli equivalents per kg of oil. A high peroxide value (> 20 meq. O2 kg-1) indicates a strong potential towards rancidity.

Materials used were: Glacial acetic acid, Chloroform**,** Potassium iodide**,** Distilled Water, Sodium thiosulfate, Starch.

By using the AOAC method number965.33, about 5g of oil were weighed into 250 ml glass-stoppered conical flask, then 30 ml of glacial acetic acid-Chloroform solution (3:1 by volume) were added with swirling to dissolve oil completely, then 1 ml of saturated potassium iodide solution was added (Potassium iodide has a solubility of 144 g/ 100 mL of water at room temperature. Therefore, at room temperature more than 144 g of potassium iodide were dissolved in 100 mL of water, creating a saturated solution. Anything more than 144 grams will not dissolve), then the flask was quickly stoppard and let to st and with occasional shaking for 1 minute in the dark, thereafter, 30 ml of freshly boiled and cooled water were added and flask contents were titrated with 0.01 N sodium thiosulfate solution with vigorous shaking until yellow colour had almost gone, about 0.5 ml of starch solution was added and titration was continued with vigorous shaking to release all iodine from chloroform layer, until the blue color just disappeared. Blank determination is conducted in the same way without the sample (Blank is composed of all additions except oil sample).

Calculation

peroxide value is the number of milliequivalents of peroxide found in1000 grams of sample.

$$\text{Peroxide value} = \frac{(V1 - V2) \times N \times 1000}{W}$$

Where :

V1 = Volume (in ml) of solution thiosulfate used in test.

V2 = Volume (in ml) of sodium thiosulfate used in blank test.

N = Normality of sodium thiosulfate solution.

W = Weight (in g) of sample.

3.2.6. K_{232}, K_{270} extinction coefficients

This test consists of measuring two parameters (K_{232}, K_{270}) determined during the same analytic procedure. The greater the value of K_{232}, the greater the concentration of conjugated dienes, whereas K_{270} is proportional to the concentratioin of conjugated trienes. However, compounds of oxidation of the conjugated dienes contribute to K_{232} while compounds of secondary oxydation (aldehydes, ketones etc.) contribute to K_{270}.

K_{232} indicates the age of oil and how long olives have been left in sacks after harvesting , milling process, storage conditions of the olive oil and the level of oxidation incurred during production and/or storage.K_{270} parameter test detects the level of adulteration .

Materials used were: Cyclohexane, UV-Vis Spectrophotometer.

By using the IOOC COI/T20/Doc. number 19/Rev.1 2001, 1% solution of oil sample at 27°C (temperature of the lab) was prepared in cyclohexane (0.25 g oil in 25 ml solvent), then the absorption was taken at 232 and 270 nm, respectively, with a UV spectrophotometer, using a path length (a cuvette width) of 1 cm.

3.2.7. Sensory evaluation (Panel test)

3.2.7.1 The panel test

P T was conducted by the Jordan Standards and Metrology Organization (JSMO) in accordance with the International Olive Council (IOC). The samples were assessed by an eight-person panel of expert tasters. Following the completion of the smell tests, the gustatory perceptions were assessed with an eye toward both good (fruity and bitter) and negative (defective) characteristics, such as fusty/muddy sediment, musty-humid-earthy, winey-vinegary, acid-sour, and rancid. Either way, the pungency tactile sense was examined. The panel leader collated the comments from each taster, using the median of the fruity and median of the faults for statistical analysis, and the results showed that the olive oils were classified as extra virgin, virgin, and lampante(IOC, 2015)

		ATTRIBUTES
Panel test	NEGATIVE	Heating/sludge Mold/moisture/ground Winey/acidic/acid/sour Frozen olives Rancid
	POSITIVE	Fruity Bitter Spicy

3.2.7.2.Panel test method

The customer took samples. The test findings then only have an impact on the test items. Reproducing the test report "except in full" is prohibited without the Jordan Standards and Metrology Organization's official consent. The International Olive Oil Council has granted the panel team accreditation (IOOC).

Procedure

Determining the Distinctive Sensory Profile:

Identify the distinctive sensory profile for the designation of origin (D.O.).

The D.O. authority will select up to ten characteristic descriptors for the designation of origin and include them in the profile sheet.

The authority will set maximum and minimum median limits for each descriptor in the profile sheet and establish limits for the robust coefficient of variation for each descriptor, following the guidelines in the D.O. profile determination guide.

These values are then entered into the IOOC spreadsheet folder-profile (software) to define the intervals of the characteristic sensory profile for the designation of origin. The D.O. authority may also evaluate the harmony of the oil as defined by the standard. Assessing How Well the Oil's Sensory Profile Matches the Distinctive Profile of Its Origin Designation:

The panel supervisor enters each taster's data into the IOOC spreadsheet folder-profile (software) for the origin designation. This process follows the guidelines in the IOOC assessment guide for evaluating the consistency of D.O. extra virgin olive oil with its distinctive sensory profile. If the sensory profile resulting from the statistical processing by the software matches the profile determined by the D.O. authority, the evaluated extra virgin olive oil complies with the sensory characteristics defined for the designation of origin.

Tasters' Use of the Profile Sheet:

Each panelist will sniff and taste the oil in the tasting glass to analyze its olfactory, gustatory, tactile, kinaesthetic, qualitative retronasal, and taste sensations. They will then indicate the intensity of each descriptor on the provided profile sheet.

Utilizing the Information by the Panel Supervisor:

The panel supervisor will collect the completed profile sheets from each taster and review the recorded intensities. If any irregularities are found, the supervisor will ask the taster to review their profile sheet and, if necessary, retake the evaluation.

3.3. Questionnaire

A questionnaire was given to the farmers, consisting of six questions regarding:

a. Whether their olive fruits were affected by olive fly.
b. The number of days of storage between harvesting and oil extraction.
c. The percentage ratio of green olives to black olives.
d. The oil percentage (the weight percentage of extracted oil relative to the weight of olive fruit before extraction).
e. The drop percentage (the percentage of olive fruit found on the ground before harvesting relative to the total olive fruit weight).

The olive yield percentage (the weight percentage of olive fruit compared to the maximum olive fruit weight ever observed).

3.4. Statistical Analysis

The results are expressed as mean ± standard deviation. All statistical analyses were carried out using SAS (SAS Institute the GLM procedure considering a fully randomized design, treating main factors . The Bonferroni procedure was employed with multiple tests in order to maintain an experiment wise of 5% ($P < 0.05$) A probability of $P < 0.05$ was considered as significant . Initially Pearson correlations were calculated to test the relation between individual quality indicators (TPC, TFC, AA, Acidity%, Peroxide value ,K232, K270) with each one of the other quality indices. . The NOMISS option was used in order to obtain results consistent with subsequent multiple regression studies**.**

CHAPTER FOUR

4.1. Results

Extra virgin olive oil (EVOO) samples were extracted from olives provided by Alreef for Investment and Agricultural Marketing. Eight distinct samples, representing various categories, were collected from several farms in Palestine, including Qarawat Bani Zaid, Kufer Qadom, Masha, Anzeh, Farkh, East Bani Zaid, Fruit Fall Trees, and Al-Yamoon. Following this, a connection was established between the chemical and sensory analyses of these samples.

4.1.1 Peroxide Value (PV)

The peroxide value (PV) is an indicator of the initial stages of oxidative change (Ruíz, et al, 2001). The PV represents the total hydroperoxide content and is one of the most common quality indicators of fats and oils during production and storage (Antolovich et al, 2002; Ruíz et al, 2001). The peroxide value is determined by measuring the amount of iodine which is formed by the reaction of peroxides (formed in fat or oil) with iodide ion (Grossi et al, 2015).
All sample's initial peroxide values (PVs) were 20 meq O2/kg oil or less. As shown in Figure 4.1, Qarawat Bani Zaid Farm (S1) had the highest PV, while Anzeh Farm (S3) had the lowest. The peroxide value, a critical quality indicator, measures the total peroxides in olive oil and is expressed as milliequivalents of O2 per kilogram of oil. Higher PVs indicate greater oxidation-related deterioration.
The results revealed that Qarawat Bani Zaid (S1) had the highest PV (9.83), indicating significant degradation and less stable olive oil. In contrast, Anzeh Farm (S3) had the lowest PV (5.22), indicating minimal degradation and more stable oil, representing high-quality

extra virgin olive oil. Bani Zaid Farm (S4) had the second-lowest PV(6.02), followed by Farkh Farm (S5) (7.21), Al-Yamoon Farm (S6) (7.97), and Masha Farm (S7) (8.37), with Qarawat Bani Zaid (S1) having the highest PV.

The peroxide values (millequivalent O_2 kg^{-1}) of Olive Oil samples were 09.83±0.06 , 7.80±0.00, 5.22±0.15, 6.02±0.20, 7.21±0.00, 7.97±0.06, 8.37±0.06 and 7.97±0.06 respectively in S1, S2, S3, S4, S5, S6, S7, S8. results are expressed as average ± SD.

According to IOC (2016) all the results less than 20 millequivalent O_2 kg^{-1} oil ,virgin or Extra virgin olive oil categories.

According to (Mansouri et al ,2013) there are many factors affecting peroxide values of olive oil like factors causing damage to the olive fruits, while it is not affected by cultivar.

Fly-infected olives were found to increase the value of peroxide (Tamendjari et al, 2009).

If the olives are healthy and processed soon after harvesting (within 24 hr), environmental conditions do not appear to have any substantial influence on the peroxide number of the oil, which allow the classification of the oils as EVOOs (Pannelli et al, 1990; Ripa et al, 2008).

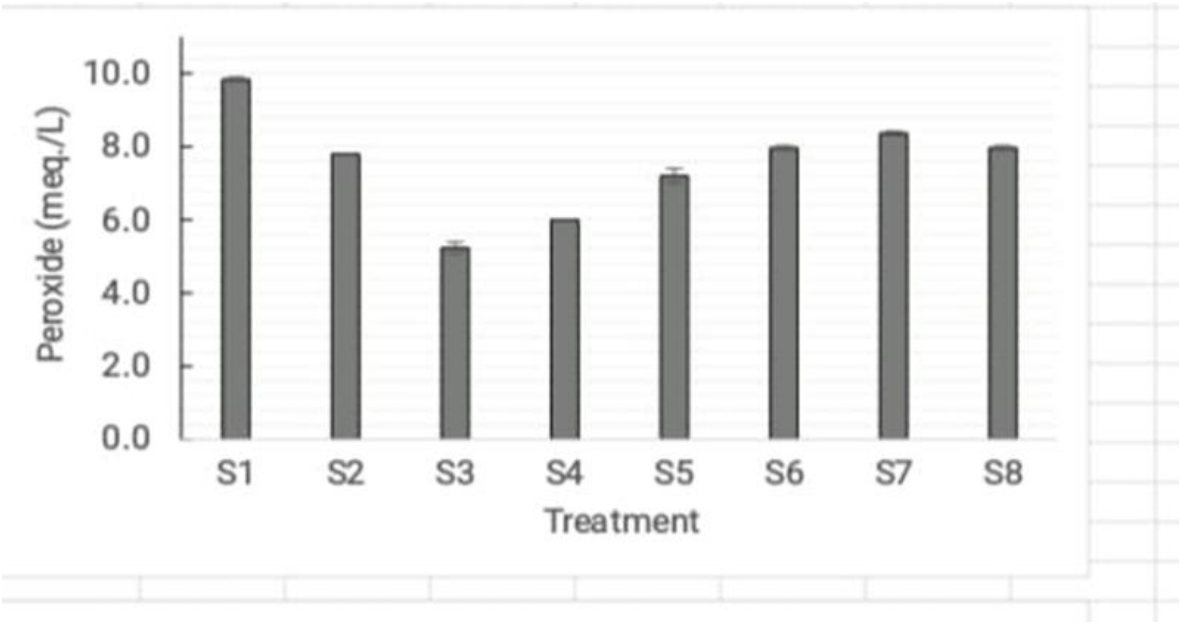

Figure 4.1 Mean values of Peroxide Value assay (millequivalent O_2 kg^{-1}) in the different Farms

4.1.2. Acidity %

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat or oil (Hamid, F., & Hamid, F.

H.2016). Figure 4.2 shows that Qarawat Bani Zaid Farm (S1) had the highest acidity (0.48), while Anzeh Farm (S3) had the lowest(0.32).

The Acidity% Values(expressed as % oleic acid)of Olive Oil samples were 0.48±0.04 ,0.40±0.02, 0.32±0.01, 0.40±0.00, 0.44±0.01, 0.40±0.01, 0.36±0.03 and 0.36±0.02 respectively in S1, S2, S3, S4, S5, S6, S7, S8. results are expressed as average ± SD.
According to (Figure 4.2) we observed a decrease in Acidity% Values in the Olive oil in Anzeh and there was an increase in Qarawat Bani Zaid. According to IOC (2016), it can be observed that all our acidity results in categorized our oil samples as Extra virgin olive oil.

Mansouri et al, (2013) stated that factors causing damage to the olive fruits affect acidity of olive oil, while (Salvador et al., 2001) considered that ripening stages affect acidity. Tamendjari, et al (2009) found that olive oils obtained from infested olives had higher acidity values than non infested olives. Méndez & Falqué (2002) found that during olive oil storage, acidity increased slightly in almost all oils tested and showed that the lowest degree of acidity was obtained with h and harvested olives and the highest level was obtained with olives fallen into the ground. Arafat et al, (2016) observed that the oils from 100m altitude were higher in free acidity compared to 400 m elevation.
If the olives are healthy and processed soon after harvesting (within 24 hr), environmental conditions do not appear to have any substantial influence on the free acidity of the oil, which allow the classification of the oils as EVOOs (Pannelli et al. 1990a; Ripa et al. 2008).
There was no significant difference between Acidity% Values in farms
Differences in our Acidity% values results can not be explained according to the difference in farms alone.

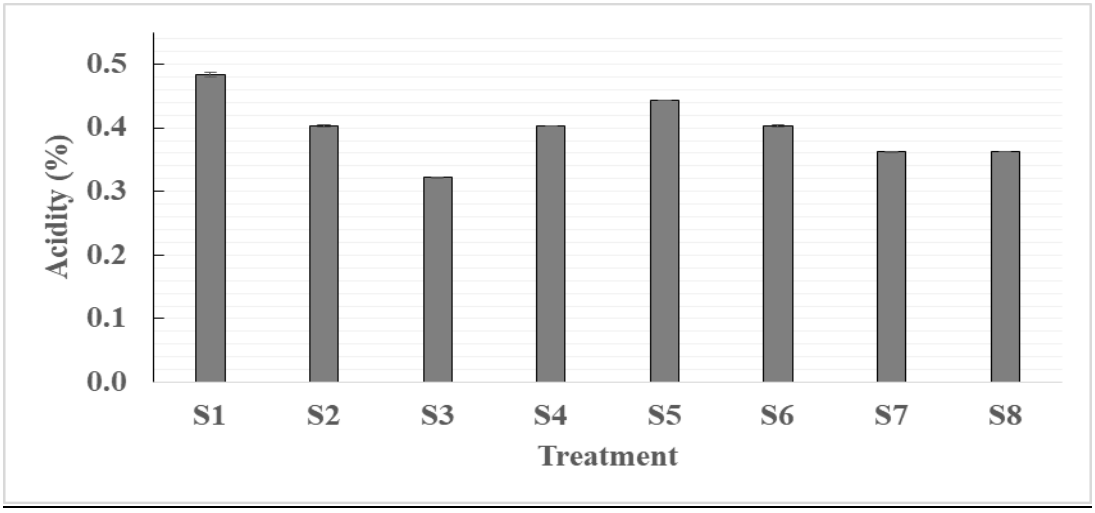

Figure 4.2 Mean values of Acidity% assay (expressed as % oleic acid) in different samples

4.1.3. Total Phenolic Content (TPC)

The results showed that Qarawat Bani Zaid Farm (S1) had the highest total phenolic content (TPC) among the oil samples (217.3), while Farkh(S5) Farm had the lowest values (179.3), as depicted in Figure 4.3. Phenolic compounds are key components with redox properties responsible for antioxidant activity. The hydroxyl groups in olive oil extracts enable free radical scavenging. The phenolic content of each extract was measured using the Folin-Ciocalteu reagent, with the results expressed in gallic acid equivalents (GAE) per gram of dry extract weight, based on a calibration curve of gallic acid ($y = 0.0022x + 0.4595$, $R^2 = 0.9968$) (Figure 4.3).

The concentration of phenolic compounds in the methanol extracts ranged from 179.3 to 217.3 mg GAE/kg oil. Qarawat Bani Zaid (S1), Kufer Qadoom (S8), and Anzeh Farm (S3) had the highest phenolic contents (217.03 ± 0.643, 208 ± 0.400, and 200.40 ± 0.265 mg GAE/g, respectively). In contrast, the lowest phenolic contents were found in Masha Farm (S7), Al-Yamoon Farm (S6), East Bani Zaid Farm (S4), Fruit Fall Trees (S2), and Farkh Farm (S5) (196.43 ± 0.153, 190.47 ± 0.208, 188.40 ± 0.300, 183.50 ± 0.265, and 179.37 ± 0.379 mg GAE/g, respectively). results are expressed as average ± SD.

Some factors affect TPC of olive oil like cultivar, climate and other environmental factors, harvesting time, extraction process, conditions of packing, distribution, and storage (Servili et al, 2004).

In spite of all the data shown above there was no significant difference between TPC values in farms since the standared deviations were low.

Houshia Orwa, et al (2014) reported that the total concentration of polyphenol in some samples of Palestinian olive oil from Jerusalem, Tulkarem and Jenin ranges from 150 to 300 mg/kg while our TPC results were range from 170 t0 217

The TPC of olive oil is influenced by various factors, including the cultivar, the extraction procedure, the climate and other environmental factors, and the time of harvest. Therefore, the range of phenolic content values seen in this study could be attributed to variations in time, geographic location, or extraction techniques, all of which have the potential to affect the concentration of phenolic compounds (Servili et al, 2004).

The phenolic content values in the current investigation varied somewhat. This could be because of the length of time, regional variations, or extraction techniques, which could change the concentration of phenolics.

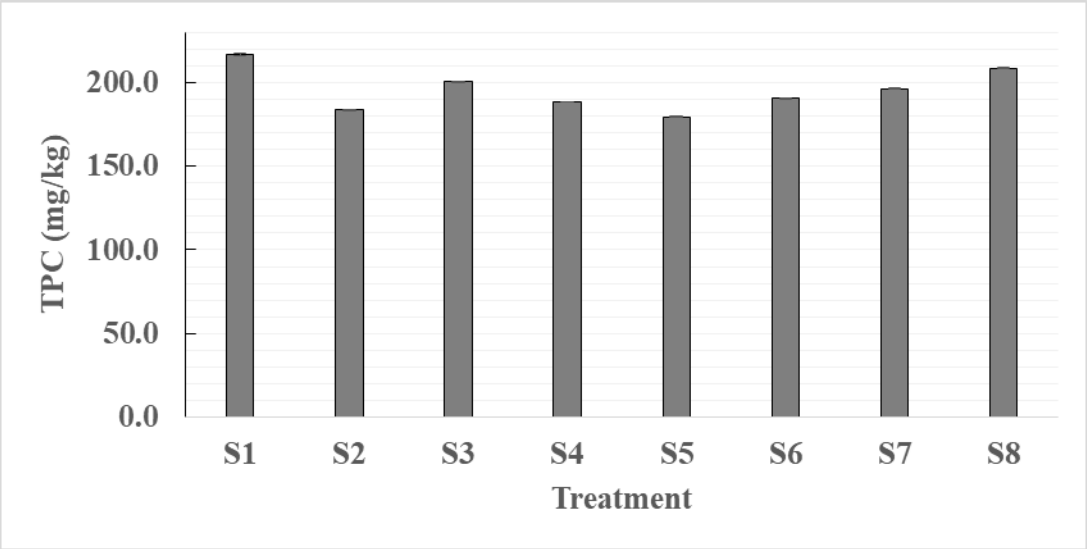

Figure 4.3 Mean values of TPC (mg GAL/Kg of oil) in the different Olive oil samples

4.1.4 . Total Flavonoids Content (TFC)

The results showed that Qarawat Bani Zaid Farm (S1) had the highest total flavonoid content (TFC) (80.54) among the oil samples, while Al-Yamoon Farm had the lowest values (21.51), as illustrated in Figure 4.4. The aluminium chloride method was used to determine the flavonoid concentration in the extracts. Results were produced using a catechin calibration curve ($y = 0.0048x + 0.0012$, $R^2 = 0.9809$) and are given in mg catechin equivalents (CE) per 100g.

The flavonoid content ranged from 21.5 to 80.54 mg CE/100g. Qarawat Bani Zaid (S1), Kufer Qadoom (S8), and East Bani Zaid (S4) had the highest flavonoid contents (80.54 ± 0.57, 53.26 ± 0.53, and 34.15 ± 0.44 mg CE/100g, respectively). In contrast, the lowest amounts of flavonoids were found in Anzeh Farm (S3), Fruit Fall Trees (S2), Masha Farm (S7), Farkh Farm (S5), and Al-Yamoon Farm (S6) (32.37 ± 0.42, 27.27 ± 0.62, 25.12 ± 0.39, 23.27 ± 0.62, and 21.51 ± 0.39 mg CE/100g, respectively). results are expressed as average ± SD.

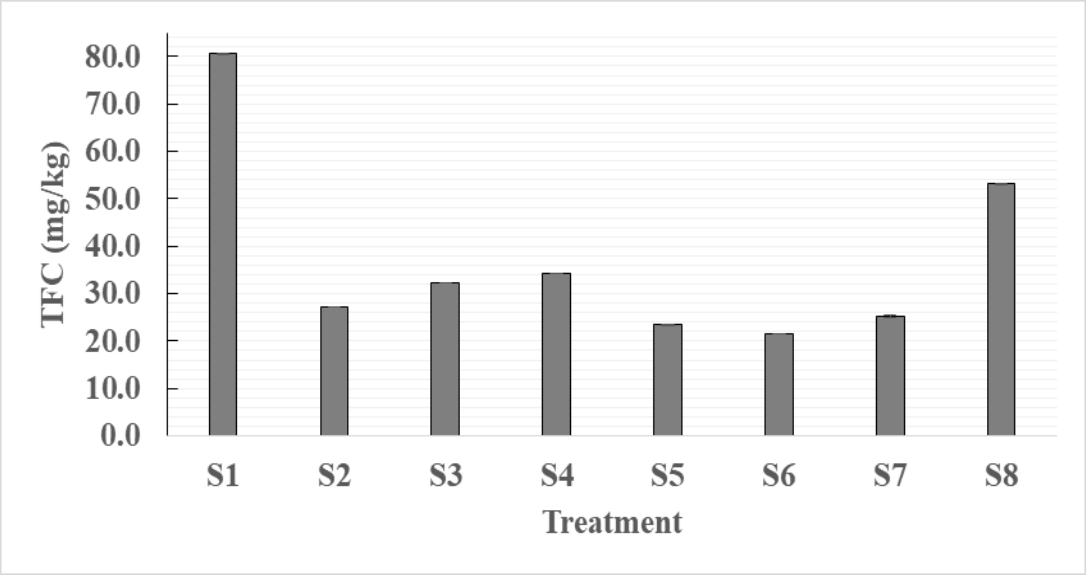

Figure 4.4 Mean values of TFC (mg catechin/Kg of oil) in the different samples

The flavonoid composition of olive oil can be influenced by different factors such as the growing climate, harvest maturity, olive cultivar, agronomic practices including irrigation or application of fertilizers, ripening hormones and the techniques employed to process and extract the oil (Rwothomio, 2011) so it is difficult to determine the specific reason for the difference in TFC values between samples since geographical origin alone is not sufficient (Kalogeropoulos & Tsimidou, 2014).

4.1.5 Determination of Antioxidant Activity(AA) by DPPH

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is based on evaluating antioxidants' capacity to scavenge the stable DPPH radical (MacDonald-Wicks et al., 2006). This antioxidant effect is assessed by monitoring the decrease in UV absorption at 517 nm (Moon et al., 2009).

Antioxidants primarily function through single electron transfer (ET) mechanisms or hydrogen atom transfer (HAT) to neutralize free radicals. Secondary antioxidants neutralize pro-oxidant catalysts. Major antioxidant vitamins include beta-carotene, vitamin C, and vitamin E. The reaction between the DPPH radical (DPPH·) and an antioxidant (AH) or radical species (R·) is described by the following equation:

$DPPH\cdot + AH \rightarrow DPPH - H + A\cdot$

Figure 4.5 shows the antioxidant values of various farms, with Qarawat Bani Zaid Farm (S1) having the highest value (98.10 ± 0.12) and Al-Yamoon Farm (S6) having the lowest (96.7 ±

0.021). Other farms included Fruit Fall Trees (S2), Anzeh Farm (S3), Masha (S7), East Bani Zaid (S4), Kufer Qadoom (S8), and Farkh (S5), with values of 97.8 ± 0.32, 97.3 ± 0.45, 96.9 ± 0.32, 96.8 ± 0.36, and 96.7 ± 0.021, respectively. results are expressed as average ± SD.

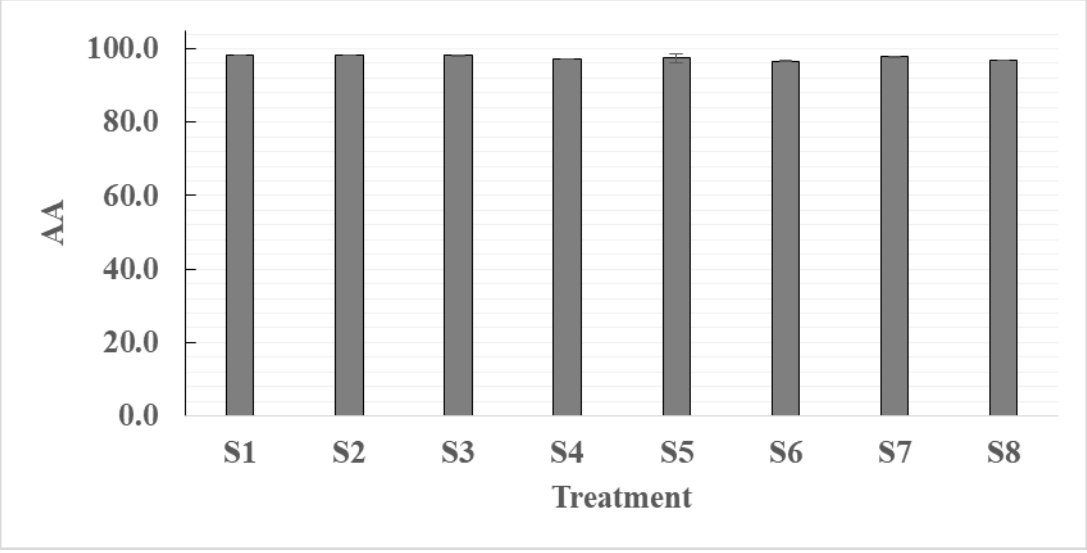

Figure 4.5 Mean value of Antioxidant Activity by DPPH method % scavenging of DPPH• = [(A0 − A1)/A0] × 100 where A0 = absorbance of the control and A1 = absorbance of the test extracts.

DPPH assay is an efficient electron donor, regardless of the reaction medium conditions and the compounds to be reduced.

There are a correlation between the total phenolic contents and DPPH$^{\bullet}$ for EVOO polar extracts.

Dabbou Samia, et al (2011) showed that there were correlation between the antioxidant capacity of virgin olive oils studied with polar components important to their shelf life.

4.1.6. Specific Absorption Coefficients (UV Absorbance Values) K270

The absorbance measured at 232 nm and 270 nm, namely K_{232} and K_{270}, provide an official method for olive oil quality control, which is capable of detecting product oxidation and adulteration by means of rectified oils, (Mignan et al, 2012; Angerosa et al, 2006) since they can give an indication of the level of oxidation to produce primary and secondary products incurred during production and/or storage (Afaneh et al.2013).

The concentration of conjugated trienes increases with higher K_{270} values. K_{270} is influenced by substances that undergo secondary oxidation, such as ketones and aldehydes. The highest

values were found in Qarawat Bani Zaid (S1), Al-Yamoon (S6), Masha (S7), and Kufer Qadoom (S8), with readings of 0.21 ± 0.0, 0.20 ± 0.0, and 0.20 ± 0.0, respectively, as shown in Figure 4.6. The lowest values were observed in Fruit Fall Trees (S2), Anzeh (S3), Farkh (S5), and East Bani Zaid (S4), with readings of 0.16 ± 0.0, 0.16 ± 0.0, and 0.17 ± 0.0. results are expressed as average ± SD.

The K_{270} parameter test is used to determine the degree of adulteration. The highest value, found in Qarawat Bani Zaid, indicates a higher content of conjugated trienes. According to (IOC, 2016) all values less than 0.22 olive oil quality extra virgin olive oil.

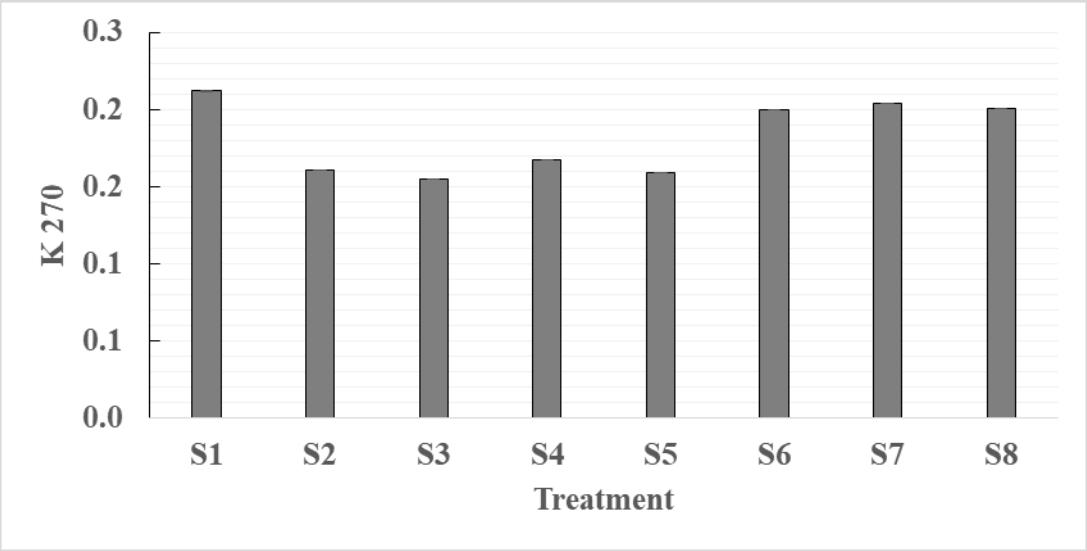

Figure 4.6 Mean values ofK_{270} assay ($K_{1\%}$/1cm) in the different samples

Mansouri et al (2013) reported that K_{270} as one of the quality indices is affected by variety and factors causing damage to the olive fruits, while Abbadi et al (2014) reported that absorption coefficient K_{270} was the most sensitive determinant chemical test that determines the quality of stored olive oil and could be used as a rapid indicator test. Pannelli et al (1990a); Ripa et al (2008) reported that if the olives are healthy and processed soon after harvesting (within 24 hr), environmental conditions do not appear to have any substantial influence on the UV absorbencies of the oil, which allow the classification of the oils as EVOOs

4.1.7. Specific Absorption Coefficients (UV Absorbance Values) K232

the absorbances measured at 232 nm and 270 nm, namely K_{232} and K_{270}, provide an official method for olive oil quality control, which is capable of detecting product oxidation and

adulteration by means of rectified oils, (Mignan et al, 2012; Angerosa et al, 2006) since they can give an indication of the level of oxidation to produce primary and secondary products incurred during production and/or storage (Afaneh et al.2013).

The concentration of conjugated dienes increases as the K_{232} value rises. Conversely, K_{232} is affected by the oxidation products of conjugated dienes. K_{232} offers insights into various factors affecting olive oil, such as its age, duration stored in sacks post-harvest, milling process, storage conditions, and degree of oxidation during manufacturing or storage. (Mignan et al, 2012)

In Figure 4.7, the highest K_{232} values were recorded in Qarawat Bani Zaid (S1), Al-Yamoon (S6), Masha (S7), and Kufer Qadoom (S8), with respective values of 2.20 ± 0.0, 2.11 ± 0.0, 2.02 ± 0.0, and 2.01 ± 0.0. Conversely, Fruit Fall Trees (S2), Anzeh (S3), Farkh (S5), and East Bani Zaid (S4) exhibited the lowest values, with readings of 1.19 ± 0.0, 1.16 ± 0.0, 1.01 ± 0.0, and 1.01 ± 0.0. results are expressed as average ± SD

The highest K232 value, indicating a greater concentration of conjugated dienes, was observed in Qarawat Bani Zaid.

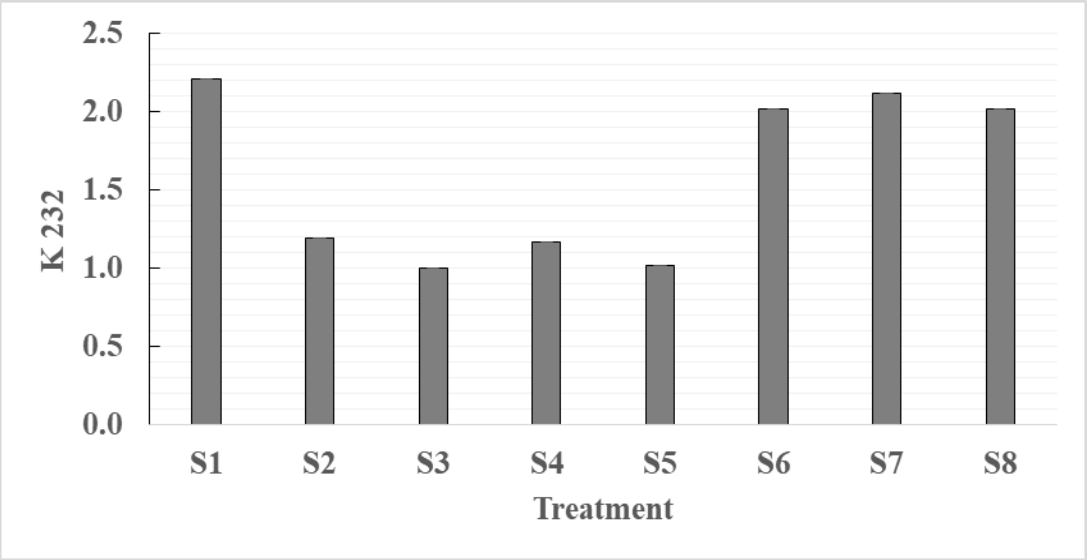

Figure 4.7 Mean values ofK_{232}assay ($K_{1\%}$/1cm) in the different sample

According to(IOC,2016) all values less than 2.5 olive oil quality extra virgin olive oil.

K_{232} as one of the quality indices is affected by variety and factors causing damage to the olive fruits (Mansouri et al, 2013).

Gharbi et al (2015) reported that K_{232} value was affected with olive storage conditions.

Pannelli et al. 1990a and Ripa et al (2008) reported that if olives are healthy and processed soon after harvesting (within 24 hr), environmental conditions do not appear to have any

substantial influence on the UV absorbencies of the oil, which are normally within the values that allow the classification of the oils as EVOOs

Table 7 Chemical parameters effects of sensory evaluation

Sample	Acidity	PV	AA	TPC	TFC	K232	K270
S1	0.48+_0.004	9.83+_0.058	98.37+_0.058	217.03+_0.643	80.56+_0.028	2.20+_0.001	0.21+_0.000
S2	0.40+_0.002	7.8+_0.000	98.30+_0.100	183.50+_0.265	27.27+_0.001	1.19+_0.000	0.16+_0.000
S3	0.32+_0.001	5.23+_0.153	98.13+_0.153	200.40+_0.265	32.37+_0.006	1.00+_0.000	0.16+_0.000
S4	0.40+_0.000	6.00+_0.000	97.27+_0.058	188.40+_0.300	34.19+_0.062	1.16+_0.000	0.17+_0.000
S5	0.44+_0.001	7.20+_0.200	97.47+_1.242	179.37+_0.379	23.38+_0.257	1.01+_0.000	0.16+_0.000
S6	0.40+_0.001	7.97+_0.058	96.67+_0.058	190.47+_0.208	21.57+_0.021	2.01+_0.000	0.20+_0.000
S7	0.36+_0.001	8.37+_0.058	97.77+_0.153	196.43+_0.153	25.26+_0.245	2.11+_0.000	0.20+_0.000
S8	0.36+_0.000	7.97+_0.058	96.87+_0.058	208.50+_0.400	53.14+_0.061	2.02+_0.000	0.20+_0.000

Table 7 Descriptive statistics of the chemical parameters data set
Results are expressed as mean ± SD of eight sample replicates $P < 0.05$

4.1.8. Sensory Evaluations of olive oil samples

Table 7 provides a summary of the chemical characteristics that influence the sensory perception of olive oil. The samples were taken by the customer, and the test results are applicable only to the tested items. Reproduction of the test report in its entirety is prohibited without official consent from the Jordan Standards and Metrology Organization. The panel team has received accreditation from the International Olive Oil Council (IOOC).

4.1.8.1 Sensory Test of Qarawat Bani Zaid (S1)

Table 8 presents the sensory evaluation conducted at the Qarawat Bani Zaid farm's testing lab by the Jordan Standards and Metrology Organization. The results of the test showed that the olive oil (VOO) type exhibited faults, with a median of 3 for musty characteristics.

Table 8 Sensory Test of Qarawat Bani Zaid (S1) oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	22/SEL/24	Sample code/source	Qarawat Bani Zaid	Opening Date	6/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.12.1/SEL/24	Session Date	6/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/ sensory lab evaluation	Session Time	At 10 am	Issuing Date	6/12/2022
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Virgin Olive Oil
Defects Median	Musty: 3
Remarks	----------

4.1.9.1. Sensory Test of Fruit Fall Trees (S2):

Table 9 presents the sensory evaluation conducted at the Fruit Fall 'Trees farm's testing by the Jordan Standards and Metrology Organization. The results of the test showed that the Extra virgin olive oil (EVOO) type has a median defect count of zero and a fruity count of three.

Table 9 Sensory Test of Fruit Fall Trees of oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	22/SEL/23	Sample code/source	Fruit fall tree	Opening Date	6/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.12.1/SEL/23	Session Date	6/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/ sensory lab evaluation	Session Time	At 10 am	Issuing Date	6/12/2022
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Extra Virgin Olive Oil Fruity: 3
Defects Median	----------
Remarks	----------

4.1.9.3. Sensory Test of Anzeh (S3)

Table 10 displays the sensory evaluation conducted at Anzeh Farm, revealing that it is Extra Virgin Olive Oil (EVOO) with a median of zero for defects and a fruity score of 4.

Table 10 the sensory test of Anzeh farm oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	22/SEL/26	Sample code/source	Anzah	Opening Date	6/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.12.1/SEL/26	Session Date	6/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/ sensory lab evaluation	Session Time	At 10 am	Issuing Date	6/12/2022
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Extra Virgin Olive Oil Fruity: 4
Defects Median	----------
Remarks	----------

4.1.9.4. East Bani Zaid (2) (S4)

Table 11 presents the sensory evaluation conducted at East Bani Zaid Farm, indicating that the oil is classified as Extra Virgin Olive Oil (EVOO) with a median of zero for defects and a fruity score of 2.

Table 11 the sensory test of East Bani Zaid farm of oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	22/SEL/22	Sample code/source	East Bani Zaid (2)	Opening Date	1/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.10.1/SEL/22	Session Date	1/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/	Session Time	At 2 Pm	Issuing Date	1/12/2022

	sensory lab evaluation				
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Extra Virgin Olive Oil Fruity:2:0
Defects Median	----------
Remarks	----------

4.1.9.5. Sensory Test of Farkh (S5)

Table 12 presents the sensory evaluation conducted at Farkh farm, indicating that the oil is classified as Extra virgin olive oil (EVOO), with a median of zero defects and a fruity score of 3:5

Table 12 the sensory test of Farkh farm of oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	22/SEL/20	Sample code/source	Farkah	Opening Date	1/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.10.1/SEL/20	Session Date	1/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/ sensory lab evaluation	Session Time	At 2 Pm	Issuing Date	1/12/2022
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Extra Virgin Olive Oil Fruity:3:5
Defects Median	----------
Remarks	----------

4.1.9.6. Sensory Test of AL-yamoon (S6)

Table 13 presents the sensory evaluation conducted at Alyamoon farm, indicating that the oil is classified as virgin olive oil (VOO), with a median defects: Rancidity :2.5, musty:1, fruity :1

Table 13 the sensory test of Alyamoon farm of oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	22/SEL/19	Sample code/source	AL-yamoon	Opening Date	1/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.10.1/SEL/19	Session Date	1/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/ sensory lab evaluation	Session Time	At 2 Pm	Issuing Date	1/12/2022
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Virgin Olive Oil fruit: 1
Defects Median	Rancid 2.5 musty: 1.0
Remarks	----------

4.1.6.7. Sensory test of Masha(2) (S7)

Table 14 presents the sensory evaluation conducted at Masha farm, indicating that the oil is classified as virgin olive oil (VOO), with a median defects: musty:2, fruitiness :2.5

Table 14 the sensory test of Masha farm of oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	23/SEL/20	Sample code/source	Masha	Opening Date	1/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.10.1/SEL/20	Session Date	1/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/ sensory lab evaluation	Session Time	At 10 am	Issuing Date	1/12/2022
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Virgin Olive Oil fruitiness:2.5
Defects Median	musty: 2.0
Remarks	----------

4.1.9.8. Sensory Test of Kofer Qadom (S8)

Table 15 presents the sensory evaluation conducted at Kofer Qadom farm, indicating that the oil is classified as virgin olive oil (VOO), with a median defects: musty: 3

Table 15 the sensory test of Kofer Qadom farm of oil samples

Standard No:	COI/T.20/DOC.NO 15/REV.10,2018	Sample Type	Olive Oil	Receiving Date	1/12/2022
Test report NO	22/SEL/25	Sample code/source	Kofer Qadom	Opening Date	6/12/2022
Customer:	Alreef fair trade	Sample code/sensory Lab	22.12.1/SEL/25	Session Date	6/12/2022
Testing Lab:	Jordan Standards and Metrology Organization/ sensory lab evaluation	Session Time	At 10 am	Issuing Date	6/12/2022
Description Of Sample :the sample was received in an airtight package					
Test Method: sensory evaluation					

Results:

Oil Classification	Virgin Olive Oil
Defects Median	musty: 3.0
Remarks	----------

Table 16 summarizes olive oil grading of the different samples according to the sensory evaluation for oil samples.

Table 16 Olive oil grading according to the sensory evaluation for oil samples

Cultivars	Defects	Fruity	Oil Classification
Qarawat Bani Zaid (S1)	3.0	0.0	VOO
Fruit Fall trees (S2)	0.0	3.0	EVOO
Anzeh (S3)	0.0	4.0	EVOO
East Bani Zaid (S4)	0.0	2.0	EVOO
Farkh (S5)	0.0	3.5	EVOO
Alyamoon (S6)	3.5	1.0	VOO
Masha (S7)	2.0	2.5	VOO
Kofer Qadom (S8)	3.0	0.0	VOO

EVOO is extra virgin olive oil, VOO is virgin olive oil

4.1.10 Pearson Correlation with Oil Quality Parameters

Pearson correlations among quality parameters of olive oil was performed and results are shown in Table (4.2)

Table 4.2 Pearson correlations among quality parameters of olive oil

	PV	AA	TPC	TFC	K232	K270	Defects	Fruity
Acidity	-0.725***	-0.974***	-0.790***	0.687**	0.785***	0.610**	0.210	-0.440*
PV		0.847***	0.896***	-0.647**	-0.873***	-0.835***	0.113	0.182
AA			0.986***	-0.808***	-0.999***	-0.999***	-0.206	0.491**
TPC				-0.776***	-0.987***	-0.950***	-0.151	0.453*
TFC					0.846***	0.811***	0.373	-0.739***
K232						0.969***	0.240	-0.538**
K270							0.222	-0.515*
Defects			-					-0.807***

Results are expressed as mean ± SD of three sample replicates. Significance level: ***P < 0.001; **P < 0.01; *P < 0.05; NS = not significant. acidity: P < 0.05; Peroxide value: P < 0.05; K232:P < 0.05; K270: P < 0.05; TPC: P < 0.o5, TFC: P < 0.05; AA: P < 0.o5 .

Pearson correlations show that acidity is significantly and positively correlated with TFC, (0.687**), K232 (0.785***) and K270 (0.610**). Acidity is significantly negatively correlated with PV (-0.725***), AA (-0.974***), TPC (-0.790***) and fruity (-0.440*). While Acidity was found not significantly correlated with sensory defects (0.210). Peroxide value is positively and significantly correlated with AA (0.847***) and TPC (0.896***), and negatively and significantly correlated with TFC (-0.647**), K232 (-0.873***), and K270 (-0.835***). Anti-oxidant activity is positively correlated with TPC (0.986***) and fruity only (0.491**), while its correlation with TFC (-0.808***), K232 (-0.999***), and K270 (-0.999***) was significantly negative.

Total phenolic content is positively correlated with fruity (0.453*) and negatively correlated with TFC (-0.776***), K232 (-0.987***) and K270 (-0.950***).Total flavonoid contents is positively correlated with both extinction coefficients K232 (0.847***) and 270 (0.811***) and negatively correlated with fruity. The extinction coefficient K232 is positively correlated with K270 and negatively correlated with fruity.K272 is negatively correlated with fruit, and stage of defects was negatively correlated with fruity (-0.739***).

Pearson correlations reveal significant and positive associations between acidity and TFC, K232, and K270. Conversely, acidity exhibits significant negative correlations with PV, AA, TPC, and fruity notes. However, no significant correlation was observed between acidity and defects..

Pearson correlations reveal significant and positive associations between acidity and TFC, K232, and K270, while acidity demonstrates significant negative correlations with PV, AA, TPC, and fruity notes; however, no significant correlation was observed between acidity and defects. Peroxide value exhibits significant positive correlations with AA and TPC, while displaying significant negative correlations with TFC, K232, and K270. Antioxidant activity is positively correlated solely with TPC and fruity notes, whereas it exhibits significant negative correlations with TFC, K232, and K270. Total phenolic content shows positive correlations with fruity notes and negative correlations with TFC, K232, and K270. Total flavonoid content demonstrates positive correlations with both extinction coefficients (K232 and 270) and negative correlations with fruity notes. The extinction coefficient K232 shows positive correlations with K270 and negative correlations with fruity notes, while K270 exhibits negative correlations with fruity notes, and the stage of defects is negatively correlated with fruity notes.

4.2 Discussion

The chemical composition of olive oil is closely linked to its sensory, nutritional, and qualitative attributes. Extra virgin olive oil (EVOO) holds significant value due to its high oleic acid content and the presence of phenolic compounds, which contribute to its distinctive aroma and antioxidant properties (Servili et al, 2004), . This dual benefit, encompassing both commercial appeal and health advantages, underscores its importance in various contexts.

However, the quality and stability of olive oil can be compromised by lipid oxidation, which is a primary concern. This process leads to rancidity, resulting in off-flavors and a reduction in the oil's nutritional value. Such deterioration not only undermines consumer satisfaction but also poses health risks. Thus, ensuring rigorous quality control measures throughout production and storage is imperative to meet consumer expectations and uphold the integrity of olive oil products(Caballero ,1994)

Olive oil is assessed through analytical parameter measurements, with established limit values. Alongside sensory evaluation, key criteria for evaluating olive oil quality in

commercial transactions include acidity, peroxide value, total flavonoid content, antioxidant activity, K232, K270, and total phenolic content. The International Olive Oil Council (IOOC) and European Communities Legislation (EC) define the identity characteristics of olive oil by specifying analytical methods and standard limit values for quality. These parameters have been evaluated for the Palestinian olive oil samples under investigation (IOC,2018)

The quality of parameters such as peroxide value (PV), acidity, Ultra violet (UV) absorbance values (K232 and K270) and organoleptic characteristics (odor, taste and color) for olive oils in order to improve product quality, expand international trade, and raise its consumption. Extra virgin and virgin olive oils are distinguished by their organoleptic qualities and chemical testing, which indicate the culinary quality and commercial value of the oil (Rwoth, 2011). Extra virgin olive oil is the best grade; it must have a free acidity of less than 0.8%, a peroxide value of no more than 20 milliequivalent O2 kg-1 oil, and a distinct flavor that is representative of the fruit it is made from. It must also contain zero defects and more than zero positive attributes, as determined by a certified taste panel (Rwothomio, 2011).

4.2.1. Acidity

Analysis of free fatty acid content and acidity revealed that all samples initially fell within the acceptable range of 0.8% (grams of oleic acid per 100 grams of oil). Among the farms, Anzeh (S3) recorded the lowest acidity, while Qarwat Bani Zaid (S1) had the highest. Acidity, indicative of hydrolysis and the presence of oleic acid (or free fatty acid), serves as a marker for oil quality, with lower acidity suggesting superior quality and careful olive processing. Notably, Anzeh Farm (S3) exhibited the lowest acidity, signifying minimal hydrolysis (or free fatty acid percentage). Furthermore, according to the sensory evaluation conducted by the panel test, Qarwat Bani Zaid (S1) and Anzeh (S3) farms were classified as Virgin Olive Oil (VOO) and Extra Virgin Olive Oil (EVOO), respectively, with Qarwat Bani Zaid having the highest acidity and Anzeh the lowest .

For extra virgin classification, are determined by titrating the oil with potassium hydroxide to assess its acidity. While acidity values serve as a fundamental criterion for categorizing olive oil into different grades, some researchers argue that it may not be the most accurate measure of olive oil quality. Acidity levels also reflect the oil's stability and susceptibility to rancidity. Increased acidity can result from hydrolytic rancidity, attributed to the presence of water in the oil and the catalytic action of lipase, which is often produced by microbes. (Mignan et al,

2012).This breakdown leads to the partial conversion of triglycerides into glycerol and free fatty acids, contributing to higher acidity levels.

Acidity : type of alteration Hydrolysis. Alteration Factors Mold, Fermentation ,Maturity too much advanced, Olive fly, bad storage condition such as temperature and humidity, it measure the percentage of free fatty acids Extra Virgin Maxi 0.8 %, Virgin Max 2 %.(Angerosa et al, 2006)

The average acidity% values of our samples were ranged from 0.32 – 0.48 (% as oleic acid). According to Codex Alimentarius Commission (2001) Acidity maximum % of virgin olive oil (expressed as oleic acid) equals 3.3, so our acidity% results showed that our oil in EVOO category. Amarna et al (2011) showed that average free acid value of their olive oil samples was 1.22%. Essiari & Chimi (2014) reported that acidity of olive oil was a function of geographical area and found that oils produced from olives grown on calcareous soils have a lower acidity than those obtained from olives cultivated on clay soils, while Desouky et al (2009) noticed that the acidity increased during maturation progress, especially in black stage, which had the highest acidity percentage and the reason according to Bengana et al (2013), Arslan & Schreiner (2012) and Youssef et al (2010) that free acidity increased slightly as fruit ripening progress, as during the olive ripening there is progressive activation of lipolytic activity and olives are more sensitive to pathogenic infection and mechanical damage, which result in oils with higher acidity values, while El Sohaimy et al (2016) reported that the oil showed an unstable trend in the relation between the acid value and ripening stages and concluded that the reddish ripening stage was the best stage for harvesting of the olive fruits to get the high quality of oil.

Hydrolysis of Fresh olive oil this because alteration Factors may be Mold, Fermentation ,Maturity too much advanced, Olive fly, bad condition such as high temperature and humidity, all factors causes hydrolysis before harvesting(. Angerosa et al, 2006)

Elevated Acidity suggest aged or Mold, Fermentation ,Maturity too much advanced, Olive fly, bad condition such as high temperature and humidity, all factors causes hydrolysis potentially adulterated extra virgin olive oil, possibly comprising a blend of fresh and previous harvest oils. Higher quality extra virgin olive oils typically exhibit lower Acidity with limitations for extra virgin olive oil Maxi 0.8 %, virgin olive oil Max 2 % valuable criteria for assessing olive oil quality (Mignan et al, 2012).

4.2.2 Peroxide Value (PV)

The quality of oil is primarily assessed through the peroxide value (PV), which measures the total peroxides present in olive oil (measured in meq. O2 kg^-1 oil). The standard method for determining PV involves titrating the iodine released from potassium iodide by the oil's peroxides. Essentially, PV indicates the quantity of active oxygen bound by the oil, providing insight into the extent of lipid peroxidation and reflecting the hydroxyl peroxide value. With an upper limit of 20 meq. O2 kg^-1 oil, a higher PV suggests increased degradation due to oxidation. However, levels exceeding 10 may indicate less stable oil with a shorter shelf life. Various free radicals and oxygen species, including singlet oxygen, play roles in lipid oxidation processes(Bertoncini & Testa, 2014; Amelio, 2019)

Unsaturated fatty acids and oxygen are the primary substrates involved in these reactions. The free radical mechanism of lipid oxidation is commonly explained using a three-stage chain reaction, comprising initiation, propagation, and termination steps. The initiation process within fatty acid molecules begins with the abstraction of a hydrogen atom adjacent to a double bond. This is catalyzed by factors such as light, heat, or metal ions, resulting in the formation of a free radical. Due to the high activation energy of fatty acid molecules, direct reactions with oxygen are infrequent. However, when ambient oxygen reacts with the resulting free radical, an unstable peroxy radical is formed. Subsequently, to generate a hydroperoxide, a free radical may abstract a hydrogen atom from another unsaturated fatty acid(Bertoncini & Testa, 2014).

The propagation phase of autoxidation marks the segment of the chain reaction instigated by a novel alkyl free radical, leading to further oxidation. The merging of two radical species can yield non-radical products, effectively halting the chain reaction. Within the propagation phase of the autoxidation process, an induction period ensues, characterized by minimal hydroperoxide formation. The rate of oxidation is influenced by the degree of unsaturation present in fatty acids(Amelio, 2019).

Consequently, oils containing high levels of polyunsaturated fatty acids may encounter instability issues. Breakdown products of hydroperoxides, such as alcohols, aldehydes, ketones, furans, esters, lactones, and hydrocarbons, often result in undesirable off-flavors. Moreover, they can interact with other dietary components, potentially altering their nutritional and functional attributes. Peroxide Values Type of alteration Oxidation. Alteration factors maturity too much advanced, Frost, Ageing, Ventilation, Light, Heat. It measure The hydro peroxides (-OOH) (Barnum, 1977).

Initial peroxide values (PVs) for all samples ranged from 20 meq/kg or lower. PV, a crucial quality indicator, quantifies the total peroxides in olive oil, expressed as milliequivalent of O2 per kilogram of oil. Qarwat Bani Zaid Farm (S1) exhibited the highest PV, while Anzeh Farm (S3) recorded the lowest. A higher PV indicates a greater degree of oxidation-related deterioration .Because alteration factors of Peroxide Values maturity too much advanced, Frost, Ageing, Ventilation, Light, Heat , all cause oxidation to fresh olives fruit before harvesting.

According to sensory evaluations conducted by a panel test, Qarwat Bani Zaid Farm (S1) is categorized as Virgin Olive Oil (VOO) and has the highest PV. Conversely, Anzeh Farm (S3) is classified as Extra Virgin Olive Oil (EVOO) and displayed the lowest PV.

Elevated Peroxide values suggest aged or Frost, Ageing, Ventilation, Light, Heat , all cause oxidation potentially adulterated extra virgin olive oil, possibly comprising a blend of fresh and previous harvest oils. Higher quality extra virgin olive oils typically exhibit lower Peroxide Values, with limitations for extra virgin olive oil , virgin olive oil Maxi 20.0 milliequivalents O2 kg -1 oil valuable criteria for assessing olive oil quality (Das & Gezici, 2018)

Peroxide value for our samples range between 5.2- 9.8 (milliequivalents O_2 kg^{-1} oil).

According to Codex Alimentarius Commission (2001) Peroxide value for Virgin olive oil (in milliequivalents O_2/kg oil) ≤ 20.

Peroxide values of our samples when compared with the limits fixed in the Codex Alimentarius Commission (2001), it can be seen that all the samples analysed comply with the st andard and can therefore be graded as extra virgin ($PV \leq 20$).

Amarna et al (2011) showed that average peroxide value of their olive oil samples was 19.1 meq O_2/kg which was in agreement with our results.

Fakhri & Qadir (2011) reported that in comparison between the specific gravity and IV, it was suggested that as the sp.gr. is lower represent that the IV is higher values, also when the PV is high and has abnormal range value, the IV is also high and has abnormal range, but not vice versa, while Mailer et al (2005) reported that peroxide value was shown to be higher in young olives than later in the season although that was not understood and also it was influenced by years ($p = 0.010$ to < 0.001), but from another point of view Essiari et al (2014) reported that there were a clear effect of oil extraction immediately after the olives had been harvested and they emphasize the effect of geographical origin and year on peroxide values, while El Sohaimy et al (2016) reported that peroxide values increased significantly with

developing in the ripening process for the examined varieties of olive fruits which was in agreement with Desouky et al (2009) who remarked that peroxide values in extracted oils in purple as well as in black fruits were significantly higher than those from green fruits, while Rahmani et al (1997) mentioned that peroxide values did not change significantly during the maturation periods.

4.2.3. Total Phenolic Content (TPC)

Phenolic compounds play a vital role in antioxidant activity due to their redox characteristics. The hydroxyl groups present in olive oil extracts facilitate free radical scavenging. The phenolic content of each extract was measured using the Folin-Ciocalteu reagent as a basis (Jerman ,2014).

Extra virgin olive oil stands out as one of the few oils that can be consumed unrefined. This is attributed to its high monounsaturated to polyunsaturated ratio and the presence of natural antioxidants, notably phenolic compounds, carotenoids, and tocopherols, which confer high resistance to oxidative deterioration. Consequently, it retards lipid oxidation and the formation of undesirable volatile compounds(Rodríguez et al., 2015).

The antioxidant activity of phenolic compounds primarily operates during the early stages of autoxidation by scavenging free radicals and chelating metals. However, during storage, the hydrolysis, esterification, and oxidation of oil deplete these minor components. Hence, identifying the small components of olive oil is crucial for assessing its analytical quality and self-defense capability(Manach, 2004).

In the investigated oil samples, Qarwat Bani Zaid Farm (S1) exhibited the highest Total Phenolic Content (TPC) value, while Farkh Farm (S5) had the lowest. According to sensory evaluations by a panel test, Farkh Farm (S5) and Qarwat Bani Zaid Farm (S1) were associated with the lowest and highest TPC for Extra Virgin Olive Oil (EVOO) and Virgin Olive Oil (VOO), respectively.

Total phenolic Content (TPC) for our samples range between 179.3- 217.3

Dağdelen (2016) reported that total phenolic of Edincik Su olive cultivar was found between 159.99 and 189.64 mg gallic acid equivalent/kg, and that was in agreement with our TPC values, while Houshia et al (2014)reported that the total concentration of polyphenol in some samples of Palestinian olive oil from Jerusalem, Tulkarem and Jenin ranges from 150 to 300 mg/kg which are in general lower than our results except Asira Al-Shamaliya particularly

TPC results were in agreement and some results for all farmers in Asira Al-Qibliya in both years 2013 and 2014 and for some farmers in Bayt Jala in both years too.

However, it is possible to find ranges significantly different in the literature, as in the work of Sánchez et al (2007) and Ballus et al (2015) who reported that total phenolic contents range between 1085 and 1406 mg GAE kg^{-1} were found for 39 samples of Picual EVOO in Spain and that was in agreement with some of our results like 1229, 1134 in Salfit in 2013 and 1144 in Surif in 2014. Mailer et al (2005) and Kalogeropoulos& Tsimidou (2014) reported that TPC increased progressively as olives matured and decreased in the final ripening stage,while El Sohaimy et al (2016) emphasized the previous and in addition reported that the higher the moisture content in olive fruit is the less polyphenols levels are, but Servili et al (2004) showed that some factors affect the TPC of olive oil between which cultivar, climate and other environmental factors, harvesting time, the extraction process, the conditions of packing, distribution, and storage and Baiano et al (2014) reported that there a strong positive linear correlation was observed between the phenolic content and antioxidant activity . to indicate a noticeable radical scavenging ability of phenolic compounds.

The variability in phenolic content observed in this investigation may be attributed to factors such as duration, regional disparities, or extraction techniques, which can influence phenolic concentration. Elements like cultivar, climate, harvesting season, and extraction method also impact the TPC of olive oil (Houshia & Qutit, 2014)**.**

4.2.4 . Total Flavonoids Content (TFC)

The antioxidant activity of flavonoids, which are secondary metabolites, is determined by the quantity and position of free OH groups. Pinpointing the exact cause of variation in Total Flavonoid Content (TFC) values between samples is challenging due to various factors. These factors include the growing climate, harvest maturity, olive cultivar, agronomic practices like fertilizer application or irrigation, ripening hormones, and extraction and processing methods, all of which can influence the flavonoid composition of olive oil (Das & Gezici, 2018).

In the oil samples examined in this investigation, Qarawat Bani Zaid Farm (S1) exhibited the highest TFC value, while AL-Yamoon Farm recorded the lowest values. According to sensory evaluations conducted by a panel test, Qarawat Bani Zaid Farm (S1), categorized as Virgin Olive Oil (VOO), had the highest TFC, while Alyamoon Farm (S5), classified as Extra Virgin Olive Oil (EVOO), had the lowest TFC.

The Total flavonoids content values of our study ranged from 21.6-80.5 mg catechin/Kg of oil. El Sohaimy et al (2016) reported that the Total flavonoids content of extracted oil from Manzanilla variety ranged from 61.62±1.74 to 139.43±1.63 mg catechin/Kg, while the flavonoids content of Kalamata oil was varied from 56.33±1.93 to 134.60±0.94 and reported that flavonoids level in early maturation stages washigher than late maturation stages since high levels of total phenolics and flavonoids in the early maturation stages might refer to the accumulation of these compounds in metabolic processes with the maturation developments and in late stages the phenolase enzyme may cause degradation of phenolic compounds and decreasing their concentrations

The variability in flavonoids content observed in this investigation may be attributed to factors such as duration, regional disparities, or extraction techniques, which can influence flavonoids concentration. Elements like cultivar, climate, harvesting season, and extraction method also impact the TFC of olive oil (Das & Gezici, 2018).

4.2.5 Antioxidant Activity(AA) by DPPH

The DPPH assay stands out as the most widely used and user-friendly method for evaluating the efficacy of olive oil extracts in scavenging free radicals. Antioxidants, capable of visually quenching the stable purple-colored DPPH radical and transforming it into a yellow-colored form, are pivotal in this assay. Other techniques employed to assess antioxidant capacity and activity include the oxygen radical absorbance capacity (ORAC), ferric reducing/antioxidant power (FRAP), and total reactive antioxidant potential (TRAP) assays (Wicks et al., 2006).

At the core of the DPPH (2,2-Diphenyl-1-picrylhydrazyl) experiment lies the evaluation of antioxidants' capability to neutralize the stable DPPH radical. The primary types of antioxidants function through either a single electron transfer (ET) mechanism or hydrogen atom donation (HAT) to counteract free radicals. Secondary antioxidants serve to neutralize pro-oxidant catalysts. The decline in UV absorption at 517 nm serves as a straightforward indicator of antioxidant action (Moon and others, 2009).

All the values have high Antioxidant Activity, with slight difference. This result because fresh olive oil samples , it was not affected by the surrounding factors. Qarawt Bani Zaid Farm (S1) exhibited the highest antioxidant activity (AA) value, while AL-Yamoon Farm (S6) recorded the lowest. As the stable organic free radical DPPH interacts with an odd electron, its absorption spectrum band, typically between 515 and 528 nm, diminishes.

The DPPH values of our study ranged from 96.87- 98.37 mg Torolox/kg oil.

Minioti et al (2010) reported that antioxidant capacities determined in the hydrophilic fraction range between 1.29 - 9.95 mM Kg^{-1} for the DPPH method and El Sohaimy et al (2016) and Ninfali et al (2001) reported that olive oil obtained from mid-period of maturation and stored for two weeks had an antioxidant capacity significantly lower than the top level. low AA have been attributed to a range of reasons such as incorrect variety, immature trees, harvesting too early, high fruit moisture contents and poor extraction efficiency. Salvador et al (2001) and Beltran et al (2004) showed that numerous studies in the mediterranean have shown that during the ripening period, oil percentage increases dramatically during early fruit ripeningthen slows as full ripeness approaches and declines slightly as fruit becomes over ripe.

4.2.6. Specific Absorption Coefficients (UV Absorbance Values) K270

The K270 test provides valuable insights into the quality and oxidative status of the oil. By measuring UV absorption at 270 nm, it offers indications of secondary oxidation products, particularly aldehydes and ketones, shedding light on the oil's quality and oxidative changes.

K232 serves as a pivotal indicator of high-quality extra virgin olive oil. Natural aging, improper handling, or overheating during refining may lead to oxidation, compromising oil quality (Kiritsakis et al.2002). Cases of fraud may involve the addition of refined oils to extra virgin olive oil, as dienes and trienes produced during refining also absorb light at 232 and 270 nm. Consequently, a high K270 index could signal potential fraud in extra virgin olive oil.(Kiritsakis et al.2002).

The concentration of conjugated trienes increases with rising K270 values, influenced by substances undergoing secondary oxidation like ketones and aldehydes. The K270 parameter test aids in identifying adulteration, with higher values indicating a greater content of conjugated trienes. Sensory evaluations by a panel test classified Farkh Farm and East Bani Zaid as Extra Virgin Olive Oil (EVOO) and Qarawatt Bani Zaid Farm as Virgin Olive Oil (VOO), with the latter exhibiting the highest K270 value.

Our K_{270} values were between 0.16 -0.21 ($K_{1\%}$/1cm).

According to Codex Alimentarius Commission (2003) the absorbency in ultraviolet at 270 nm for Virgin olive oil ≤ 0.25, so our oil samples are in EVOO category.

Ranalli et al (1996) and Kiritsakis (1998) reported that geographical origin has no significant influence on K_{232} and K_{270} which are basically affected by factors that cause fruit damage such as attacks from olive fruit fly or damage from harvest equipment or during fruit

transportation and storage and said that K values significantly decreased from Intense green stage to black stage but still within standard limit and found that K_{270} was between 0.10 and 0.18 for Manzanilla oil ($p<0.005$). while K_{270} was varied from 0.116-0.140 for Kalamata oil ($p<0.005$), and the obtained results confirmed the high purity and freshness of the oil especially in reddish maturation stage but these findings disagreed with the study of Desouky et al (2009) who reported that the K_{232} or K_{270} values increased significantly from purple to black fruits

Elevated K270 values suggest aged or potentially adulterated extra virgin olive oil, possibly comprising a blend of fresh and previous harvest oils. Higher quality extra virgin olive oils typically display lower K232 and K270 values, with set limitations for extra virgin olive oil at less than 2.5 and 0.22, respectively. K270 serves as a pertinent metric for assessing olive oil quality (Nouros, et al. 1999).

4.2.7. Specific Absorption Coefficients (UV Absorbance Values) K232

This is a more sensitive measure of oxidation, particularly in hot oils used in refining. It quantifies the amount of specific oxidized chemicals resonating at 232 and 268 nanometers (nm) in the ultraviolet spectrum using a spectrophotometer, offering insights into oxidative changes and quality. Specifically, the primary oxidation products, conjugated peroxides, exhibit absorption at 232 nm (Kiritsakis et al. 2002).

K232 serves as a crucial indicator of superior extra virgin olive oil, with oxidation potentially signaling improper handling, overheating during refining, or natural aging. Cases of fraud may involve the addition of refined oils to extra virgin olive oil, as dienes and trienes produced during refining also absorb light at 232 and 270 nm. Hence, elevated K232 and K270 indices could suggest potential fraud in extra virgin olive oil, necessitating peroxide index examination to discern fraud or rancidity(Kiritsakis et al, 1998)

Conjugated dienes' concentrations increase with higher K232 values, influenced by products of oxidation of the conjugated dienes. K232 provides insights into olive oil's age, storage conditions, milling process, and degree of oxidation during manufacturing or storage (Butnariu & Grozea, 2009). The highest K232 value, observed in Qarawat Bani Zaid, indicates a higher conjugated diene content. Sensory evaluations classify Qarawat Bani Zaid Farm (S1) as Virgin Olive Oil (VOO), with the highest K232 value, and Farkh Farm, East Bani Zaid (EVOO), with the lowest. Factors effecting of K232 before harvesting are

Ageing, Ventilation, Light, Heat, Olive fly. K232 hydroperoxides of C18 :2 and conjugated diene decomposition.

Our K_{232} values were between 1.00- 2.20 ($K_{1\%}$/1cm).

According to Codex Alimentarius Commission (2003) absorbency in ultraviolet at 232 nm for Virgin olive oil ≤ 2.60, so our oil samples are in the EVOO category.

Ranalli et al (1996) and Kiritsakis (1998) reported that geographical origin has no significant influence on K_{232} and K_{270} which are basically affected by factors that cause fruit damage such as attacks from olive fruit fly or damage from harvest equipment or during fruit transportation and storage and said that K values significantly decreased from Intense green stage to black stage but still within standard limit and found that K_{232} value was ranged between 1.65 and 2.41. K_{232} was 1.50 -2.17 for Kalamata oil ($p<0.005$).

The obtained results confirmed the high purity and freshness of the oil especially in reddish maturation stage but these findings disagreed with the study of Desouky et al (2009), who reported that the K_{232} or K_{270} values increased significantly from purple to black fruits.

Elevated K232 values suggest aged or potentially adulterated extra virgin olive oil, possibly comprising a blend of fresh and previous harvest olives. Higher quality extra virgin olive oils typically exhibit lower K232 and K270 values, with limitations for extra virgin olive oil set at less than 2.5 and 0.22, respectively. K270 and K232 serve as valuable criteria for assessing olive oil quality(Kiritsakis et al. 2002).

4.2.8 Pearson coefficients between quality parameters : Acidity, PV, TPC, TFC, AA, K232, K270, Defects and Fruity

The indices used to assess olive oil quality are interconnected. Acidity correlated with the K232 value, as both indicate oxidation products. A positive correlation between Acidity and K232 is expected, given K232's measurement of primary oxidation products and Acidity's indication of hydrolysis. Similarly, a positive correlation exists between K270 and acidity, as K270 reflects secondary oxidation products. Also, a positive correlation with Total Flavonoid Content (TFC) is observed, indicating that as acidity increases, TFC, K232, and K270 also increase due to higher hydrolysis. Conversely, acidity exhibits significant negative correlations with Peroxide Value (PV), reflecting oxidation, and Antioxidant Activity (AA), which limits oxidation.

Peroxide Value (PV) serves as a crucial guide of oil quality, measuring the degree of lipid peroxidation. It correlates positively with AA, which inversely affects oxidation, and negatively with Total Phenolic Content (TPC), TFC, K232, and K270, indicating higher degradation with increased PV. Phenols and PV show a similar negative correlation, aligning with the expectation that decreased phenolic content leads to higher PV. However, no significant correlated exists between PV and defects.

Antioxidant Activity (AA) is positively correlated with Total Phenolic Content (TPC), indicating increased phenolic content boosts AA. It also correlates positively with fruity notes, signifying higher olive oil quality. Conversely, AA exhibits negative correlations with TFC, K232, and K270, reflecting the adverse effects of oxidation. No significant correlation is found between AA and defects.

Total Phenolic Content (TPC) correlates positively with fruity notes, indicating higher-quality olive oil, and negatively with TFC, K232, and K270, aligning with the expectation of decreased phenolic content with oxidation. Similarly, Total Flavonoid Content (TFC) shows positive correlations with extinction coefficients (K232 and K270) and negative correlations with fruity notes, without significant correlation with defects.

Extinction coefficients K232 and K270 exhibit positive correlations with each other and negative correlations with fruity notes. K272, however, shows negative correlations with fruity notes, indicating lower quality when K232 and K270 increase. No significant correlations exist between K232, K270, and defects, while the stage of defects correlates negatively with fruity notes.

4.2.9 Sensory Evaluation

Extra virgin olive oil (EVOO) stands out as the pinnacle of olive oil quality, adhering to stringent chemical and sensory standards. Renowned for its exquisite taste and unparalleled health benefits, EVOO undergoes rigorous evaluation based on sensory attributes like mildness, fruitiness, and aroma. This grade of olive oil is prized for its distinct fragrance and vibrant green, fruity notes, a result of enzymatic reactions during extraction. Volatile compounds, including aldehydes, alcohols, esters, and ketones, contribute to its aroma profile, while non-volatile phenolic compounds enhance its bitterness and pungency. (International Olive Council, 2015a; Bertoncini & Testa, 2014; Amelio, 2019)

Factors such as soil composition, climate, and extraction methods influence the sensory profile, yet enzymatic activity primarily dictates volatile compound level (Bertoncini & Testa, 2014). Chemical indices like absorption coefficients K232 and K270, coupled with sensory evaluations, determine olive oil quality. Total flavonoid concentration emerges as a critical chemical marker, impacting quality, while acidity and sensory defects play lesser roles.

Total phenolic content significantly correlates with antioxidant activity, underscoring its importance in enhancing olive oil quality. Sensory evaluation alone might be insufficient for accurate quality assessment, necessitating complementary chemical analysis. K270 and K232 absorption coefficients prove particularly sensitive in determining olive oil quality, while peroxide value shows no significant association with EVOO. Strong correlations between antioxidant activity, total phenolic content, and sensory defects highlight their pivotal roles in defining olive oil quality.

CHAPTER FIVE

Conclusion

Extra virgin olive oil (EVOO) stands as the pinnacle of olive oil quality, subject to stringent chemical and sensory regulations. Renowned for its superior taste and health benefits, EVOO represents the highest grade among olive oil varieties. Despite being the most challenging to produce, the effort is well justified by its exceptional quality.

The criteria for defining extra virgin olive oil include a median score of zero for faults and a positive median score for fruitiness. Antioxidant activity and polyphenol concentration exhibit strong positive correlations with fruitiness, underscoring their significance in olive oil quality assessment. Moreover, Total flavonoid concentration emerges as a critical chemical marker, impacting quality, while acidity and sensory defects play lesser roles.

the absorption coefficients K270 and K232 emerge as sensitive chemical indicators for determining fresh olive oil quality, with fruitiness showing a negative correlation with both, while peroxide value shows no significant association with EVOO.

This study highlights the insufficiency of sensory evaluation alone, emphasizing the importance of complementing it with chemical analysis. However, there remains a lack of comprehensive research on Palestinian olive oils, particularly regarding their volatile profiles and organoleptic properties, as well as their correlations with chemical factors.

Recommendations:

Given the limited research on Palestinian olive oils, further investigation is warranted to explore their chemical and sensory characteristics comprehensively. Specifically, future studies should focus on elucidating volatile profiles, organoleptic properties, and correlations between chemical and sensory factors, utilizing robust statistical methods such as Pearson's correlation analysis. This will provide valuable insights into enhancing the quality and market competitiveness of Palestinian olive oils.

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Appendix

Appendix A

Concentration of Gallic acid (ppm)	Absorbance at λ= (765 nm
100	0.132
200	0.426
350	0.830
450	1.070
500	1.199

Table1: Absorbance of different concentration of Gallic acid

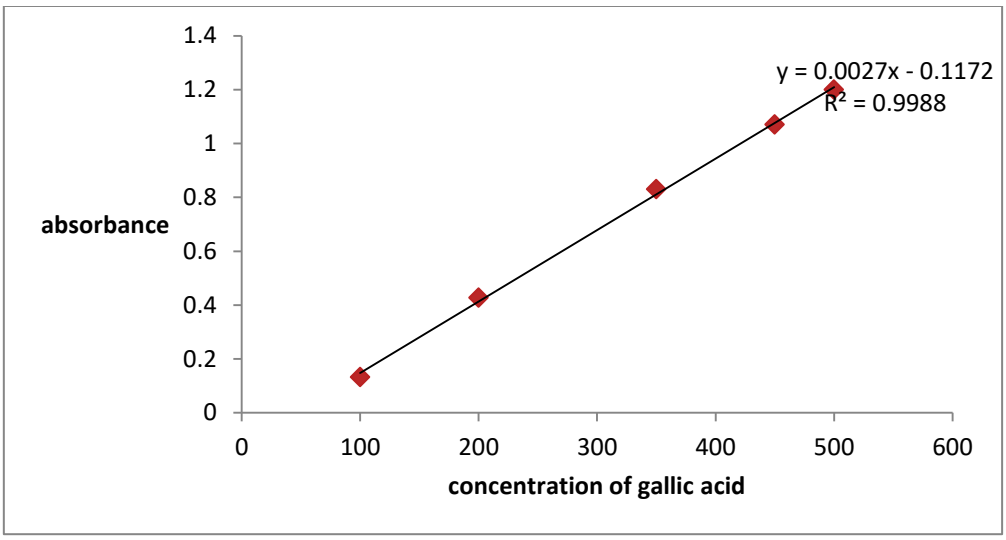

Figure 1: Calibration curve for total phenols content.

Appendix B:

Concentration of catechin (ppm)	Absorbance λ= (510 nm
50	0.255
60	0.282
75	0.353
86	0.396
100	0.496

Table2: Absorbance for different concentration of Catechin.

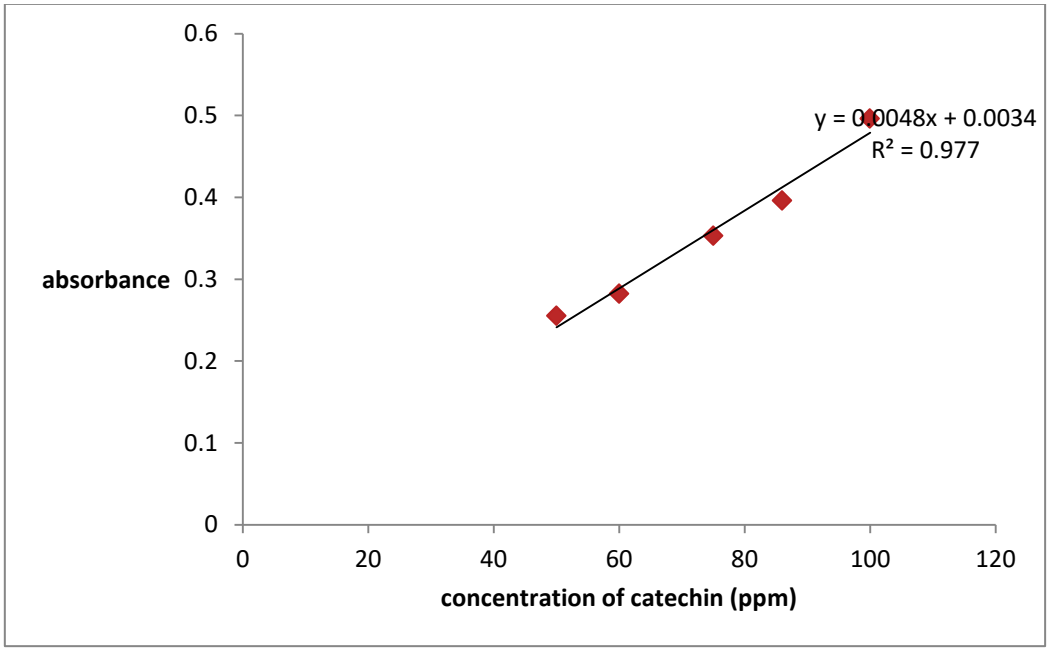

Figure 2: Calibration curve for total flavonoid content. From the calibration curve

Appendix C:

Concentration ofFe^{+2} (mM)	Absorbance λ= (593 nm
2	0.279
2.5	0.299
3	0.400
3.5	0.511

4	0.627
4.5	0.745
5	0.848

Table 3: Absorbance of different concentration of Ferric

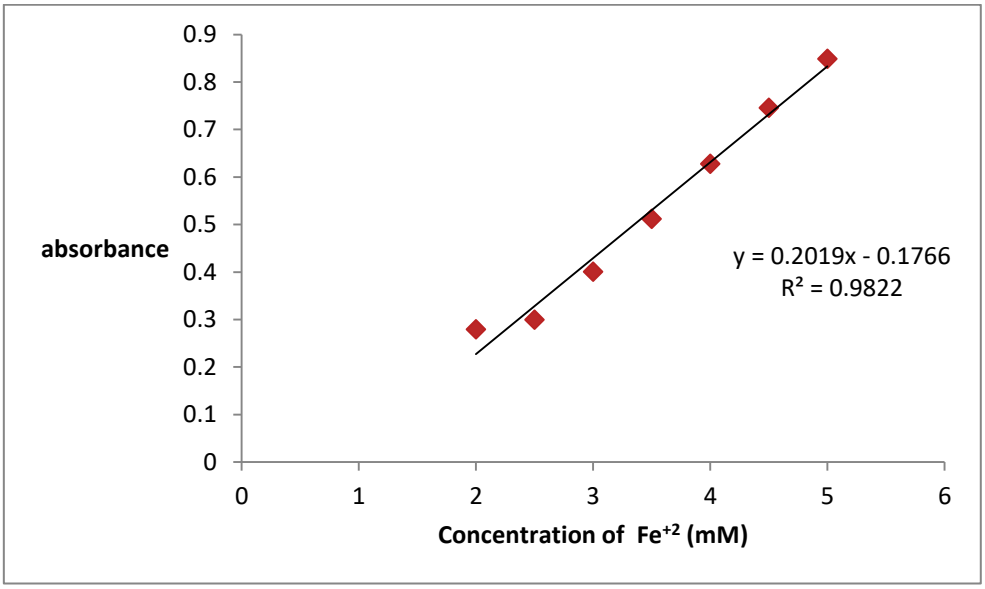

Figure 3: Calibration curve for FRAP antioxidant.

Appendix D:

concentration of trolex	Absorbance at λ= (450nm
20	0.032
40	0.059
60	0.077
80	0.098
100	0.118
120	0.142
140	0.168

Table4: Absorbance for different concentration of trolox

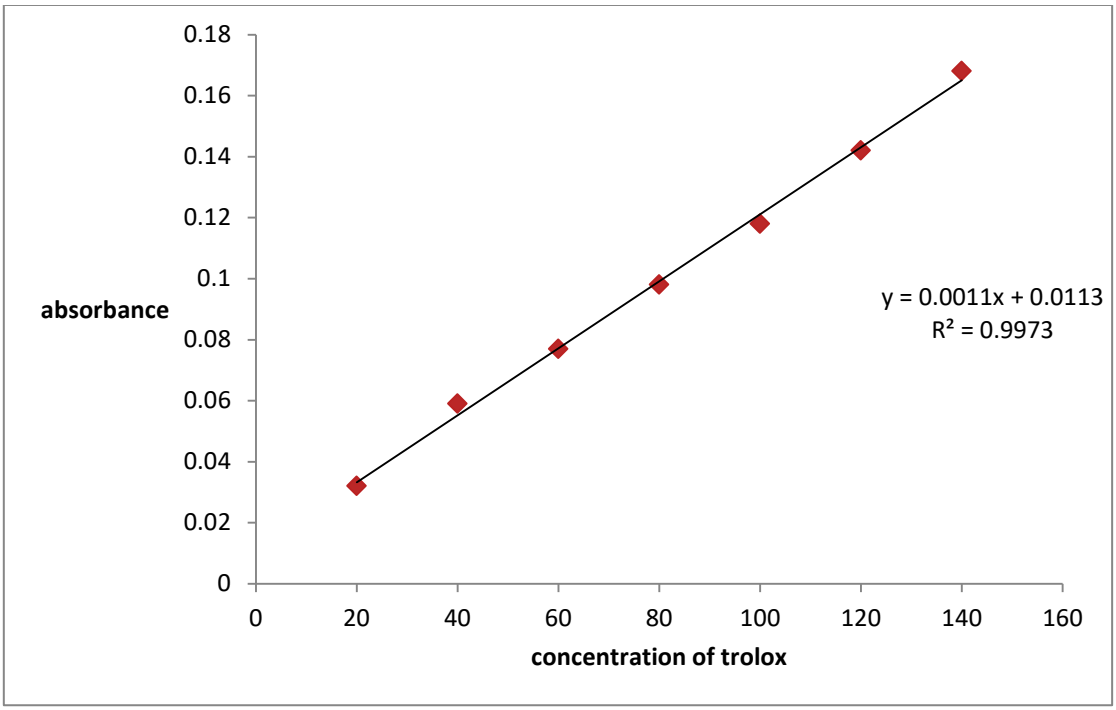

Figure 4: Calibration curve for CUPRAC antioxidant powder

Appendix E:

concentration of Trolex	Absorbance λ= (515 nm
20	0.729
40	0.677
60	0.623
80	0.580
100	0.523
120	0.470

Table 5: Absorbance for different concentration of Trolox

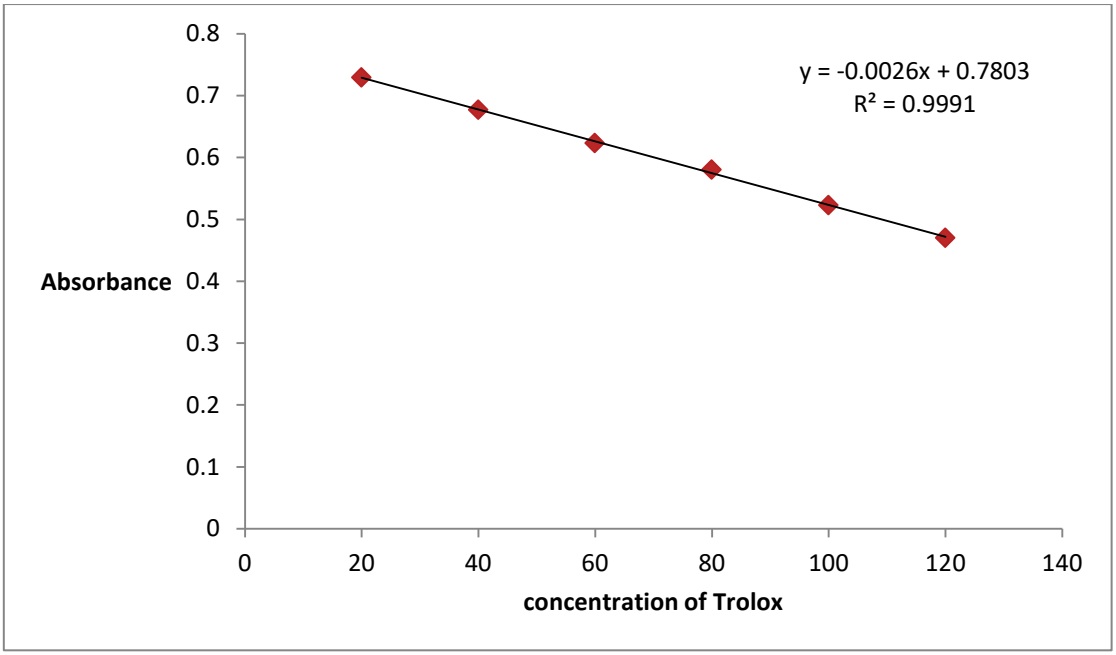

Figure 5: Calibration curve for DPPH

Appendix F

Conc. Ppm	Abs. (734 nm)
5	0.571
10	0.500
15	0.426
20	0.361
25	0.289
30	0.199
35	0.120
40	0.027

Table 6: Absorbance for different concentration of Trolox

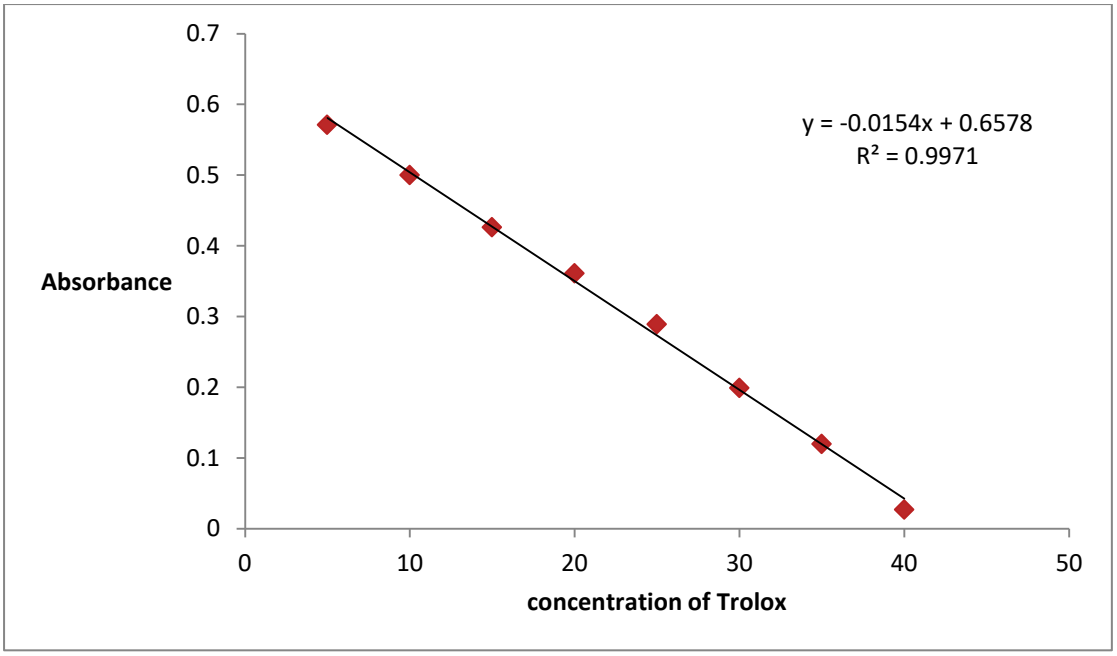

Figure 6: Calibration curve for ABTS.

الملخص

العلاقة بين الخصائص الكيميائية والحسية لزيت الزيتون البكر

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تعتبر فلسطين دولة ناشئة في إنتاج زيت الزيتون البكر، ومع ذلك لم يتم إجراء سوى القليل من الأبحاث حول زيت الزيتون لديها حتى الآن. ومن الجدير بالذكر أن هناك فجوة في الأدبيات المتعلقة بالخصائص الكيميائية والعضوية للزيوت وارتباطات بيرسون بين المعلمات الكيميائية والحسية. تُعرف مجموعة الخصائص التي تحدد مدى جاذبية زيت الزيتون للمستهلكين بجودته، والتي يمكن وصفها أيضًا من وجهات نظر تجارية أو غذائية أو حسية. يستمد زيت الزيتون البكر الممتاز (EVOO) فوائده الغذائية من المكونات الثانوية التي تركز على مضادات الأكسدة والمحتوى العالي من حمض الأوليك. ولذلك، فإن عوامل الجودة هذه تدعم تسعير زيت الزيتون ومتطلبات استهلاكه مقارنة بزيوت الطعام الأخرى.

في هذه الدراسة، تم توفير زيت الزيتون المستخدم مباشرة من قبل المزارعين في معاصر زيت الزيتون. تم جمع ثماني عينات من كل مزارع، وتم إغلاقها في حاويات محكمة الإغلاق، وتخزينها في الثلاجة لحين التحليل. وتم جمع العينات في ظل ظروف مختلفة في أواخر أكتوبر ٢٠٢٢. هدف هذا العمل إلى تقييم المعايير الكيميائية، بما في ذلك محتوى الفينول الكلي (TPC)، ومحتوى الفلافونويد الكلي (TFC)، والنشاط المضاد للأكسدة (AA)، والحموضة، وقيمة البيروكسيد (PV)، بالإضافة إلى الجودة الحسية لثمانية عينات من زيت الزيتون من مزارع مختلفة في فلسطين: مزرعة قراوة بني زيد (S1)، مزرعة مسحة (S2)، عنزة (S3)، فرخ (S4)، شرق بني زيد ٢ (S5)، أشجار الفاكهة المتساقطة ١ (S6)، اليامون (S7)، وكفر قدوم (S8). تم إجراء التقييم الحسي من قبل لجنة معتمدة من قبل هيئة المواصفات والمقاييس الأردنية كمجلس الزيتون الدولي (IOC). كما تم تحليل الارتباط بين التقييمات الكيميائية والحسية. لكي يتم تصنيف زيت الزيتون على أنه بكر ممتاز، يجب أن يكون متوسط درجة العيب فيه صفرًا أو متوسط درجة أحد عيوب زيت الزيتون الخمسة (المتعفن، والعفن، والزنخ، والخل الخمري وغيرها التي يتم إدراكها بأكبر قدر من الشدة على أنها صفر، ومتوسط درجة الفاكهية أعلى من الصفر. إن نشاط مضادات الأكسدة وتركيز البوليفينول، وكلاهما له ارتباط إيجابي قوي بالفاكهة، يدلان بشكل كبير على جودة زيت الزيتون. علاوة على ذلك، تم تحديد معاملي الامتصاص K270 وK232 على أنهما الاختبارات الكيميائية الأكثر حساسية لتحديد جودة زيت الزيتون الطازج.

ترتبط السمات الفاكهية سلبًا بقيم K270 وK232. كما ترتبط السمات الفاكهية سلبًا بالعيوب. تحدد المؤشرات الكيميائية مثل معامل الامتصاص K232 و K270، إلى جانب التقييمات الحسية، جودة زيت الزيتون. يظهر تركيز الفلافونويد الكلي كعلامة كيميائية حاسمة تؤثر على الجودة، في حين تلعب الحموضة والعيوب الحسية أدوارًا أقل. يرتبط محتوى الفينول الكلي بشكل كبير بنشاط مضادات الأكسدة، مما يؤكد أهميته في تحسين جودة زيت الزيتون. أثبتت معامل الامتصاص K270 و K232 حساسيتها بشكل خاص في تحديد جودة زيت الزيتون، في حين لا تظهر قيمة البيروكسيد أي ارتباط كبير مع زيت الزيتون البكر الممتاز.

تسلط الارتباطات القوية بين نشاط مضادات الأكسدة ومحتوى الفينول الكلي والعيوب الحسية الضوء على أدوارها المحورية في تحديد جودة زيت الزيتون. التقييم الحسي وحده غير كافٍ لتقييم الجودة بدقة، مما يستلزم تحليلًا كيميائيًا

تكميليًا. تشمل معايير تعريف زيت الزيتون البكر الممتاز درجة متوسطة صفرية للعيوب ودرجة متوسطة إيجابية للفاكهة. تظهر النشاط المضاد للأكسدة وتركيز البوليفينول ارتباطات إيجابية قوية مع الطعم الفاكهي، مما يؤكد أهميتها في تقييم جودة زيت الزيتون. علاوة على ذلك، تظهر معامل الامتصاص K270 وK232 كمؤشرات كيميائية حساسة لتحديد جودة زيت الزيتون الطازج، حيث تظهر الطعم الفاكهي ارتباطًا سلبيًا بكليهما.

تسلط هذه الدراسة الضوء على عدم كفاية التقييم الحسي وحده، مؤكدة على أهمية استكماله بالتحليل الكيميائي. ومع ذلك، لا يزال هناك نقص في البحث الشامل حول زيوت الزيتون الفلسطينية، وخاصة فيما يتعلق بملفات مكوناتها الثانوية وخصائصها الحسية، فضلاً عن ارتباطها بالعوامل الكيميائية.