An-Najah National University

Faculty of Graduate Studies

Analysis of Palestinian Olive Oil of Different Storage Ages by Fluorescence Spectroscopy Technique

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Dedication

This thesis is dedicated to my husband Mr. Omar Abu Subha, my children, my parents, my sisters and brothers, with love and respect.

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I would like to express my sincere appreciation to my supervisor Prof. Dr. Issam Rashid Abdelraziq for his guidance, support and continual encouragement. I will always be thankful for his wisdom and knowledge; it has been an honor to work with him. I would like to thank my co-supervisor Dr. Mohammed Abu-Jafar for his cooperation and valuable suggestions which helped me to complete this work.

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الاقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Analysis of Palestinian Olive Oil of Different Storage Ages by Fluorescence Spectroscopy Technique

أقر بأن ما اشتملت عليه هذه الرسالة, انما هو نتاج جهد الباحث نفسه, باستثناء ما تمت الاشارة اليه حيثما ورد, وأن هذه الرسالة ككل, أو أي جزء منها لم يقدم من قبل لنيل أي درجة علمية أو بحث علمي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:** جيرن معروف تركي أبو صنوبر

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Date: **التاريخ:** ٢٠١٦/٥/٣م

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List of Abbreviation

Symbol	Abbreviation
cP	Centipoise
cSt	Centistokes
EVOO	Extra Virgin Olive Oil
FFA	Free Fatty Acids
Fig.	Figure
IOOC	International Olive Oil Council
N	Newton
P	Poise
Pa	Pascal
R^2	Coefficient of Determination
RPM	Revolution Per Minute
VOO	Virgin Olive Oil
S_1	Allar
S_2	Saida
S_3	Yasid
P	Density
η_D	Dynamic Viscosity
η_K	Kinematic Viscosity
G	Gram
J	Joule
S	Second
$\lambda_{ex.}$	Excitation wavelength
Nm	Nanometer
WLF	Williams-Landel-Ferry
$\lambda_{em.}$	Emission wavelength
UV-VIS-NIR	Ultraviolet-Visible-Near Infrared
$\lambda_{abs.}$	Absorption wavelength

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Abstract

This work focuses on the effect of storage age of Palestinian olive oil on the emission and absorption wavelengths using the fluorescence spectroscopy technique. In addition, the effect of storage age of olive oil on the physical properties: viscosity, refractive index, acidity, and mass density are studied.

The results of the emission and absorption spectra of olive oil samples at different storage ages give indication about how the amounts of the fluorescent components of olive oil affected by storage age. It was noticed that all vitamin E components, chlorophylls and phenolic compounds decrease as the storage age increases.

The viscosity, refractive index, acidity (FFA%) and mass density for the sample collected from Saida (S_2) of 5 years storage age at 25^{o}C are found to be 58.9 cP, 1.4672, 2.98% and 0.90922 g/cm^3, respectively.

The viscosity, refractive index and mass density of olive oil samples at different storage ages decrease as the storage age increases, whereas the acidity increases as the storage age increases. The measured viscosity, refractive index, acidity and mass density of olive oil samples at 5 years storage ages agree with the standard values. The olive oil of storage age less than 5 years is considered as an edible olive oil.

The Palestinian standard values of olive oil for viscosity, refractive index, acidity and mass density are regarded as; 84.0 cP – 63.6 cP, 1.4677 – 1.4705, 0.8 % - 3.3 % and 0.910 g/cm^3 – 0.916 g/cm^3, respectively (PS188, 1997).

Chapter One

Introduction

1.1 Olive Oil

The Mediterranean diet is recommended as a food model in order to prevent cardiovascular disease and certain types of cancerous diseases. Olive oil is main ingredient of the Mediterranean diet. Its special nutritional properties depend on the presence of monounsaturated fatty acids and unsaponifiable materials (Šarolić *et al.*, 2014).

Olive oil quality is affected by olive variety, ripeness, climate, irrigation, extraction methods and storage conditions (Gharbi *et al.*, 2015). In order to lower the loss in phenol during storage, olive oil should filled in glass bottles and kept in dark place, if the place is illuminated, high-density polyethylene (HDPE) gallons is the best (Afaneh *et al.*, 2013).

Olive oil composed of:

- Monounsaturated fatty acids are mainly oleic, linoleic and palmitic.
- Unsaponifiable materials are aliphatic and triterpene alcohols.
- Mono- and diglycerides, volatile compounds and carotenes.
- Sterols (mainly β-sitosterol), fatty alcohols, aroma compounds and waxes.
- Fluorophores:

1. Vitamin E consists of α-, β-, δ-, γ- tocopherol and α-, β-, δ-, γ- tocotrienol. Tocopherols are fat soluble antioxidants valued for their

ability to inhibit oxidation in food (Kamal-Eldin and Appelqvist, 1996).

2. Chlorophylls consist of chlorophyll a and b, Pheophytin a and b and Pheophorbide a. Chlorophyll is one of the main contributors to the colour of virgin olive oils (Caponio *et al*., 2005).
3. Phenolic compounds consist of oleuropein, vanillic acid, protocatechuic acid, p-hydroxyphenylacetic acid, homovanillic acid, syringic acid, gallic acid, p-coumaric acid, o-coumaric, cinnamic acid, tyrosol, caffeic acid and ferulic acid (Beltran *et al.*, 2004; Sikorska *et al*., 2012; Santos *et al*., 2013; Šarolić *et al.*, 2014).

The presence of many fluophores in olive oil urges to think about the impact of storage age, so the spectra of olive oil should be analyzed to determine the effect of storage age. Spectroscopic techniques are sufficient to give complete information for one fluophore. Olive oil have several fluophores, so a comprehensive method is needed, fluorescence spectroscopy technique is the best to show complete results (Sikorska *et al.*, 2012; Sádecká, 2007).

1.2 Previous Studies

Consumption of olive oil lowers the risk of coronary heart disease, tumors, bad cholesterol and anti-microbial effects (Carralafuente, 2003). Mailer and his group studied some Australian olive oil samples stored in different types of containers after 2, 4, 8, 16, 32 and 52 weeks. They found that phenols, chlorophylls and α-tocopherol decrease with age (Mailer *et al*., 2012). Mailer and Graham also studied the effect of containers type on the stored olive oil.

After 16 weeks they found that the loss of phenols, chlorophylls and α-tocopherol in olive oil samples stored in plastic containers is higher than that in those stored in dark in glass and stainless containers (Mailer and Graham, 2009).

Zandomeneghi and his group used the right angle fluorescence to study the undiluted olive oil samples. The absorption wavelengths was ranged in the interval (325 - 260) nm, this band belongs to vitamin E and phenolic compounds (Zandomeneghi *et al.*, 2005).

Zandomeneghi and his team used the right angle fluorescence method to differentiate between extra virgin olive oil and olive oil depending on their fluorescence spectra in the interval (400 - 600) nm. They found the maximum absorbance was at 522 nm, this wavelength belongs to vitamin E (Zandomeneghi *et al.*, 2006).

Sikorska's group measured the spectrum of virgin olive oil and refined olive oil by using right angle geometry. They noticed a band in spectra of refined olive oil with excitation at 280 - 330 nm and emission at 372 - 480 nm. They also observed that the long wavelength band has a lower intensity in refined olive oil. This interval belongs to phenolic compounds, so the amount of phenolic compounds in virgin olive oil is higher than that in refined olive oil (Sikorska *et al.*, 2012). In another work, Scott's group measured the spectra of undiluted olive oil samples (extra virgin and non-virgin olive oil). The excitation range was (350-450) nm, and the emission range was (400-720) nm. Luminescence spectroscopy technique detect the adulteration of olive oil (Scott *et al.*, 2003).

Kyriakidis and Skarkalis suggested that the bands in the undiluted olive oil emission spectrum was (λ_{ex} = 365 nm), with the maximum at 525 nm came from vitamin E (Kyriakidis and Skarkalis, 2000).

Tena's group studied the fluorescence for phenolic components of olive oil samples at ($\lambda_{ex.}$ = 270 nm), the fluorescence maxima was between 362 nm and 420 nm (Tena *et al.*, 2009). Cert who measured the amount of phenolic compounds in virgin and refined olive oil (Cert *et al.*, 2000). Kyriakidis reported the emission spectra of tocopherols bands of several oils at ($\lambda_{ex.}$ = 365 nm). Tocopherols amount in refined olive oil is less than that in virgin olive oil (Kyriakidis *et al.*, 2004).

Kongbonga measured the spectra for many vegetable oils at excitation wavelength $\lambda_{ex.}$ = 370 nm, and the intensities at λ = 525 nm by using fluorescence spectroscopy (Kongbonga *et al.*, 2011). Dankowska and Małecka studied the spectra of olive oil in the interval 240 – 700 nm with band width of 10, 30, 60 and 80 nm. Fluorescence spectroscopy technique is useful for determination the adulteration of olive oil (Dankowska and Małecka, 2009).

Fasina and Colley noticed that viscosity of vegetable oils decreases exponentially with temperature. They used three models to describe the viscosity- temperature relation; the best model was the modified Williams-Landel-Ferry (WLF) (Fasina and Colley, 2008).

El-hefian determined the viscosity of pure virgin olive oil and virgin olive oil at temperature range (15^{o}C - 40^{o}C). His results showed that acidity

increases and viscosity decreases as period of storage increases (El-hefian *et al.*, 2007).

Diamante and Lan found that the absolute viscosity of the vegetable oil inversely proportional to the temperature, they proposed that Arrhenius model describes the relation (Diamante and Lan, 2014). Nierat measured the dynamic viscosity of Palestinian olive oil samples of different storage ages from different locations as a function of temperature. She noticed that dynamic viscosity decreases as a function of storage age. Dynamic viscosity depends on the Fatty acids and waxes composition of olive oil. These substances are affected by the storage age (Nierat, 2012).

Ariponnammal determined the refractive indices of fifteen oils. He found that the percentage of adultration of these oils has been deduced by using refractive index as a tool (Ariponnammal, 2012).

Abdalla analyzed olive oil for fatty acids. He found oliec acid percentage (64.80 to 72.80), then palmitic, Linoleic, palmitoleic, stearic and linolenic (Abdalla *et al.*, 2000).

Odeh, Bahti and Fuqaha measured the viscosity, refractive index, density and acidity of olive oil of different regions in Palestine. Odeh concluded that the acidity for olive oil samples collected from trees irrigated by waste water is higher than the olive oil samples from those irrigated by rain water. Bahti found that the acidity of some olive oil samples (storage age $\leq$ 12 years) are in good agreement with the standard values (< 3.3%). Fuqaha determined that viscosity, acidity, density and refractive index of olive oil from different geographical locations and heights in Palestine are within the standard values

(Odeh *et al*., 2015; Bahti *et al*., 2015; Fuqaha *et al*., 2015). Nierat studied the dynamic viscosity of olive oil at different storage ages in Palestine in weekly basis. The dynamic viscosity is inversely proportional to the temperature. In another study, Nierat studied the acidity of olive oil samples at different storage ages in yearly and weekly basis. Olive oil can be stored for a period not more than 12 years without deterioration (Nierat *et al*., 2013; Nierat *et al*., 2014).

1.3 Objectives of the Study

The main objectives are to:

- Measure the emission spectra of Palestinian olive oil of different regions and different storage ages using the fluorescence spectroscopy technique. The goal is to determine the percentage of fluorescent components (vitamin E, chlorophylls and phenolic compounds) in olive oil at different storage ages using the emission spectra.
- Study the dependence of the intensity at different emission wavelengths of different storage ages on the storage age.
- Measure the absorption spectra of Palestinian olive oil of different regions and different storage ages using the fluorescence spectroscopy technique.
- Study the dependence of the absorbance at different absorption wavelengths of different storage ages on the storage age.
- Measure the physical properties: viscosity, refractive index, acidity, and density of olive oil of different regions and different storage ages.

- Study the relationship of viscosity, refractive index, acidity, and mass density of olive oil of different storage ages and storage age.

The samples of olive oil used in this study were collected from different Palestinian regions and different storage ages as shown in Table 1.1.

Table (1. 1): Samples of different storage ages (years) of Palestinian olive oil

Sample	**Storage Ages in (years)**							
S_1	3	5	16	17				
S_2	0	1	3	5	7	16	17	18
S_3	3	4	5	8				

Where,

S_1: Allar village, samples were taken from 1998 to 2012,

S_2: Saida village, samples from 1997 to 2015,

S_3: Yasid village, samples were collected from 2007 to 2012.

Chapter Two

Theory

2.1 Olive Oil

2.1.1 Olive Oil Structure

Olive oil consists of the mixed triglyceride esters of oleic acid, palmitic acid and of other fatty acids, with traces of squalene and sterols, as shown in Fig.2.1 (Tripoli *et al.*, 2005).

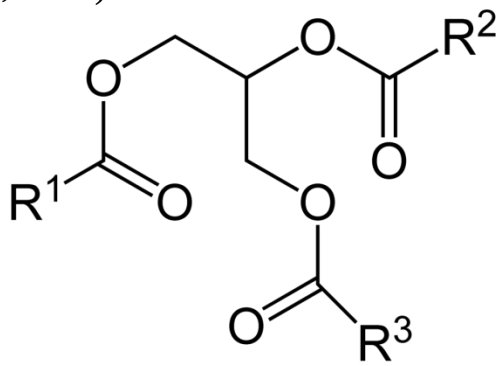

Figure (2. 1): Structure of olive oil (triglyceride), R^1, R^2 and R^3 are alkyl groups

The symbol R is used to designate a generic (unspecified) alkyl group. The smallest alkyl group is methyl, with the formula CH_3—, an alkyl substituent is an alkane missing one hydrogen atom. An alkyl has the general formula C_nH_{2n+1} (Carey, 2000; Daley R. and Daley S., 2005).

2.2 Fluorescence

Stokes noticed that the fluorite (fluorspar) emits light when exposed to ultraviolet light, then he described and coined the term "Fluorescence" in 1852 (Valeur and Berberan-Santos, 2011).

Luminescence is the emission of light from an electronically excited states substance (Demchenko, 2009). Luminescence divided into two classes according to the nature of excited states:

- Phosphorescence: is the excited triplet states, the electron in the excited orbital is paired by same spin to the electron in the ground state orbital. The electron in the excited state slowly return to the ground state in forbidden transition by emission of a photon, with lifetime about 10^{-3} s to 1 s. The lifetime of a substance is the time taken in the transition between its excitation and relaxation (Lakowicz, 2006; Tully and O'kennedy, 2014).
- Fluorescence: is the emission of light from singlet excited states. The electron in the excited orbital has opposite spin to the electron in the ground state orbital. The electron in the excited state rapidly return to the ground state in spin allowed transition by emission of a photon; with a lifetime of 10^{-8} s. This emission called fluorescence, when the matter absorbs photons in ultraviolet region, then emits light in visible region. Conjugated polycyclic aromatic molecules are capable of showing fluorescence (Lakowicz, 2006; Periasamy and Day, 2005).

Fluorescence steps: firstly, excitation of valence electrons of susceptible molecules into higher electronic and vibrational levels by absorbing photons, in about 10^{-15}s. Then the electrons relax to the lowest vibrational levels of S_1 (singlet excited state), in about 10^{-12} s. This transition called internal conversion. Finally, the electrons return to the ground state S_0 (singlet ground

state) by emission photons in about 10^{-9} s (Al-Rawashdeh, 2012), as shown in Jablonski diagram in Fig. 2.2.

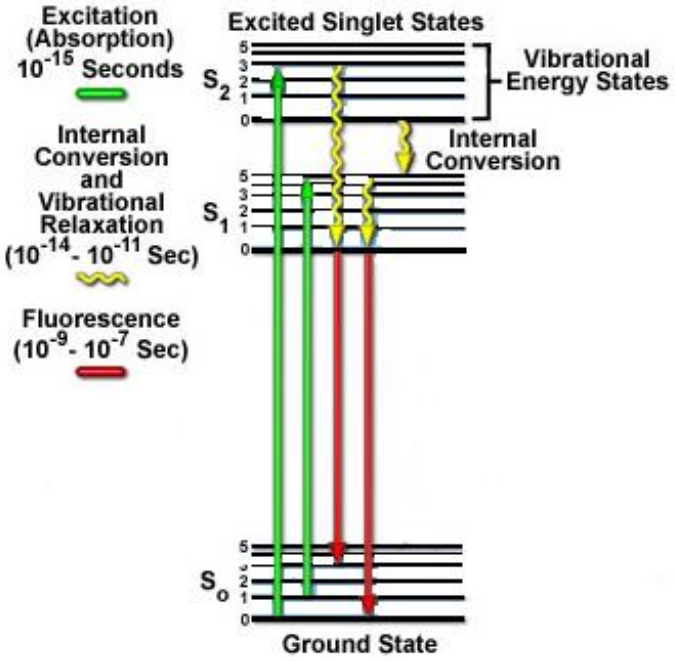

Figure (2. 2): Jablonski Energy Diagram (Meur, 2010)

There are several possible electronic transitions in the molecular orbitals, each of a different relative energy, as shown in Fig. 2.3

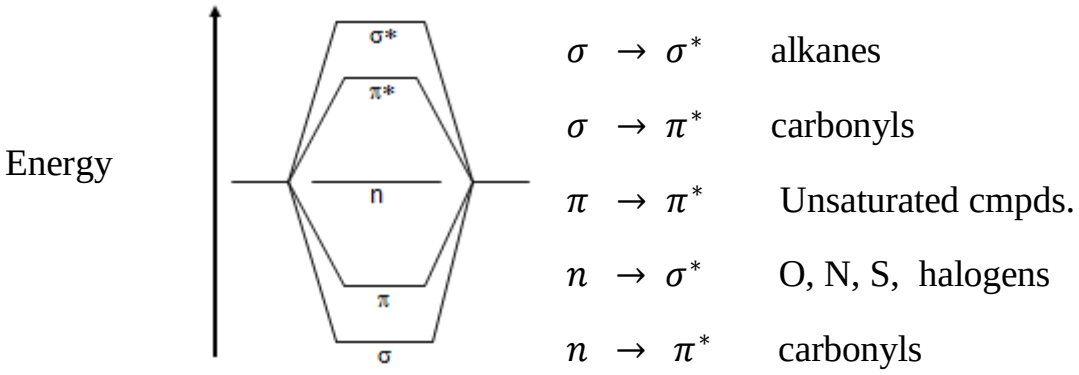

Figure (2. 3): Molecular orbital diagram representing the electronic transitions

Sigma bond (σ): is a covalent bond resulting from the formation of a molecular orbital by the end-to-end overlap of atomic orbitals.

Pi bond (π): is a covalent bond resulting from the formation of a molecular orbital by side-to-side overlap of atomic orbitals along a plane perpendicular to a line connecting the nuclei of the atoms.

Types of possible electronic transition in molecules:

- $\sigma \rightarrow \sigma^*$ Transition: This transition has the largest energy among the electronic transitions, in which, the electron transition occurs from the bonding orbital σ to the antibonding orbital σ^*. This absorption occurs in alkanes. Methane has single bonds between carbon and hydrogen atoms (C-H) only. $\sigma \rightarrow \sigma^*$ is the only allowed transition with $\lambda_{max.}$ equals 125 nm.
- $\sigma \rightarrow \pi^*$ Transition: Carbonyls contain double bond between carbon and oxygen (C=O). This group is capable of showing this type of transition.
- $\pi \rightarrow \pi^*$ Transition: Unsaturated compounds contain molecules with π electrons, such as alkenes and aromatics, which are capable of offering this transition, and $\lambda_{max.}$ equals 343 nm.
- $n \rightarrow \sigma^*$ Transition: Energy needed for this transition is generally less than $\sigma \rightarrow \sigma^*$ transition. Saturated amines and alcohols, which contain atoms with non-bonding electrons, can show this transition. Absorption in this transition ranges between 150-250 nm.
- $n \rightarrow \pi^*$ Transition: This type of transition occurs in unsaturated compounds which consist of atoms with non-bonding electrons, and $\lambda_{max.}$ equals 512 nm (Hamid, 2007; Dash, 2011).

Fluorescent materials divided into two classes:

- Primary fluorescence or autofluorescence materials: Materials that fluoresce in its natural form when irradiated with ultraviolet light, such as butter, chlorophyll and vitamins (Meur, 2010; Abramowitz, 2010). Olive oil is classified as autofluorescence material, due to the minor components species like tocopherols, phenols and chlorophylls (Diaz *et al*., 2003).
- Secondary fluorescence materials: These materials can't fluoresce in its natural form, like pathogens. Fluorescence microscope detects such pathogens after processed with fluorescing chemicals (Meur, 2010).

The two kinds of spectra describe the fluorescent properties, excitation and emission spectra, which are fluorescence intensity, plotted as a function of excitation and emission wavelengths (Guilbault, 1999; Sikorska *et al*., 2012). Fluorescence for a given compound that contains fluorophores (chromophores) are affected by many factors, such as temperature, other chemicals in the compound, and fluorophores nature and concentrations (Meur, 2010; Sauer *et al*., 2011).

2.2.1 Properties and Principles

Stokes Shift: The energy of the emission is less than that of absorption, fluorescence happens at lower energies. This property is known as Stokes shift (Sanborn *et al*., 2005). Kasha's rule: Emission spectra are usually independent of the excited wavelength. This rule is called Kasha's rule (Lakowicz, 2006).

Mirror Image Rule: For many fluorophores, the spacing between vibrational states in the ground state and excited state are equal, besides, the appropriate transitions in emission and absorption are the same. These two facts explain why the fluorescence emission spectrum and absorption spectrum are mirror images to each other (Meur, 2010; Al-Rawashdeh, 2012).

Quenching: Quenching occurs when a non-fluorescent molecule collides with an excited state fluorophore molecule; the fluorophore relaxes to the ground state without fluorescence. Another form of quenching happens when the two molecules collide in the ground state; the quencher molecule reduces the population of active molecules in order to limit the absorption. Oxygen, halogens and amines are behaving as quenching factors in the compounds (Meur, 2010).

2.2.2 UV-VIS-NIR Spectrophotometry

The last three decades witnessed a noticeable growth in the use of UV-VIS-NIR Spectrophotometry technique in chemical and physical sciences. UV-VIS-NIR Spectrophotometry technique is now the quick, inexpensive and easy method used largely in biotechnology, medical diagnostics, forensics, cell biology and food analysis (Lakowicz, 2006; Amiot *et al.*, 2008).

UV-VIS-NIR Spectrophotometry belongs to absorption spectrum or reflectance spectrum in the ultraviolet-visible-near infrared spectral region. This technique is a branch from fluorescence spectroscopy. Spectroscopy means light interacting with matter. Fluorescence deals with transitions from the excited state to the ground state, while absorption deals with transitions from the ground state to the excited state (Skoog *et al.*, 2007; Fanun, 2011).

The quantitative way to find out the concentrations of the chemicals that absorbing light are the using of Beer-Lambert law (Christopher, 2004).

$$I = I_O e^{-A} \quad (2.1)$$

Where,

$$A = C\varepsilon l, \quad (2.2)$$

I_O: The intensity of the incident wave.

I: The intensity of the transmitted wave.

C: Concentration of substance in solution in moles/liter.

ε: Molar absorptivity (liter mol^{-1} cm^{-1} or M^{-1} cm^{-1}) where, M = mol L^{-1}.

l: Length of the cell in cm.

The molar absorptivity of a compound is a constant that is characteristic at a particular wavelength.

2.2.3 Chromophores

Chromophores: The energy absorbed during excitation of electrons depends on the nuclei that hold the electrons together in a bond. The group of atoms possessing electrons responsible for the absorption is chromophores, for example, C-H and C=O. Substituents effects on chromophore as shown in Fig. 2.4 (Field, 2013):

- Bathochromic shift: a shift to longer λ.
- Hypsochromic shift: a shift to shorter λ.
- Hyperchromic effect: an increase in absorbance.
- Hypochromic effect: a decrease in absorbance.

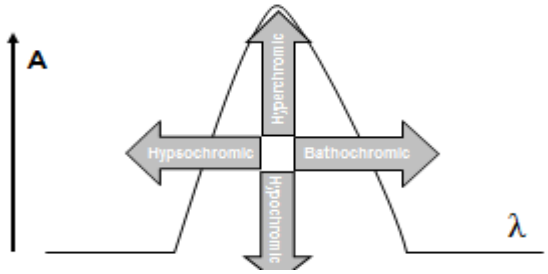

Figure (2. 4): Substituents effects on a chromophore

2.3 Viscosity

Viscosity is a distinctive feature of all liquids. Liquids have an internal resistance to flow or to shear. This resistance is called viscosity. Viscosity of liquids depends on shear rate, temperature, pressure and concentration (Viswanth *et al.*, 2007; Giap, 2010). Viscosity is expressed in two forms:

- Dynamic viscosity (η_D)
- Kinematic viscosity (η_K)

Dynamic viscosity is defined as the ratio of shear stress to the rate of deformation, where, shear stress is force over cross section area, and the rate of deformation is the difference of velocity over a sheared distance.

Dynamic viscosity presented as:

$$\eta_D = \frac{\tau}{\frac{\partial u}{\partial x}} \tag{2.3}$$

Where,

η_D is the dynamic viscosity in Pascal-second (Pa.s),

τ is the shear stress (N/m^2),

$\frac{\partial u}{\partial x} = \gamma$ is the rate of deformation or velocity gradient or shear rate (1/s).

Kinematic viscosity is defined as:

$$\eta_K = \frac{\eta_D}{\rho} \tag{2.4}$$

Where,

η_K is the kinematic viscosity in centistokes (cSt),

ρ is the mass density of the liquid in g/cm^3.

The liquids flow depends on viscosity, so the liquids divided into two categories: Newtonian and non-Newtonian liquids.

- Newtonian liquid: is defined the viscosity of the liquid does not change, and it is independent on the applied shear stress. Shear stress and shear rate are directly proportional to each other (Akhtar *et al*., 2009).
- Non-Newtonian liquid: is defined when the viscosity depending on the applied shear force and time, it changes at different shear rates (Viswanth *et al*., 2007; Chhabra, 2006).

Non-Newtonian liquids divided into two groups:

- The time independent: in which the viscosity does not change as a function of time when it is measured at a specific shear rate (James, 1996; Chhabra, 2006).
- The time dependent: where the viscosity of the liquid changes as a function of time (Chhabra, 2006).

In the measurement of viscosity, an increase in shear stress leads to a greater portion increase in shear rate, and therefore, reducing viscosity value as measured by viscometer. This phenomenon called shear- thinning. Olive oil exhibit non-Newtonian behavior by showing a shear-thinning relationship between viscosity and shear rate, which is known as pseudoplastic behavior, olive oil is time independent oil (Giap *et al*., 2009).

The Arrhenius type relation was used to fit the effect of temperature on dynamic viscosity which is given as (Giap, 2010).

$$\eta_D = \eta_{\infty,T} e^{\frac{E_a}{RT}} \quad (2.5)$$

Where,

$\eta_{\infty,T}$ is the viscosity at infinite temperature in unit (Pa.s),

E_a is the activation energy in unit (J/mol),

R is the gas constant in unit (J/mol.K),

and T is the absolute temperature in Kelvin.

Arrhenius relationship has failed to give real representation phenomena for all fluids, because of the complex of the nature of the fluid. There are large deviation in the measured viscosity and theoretical calculation of viscosity (Viswanth *et al*., 2007).

De-Guzman proposed a relation for the viscosity as a function of temperature, which is (De-Guzman, 1913)

$$\eta_D = A e^{\frac{B}{T}} \quad (2.6)$$

Where A and B are positive constants.

Equation (2.4) was used in logarithmic form by Dohan, which is (Dohan, 1979).

$$\ln \eta_D = \acute{A} + \frac{\acute{B}}{T} \quad (2.7)$$

Where $\acute{A}$ and $\acute{B}$ are constants.

The effect of temperature on dynamic viscosity is also studied by Abramovic, that he proposed the following relationship (Abramovic and Klofutar, 1998).

$$\log \eta_D = \frac{A}{T} - B \quad (2.8)$$

Where A and B are constants evaluated for olive oil to be A = 1558.2K and B = 3.433K. Abramovic used Andrade relationship to form three constants equation to describe the relation between viscosity and temperature, which is represented mathematically by the following equation.

$$\ln \eta_D = A + \frac{B}{T} + CT \quad (2.9)$$

A, B and C are constants evaluated by Andrade for olive oil to be A is 32.72, B is 7462.27 K and C is 0.04 K^{-1} (Abramovic and Klofutar, 1998; Andrade, 1930).

Nierat proposed formulas of three-constant and of multi-constant, fit her experimental data of dynamic viscosity, which are represented by the following equations.

$$\ln(\eta) = A - \frac{B}{T+C} \quad (2.10)$$

$$\eta = A + \frac{B}{t} + C\ln(t) + Dt^E \quad (2.11)$$

A, B, C, D and E are constants evaluated by Nierat for olive oil to be A is -136.6100 (cP), B is 3822.114 (cP.°C), C is 23.21082 (cP), D is 694.2263 ($cP/°C^E$) and E is -2624.33 (Nierat, 2012).

2.4 Refractive Index

Refractive index is an important optical feature to analyze the light rays passing through materials medium. In laboratory, the refractive index of oils can be used to test adultration (Ariponnammal, 2012).

Refractive index (n) of a medium is defined as the ratio of the speed of light in the vacuum to the speed of light traveling through this medium (Mills *et al.*, 1993), and mathematically is written as:

$$n = \frac{c}{v} \tag{2.12}$$

Where,

c is the speed of light in the vacuum,

v is the speed of light in the medium.

Refractive index for virgin olive oil extends from 1.4677 to 1.4705 at 20°C (Codex, 2001). Refractive index for Palestinian virgin olive oil extends from 1.4677 to 1.4705 (PS188, 1997).

2.5 Acidity

The acidity of olive oil is affected by different factors, for example, ripeness, extraction processes, altitude, climate, and storage age. Free fatty acid contents (FFA %) were often determined to classify and evaluate oil (Mariotti and Mascini, 2001).

2.6 Mass Density

The mass density of a substance is the value of the mass in gram over the volume in cm^3 (Mills *et al.*, 1993). Palestinian Standard determines the density of virgin olive oil is 0.910-0.916 g/cm^3 at 20°C (PS188, 1997; Codex, 2001).

The mass density of a substance is given by

$$\rho = \frac{m}{V} \tag{2.13}$$

where,

ρ : the mass density (g/cm^3),

m : the mass in grams,

V : the volume in cm^3.

Chapter Three

Methodology

Olive oil samples were collected from three regions Allar (S_1), Saida (S_2) and Yasid (S_3) of different storage ages. All the samples were kept in bottles, and stored in dark place at room temperature.

The following experimental steps will be done:

- The emission and absorption spectra of the samples will be measured.
- The intensity of different emission wavelengths ($\lambda_{em.}$) of the samples will be measured.
- The absorbance of different absorption wavelengths ($\lambda_{abs.}$) of the samples will be measured.
- The viscosities of the samples will be measured at 25°C.
- The refractive indices of the samples will be measured at 25°C.
- Titration method will be used to measure the free fatty acid as oleic acid percentage (FFA%) of the samples.
- The mass densities of the samples will be measured at 25°C.

3.1 Experimental Apparatus

3.1.1 Emission Spectra Apparatus

The luminescence Spectrometer LS50B which is shown in Fig.3.1 will be used for measuring the emission spectra of olive oil samples. The wavelength accuracy of this apparatus is +1.0 nm, and the range is 200 - 900 nm.

Figure (3. 1): Luminescence Spectrometer LS50B

This apparatus consists of spectrometer linked to computer, the spectrometer consists of:

- Source: xenon discharge lamp source of ultraviolet and visible range.
- Sample container: where the sample is illuminated by the focused excitation light and emits fluorescence.
- Optical filters on the emission path for preventing interfering wavelengths.
- Sample detector and reference detector with gated photomultipliers.
- Monochromators: quartz prism or gratings for splitting the excitation light into its components.
- Readout system (recorder and printer), the output printouts is unsuitable for publication. This system save data and convert it to Excel for processing.
- Computer.

3.1.2 Absorption Spectra Apparatus

UV-VIS-NIR Scanning Spectrophotometer UV-3101PC which is shown in Fig.3.2 was used to measure the absorption spectra of olive oil samples. The wavelength range of this apparatus (190-3200) nm, resolution 0.1 nm, wavelength accuracy ±0.3nm in ultraviolet-visible region and ±1.6 nm in near-infrared region.

Figure (3. 2): UV-VIS-NIR Scanning Spectrophotometer UV-3101PC

UV-VIS-NIR Scanning Spectrophotometer UV-3101PC consists of:

- Double monochromators: each monochromator consists of three diffraction gratings so as to minimize the amount of stray light. The diffraction grating has a reflective surface with hundreds grooves/mm. Incident white light is reflected from the grating as a series of overlapping spectra. Rotation of the grating allows different wavelengths to pass through the exit slit of the monochromator. Light from second and higher orders is removed by filters.

The monochromator consist of:

- Entrance slit
- Collimating lens

- Dispersing device
- Focusing lens
- Exit slit

Polychromatic beam enters the entrance slit and collimated, and then it hits the dispersing device (a prism or a grating) at an angle and split into its components. The specific wavelength leaves through the exit slit, when moving the dispersing element or the exit slit.

- Source of radiation: Tungsten filament lamp (source of visible radiation) and tungsten halogen lamp (source of ultraviolet radiation).
- Standard rectangular cell: constructed from quartz or silica plates with volume 3.5 cm^3 and 10 mm pathlength.
- Excitation optical system.
- Fluorescence detector (photomultiplier tube) consists of a cathode, dynodes and anode.

The beam entering the tube hits the cathode and emits a number of electrons. These electrons accelerate toward the first dynode and hit it. This dynode emits a number of electrons. These electrons accelerate toward the second dynode. The dynode emits more electrons; these electrons will accelerate toward the third dynode and so on. These electrons are collected at the anode and form a current.

- Polarizers to get full reflectance at large incident angles with no impact on the polarization features.
- Signal processor and readout.
- Temperature controller with temperature range (0-80) ^oC.

- Computer.

3.1.3 Fluorescent Components Table of Olive Oil

The emission wavelengths, the molar absorption coefficients, the excitation wavelengths and the absorption wavelengths attribute to vitamin E, chlorophylls and phenolic compounds will be taken from Table 3.1, Table 3.2 and Table 3.3 (Eitenmiller *et al*., 2008; Ward *et al*., 1994; Undenfriend, 1962; Diaz *et al*., 2003; Tena *et al*., 2009).

Table (3. 1): Fluorescent components (Chlorophylls) of olive oil

Substance	**Solvent**	**ε [liter mol^{-1} cm^{-1}]**	**$\lambda_{abs.}$ (nm)**	**Solvent**	**$\lambda_{ex.}$ (nm)**	**$\lambda_{em.}$ (nm)**
Chlorophyll a	Acetone	94700, 75000	430- 663	ether	436	668
				acetone	405	669
				9:1 acetone/ water	430	669
Chlorophyll b	Acetone	131000,47100	455- 645	ether	436	648
				acetone	405	652
				9:1 acetone/ water	458	653
Pheophytin a	Acetone	101800,44500	409- 666	ether	436	673
				9:1 acetone/ water	406	671
Pheophytin b	Acetone	145000,27800	434-654	ether	436	661
				9:1 acetone/ water	435	658
Pheophorbide a	Acetone	119200,55200	409- 667	-	-	-

Table (3. 2): Fluorescent components (phenolic) of olive oil

Substance	**Solvent**	**ε [liter mol^{-1} cm^{-1}]**	**$\lambda_{abs.}$ (nm)**	**Solvent**	**$\lambda_{ex.}$ (nm)**	**$\lambda_{em.}$ (nm)**
Oleuropein	ethanol/n-hexane	-	282	ethanol/n-hexane	270	310
Vanillic acid	3% aqueous acetic acid-methanol	ε_{280}= 7471.9	260.2–291.4	methanol	270	349
Protocatechuic acid		ε_{280}= 4329.9	259.0–293.0			
P-hydroxyphenyl acetic acid		ε_{280}= 1700.5	274.4			
Homovanillic acid		ε_{280}= 2785.4	278.4			
Syringic acid		ε_{280}= 5225.2	273.4		270	361
Gallic acid		ε_{280}= 9112.6	270.6		270	382
p-Coumaric acid		ε_{280}=10989.0	308.6		270	416
O-Coumaric		ε_{280}=17352.9	276.2–321.8		270	426
Cinnamic acid					270	420
Tyrosol		ε_{280} = 151.3	275.2		270	420
Caffeic acid		ε_{280}= 5225.2	319.4		270	457
Ferulic acid		ε_{280}= 9803.9	312.4			

Table (3. 3): Fluorescent components (Vitamin E) of olive oil

Substance	**Solvent**	**ε [liter mol^{-1} cm^{-1}]**	**$\lambda_{abs.}$ (nm)**	**Solvent**	**$\lambda_{ex.}$ (nm)**	**$\lambda_{em.}$ (nm)**
α-Tocopherol	Ethanol	3265	292	n-hexane	295	320
β- Tocopherol	Ethanol	3725	296	n-hexane	297	322
δ- Tocopherol	Ethanol	3515	298	n-hexane	297	322
γ- Tocopherol	Ethanol	3809	298	n-hexane	297	322
α-Tocotrienol	Ethanol	3652	292	n-hexane	290	323
β- Tocotrienol	Ethanol	3540	292	n-hexane	290	323
δ- Tocotrienol	Ethanol	3403	297	n-hexane	292	324
γ- Tocotrienol	Ethanol	3737	297	n-hexane	290	324

3.1.4 Viscosity Apparatus

Viscosity at different temperatures will be measured by using the digital rotational viscometer, which is shown in Fig.3.3, has four spindles with rotational speeds are: 6, 12, 30 and 60 RPM. The spindles have ability to measure viscosity range from 1 to 2000000 (cP) with accuracy ±1%.

The viscosity was measured by determining the spindle zero with suitable rotational speed 60 RBM at 25ºC.

Figure (3. 3): Digital Rotational Viscometer

3.1.5 Refractive Index Apparatus

Digital Refractometer will be used to measure the refractive index, which is shown in Fig.3.4 at 25.0ºC. Its accuracy is ±0.0002.

Figure (3. 4): Digital Refractometer

3.1.6 Acidity Measurement

The acidity value defined as the mass in milligrams (mg) of potassium hydroxide (KOH) needed to neutralize the free fatty acids in 1g of olive oil. The acidity value of olive oil will be measured using the titrimetric method. The acidity of olive oil samples were measured according to these steps:

1- Steps to prepare 0.1 M of KOH:

- Weighing out 0.56 g of solid KOH and convert it to powder. Dissolving this powder in100 mL of ethanol. The molarity of KOH can be calculated using this equation:

$$\text{Molarity} = \frac{weight\ of\ KHp}{molecular\ weight\ of\ KHp} \times \frac{1000}{volume\ of\ KOH\ (ml)} \quad (3.1)$$

The number of moles can be found according to this relation:

$$\text{Number of moles} = \frac{\text{weight}}{\text{molecular weight}} \quad (3.2)$$

- Dissolving 0.204 g of solid KHP in 50.0 mL of distilled water.
- Adding up few milliliters of phenol phthalien to KOH solution until the colour changed to pink.

2- Steps to prepare Ethanol – ether mixture:

- Preparing Ethanol – ether mixture with a concentration of 1:1.
- Adding up 3 drops of phenol phthalien to the mixture.
- Adding up few drops of ethanolic KOH to get the pink color.

3- Steps to obtain acid value of olive oil:

- Weighing out 5g of olive oil sample in a conical flask.
- Adding up Ethanol – ether mixture and 3 drops of phenol phthalien to the oil.
- Titrating the prepared mixture with standard KOH until faint pink color appears.

The acid value of olive oil sample finally is given by:

$$\text{Acid value} = \frac{standerd\ solution\ KOH(ml) \times molarity\ KOH \times 56.1}{weight\ of\ sample(g)} \quad (3.3)$$

The free fatty acid (FFA) is obtained by using the equation:

$$\text{FFA \% (as oleic acid)} = \frac{\text{Acid value}}{1.99} \quad (3.4)$$

Olive oil is classified qualitatively by International Olive Council (IOC) according to its acidity into classes as given in Table 3.4 (IOC, 2015).

Table (3. 4): Classification of olive oil according to FFA%

Group	FFA%
Refined Olive Oil	≤ 0.3
Extra Virgin Olive Oil	≤ 0.8
Virgin Olive Oil	≤ 2.0
Ordinary Olive Oil	≤ 3.3
Lampante Oil	> 3.3

3.1.7 Mass Density Apparatus

The density of olive oil samples will be measured using Pycnometer, which is shown in Fig. 3.5. The density will be measured by taking the difference mass between a full Pycnometer and an empty one divided by 10 cm^3 (volume of olive oil sample), at $25.0°C$.

Figure (3. 5): Pycnometer

3.1.8 Temperature Apparatus

The temperature of olive oil samples will be measured by using Digital Prima Long Thermometer in (^{o}C), with accuracy ±1.0%, which is shown in Fig. 3.6.

Figure (3. 6): Digital Prima Long Thermometer

3.1.9 Mass Apparatus

The mass of olive oil samples and the pycnometer will be measured by using analytical balance in (g), which is shown in Fig. 3.7, with accuracy of $\pm$ 0.00005.

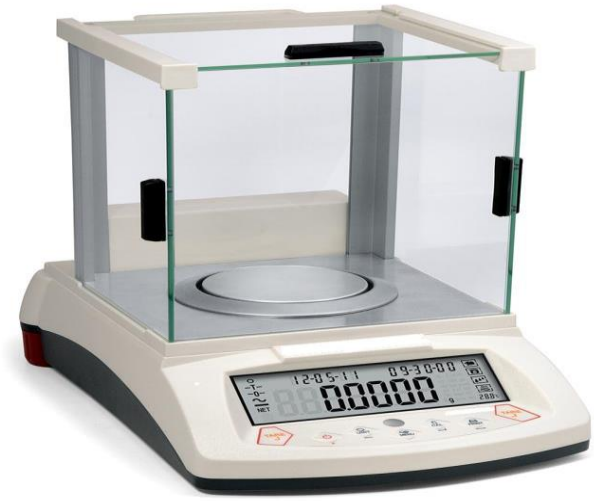

Figure (3. 7): Analytical balance

Chapter Four

Results and Analysis

4.1 Emission Wavelength Results

In this study, the emission spectra of olive oil samples of Allar (S_1), Saida (S_2) and Yasid (S_3) were measured at room temperature at different storage ages. The emission spectra of olive oil samples of Allar (S_1) at room temperature at different storage ages (3 and 5 years) with excitation wavelength ($\lambda_{ex.}$ = 295 nm) were plotted in Fig. 4.1.

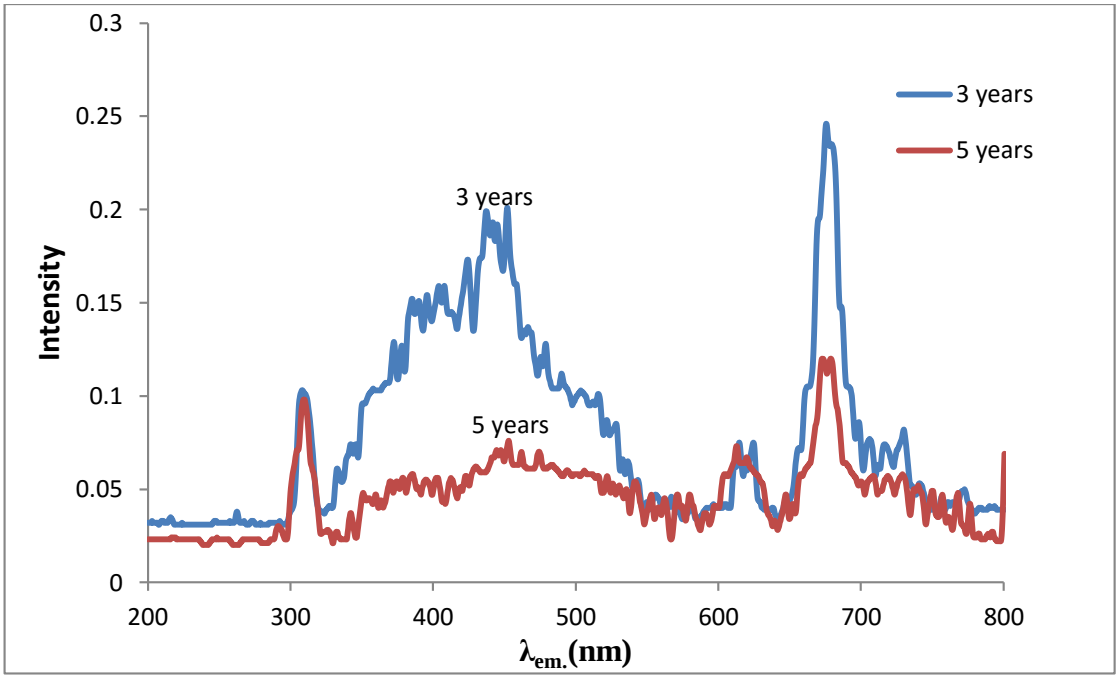

Figure (4. 1): The emission spectra of olive oil samples of Allar (S_1) at room temperature at different storage ages (3 and 5 years storage age) (λex. = 295 nm)

The values of the intensities of some emission wavelengths of olive oil samples of Allar ($S_{1)}$ at different storage ages are represented in Table 4.1.

Table (4. 1): The measured intensities of some emission wavelengths of olive oil samples of Allar (S_1)

Substance		$\lambda_{em.}$ (nm)	Storage age (years)	
			3	5
			Intensity	
Vitamin E	α-Tocopherol	320	0.039	0.033
	β-, δ- , γ-tocopherol	322	0.038	0.026
	α-, β-tocopherol	323	0.038	0.027
	δ- , γ-tocopherol	324	0.037	0.027
Chlorophylls	Chlorophyll a	668	0.16	0.084
	Chlorophyll b	648	0.043	0.040
	Pheophytin a	673	0.216	0.12
	Pheophytin b	661	0.103	0.061
Phenolic Compounds	Oleuropein	310	0.101	0.098
	Vanillic acid	349	0.084	0.039
	Syringic acid	361	0.103	0.043
	Gallic acid	382	0.136	0.051
	p-Coumaric acid, Ferulic acid	416	0.138	0.047
	o-Coumaric acid	426	0.159	0.054
	Cinnamic acid, tyrosol	420	0.152	0.05
	Caffeic acid	457	0.16	0.063

The components of vitamin E, chlorophylls and phenolic compounds in olive oil samples of Allar (S_1) decrease as the storage age increases.

The emission spectra of olive oil samples of Saida (S_2) at room temperature at different storage ages (0, 1, 3, 5 and 7 years) were plotted in Fig 4.2.

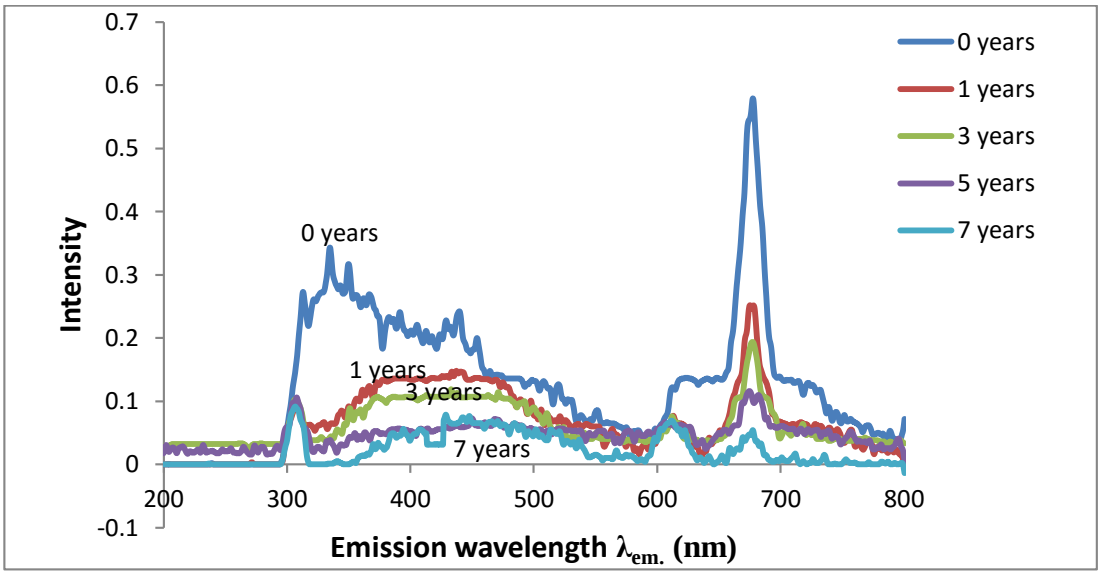

Figure (4. 2): The emission spectra of olive oil samples of Saida (S_2) at room temperature at different storage ages (0, 1, 3, 5 and 7 years storage age) (λex. = 295 nm)

The values of the intensities of some emission wavelengths of olive oil samples of Saida (S_2) at different storage ages are represented in Table 4.2.

Table (4. 2): The measured intensities of some emission wavelengths of olive oil samples of Saida (S_2)

Substance		λ em. (nm)	Storage age (years)				
			0	1	3	5	7
			Intensity				
Vitamin E	α-Tocopherol	320	0.247	0.061	0.038	0.023	0.000
	β-, δ-, γ tocopherol	322	0.261	0.055	0.038	0.020	0.000
	α-, β-tocopherol	323	0.259	0.054	0.038	0.024	0.000
	δ- , γ tocopherol	324	0.260	0.059	0.038	0.027	0.000
Chlorophylls	Chlorophyll a	668	0.357	0.140	0.106	0.090	0.029
	Chlorophyll b	648	0.137	0.039	0.038	0.046	0.003
	Pheophytin a	673	0.530	0.216	0.163	0.112	0.043
	Pheophytin b	661	0.205	0.103	0.079	0.059	0.017
Phenolic compounds	Oleuropein	310	0.212	0.085	0.094	0.094	0.079
	Vanillic acid	349	0.308	0.085	0.068	0.037	0.000
	Syringic acid	361	0.257	0.118	0.078	0.040	0.014
	Gallic acid	382	0.232	0.132	0.102	0.051	0.032
	p-Coumaric acid, Ferulic acid	416	0.194	0.136	0.107	0.053	0.030
	o-Coumaric acid	426	0.198	0.14	0.108	0.056	0.031
	Cinnamic acid, tyrosol	420	0.187	0.136	0.107	0.054	0.043
	Caffeic acid	457	0.167	0.136	0.107	0.061	0.055

The components of vitamin E, chlorophylls and phenolic compounds in olive oil samples of Saida (S_2) decrease as the storage age increases.

The emission spectra of olive oil samples of Yasid (S_3) at room temperature at different storage ages (3, 4, 5and 8 years) were plotted in Fig 4.3.

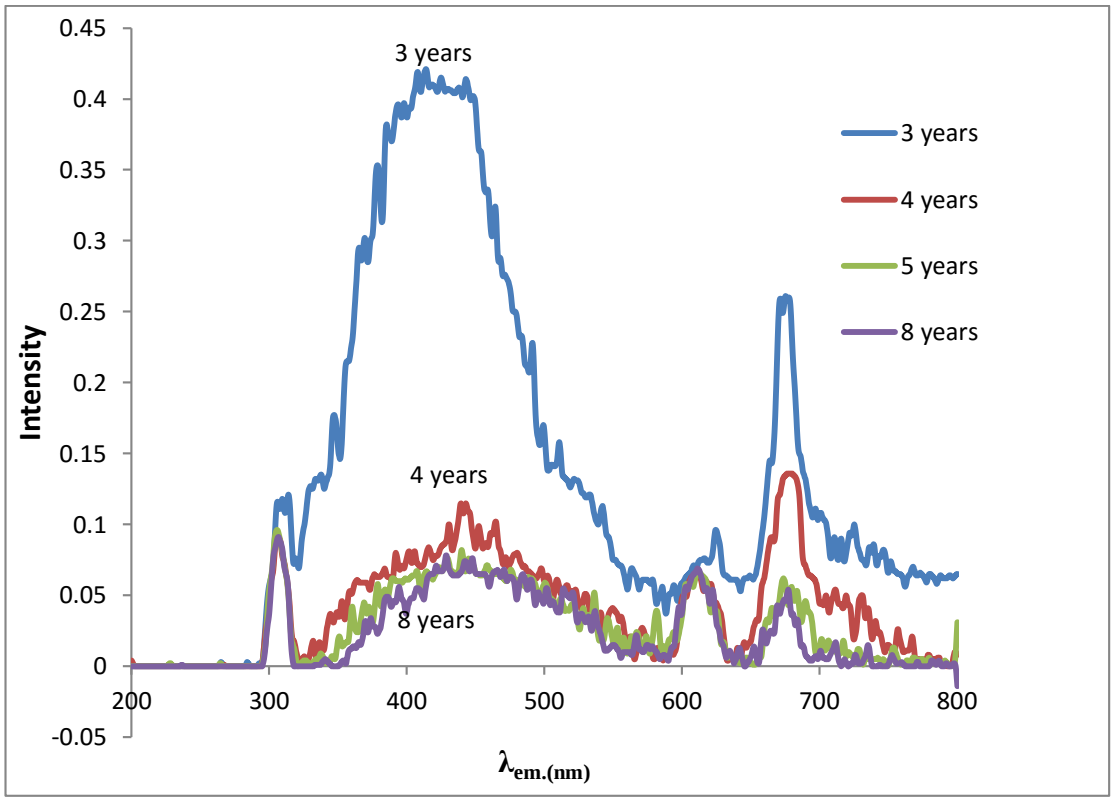

Figure (4. 3): The emission spectra of olive oil samples of Yasid (S_3) at room temperature at different storage ages (3, 4, 5 and 8 years storage age) (λ ex. = 295 nm)

The values of the intensities of some emission wavelengths of olive oil samples of Yasid ($S_{3)}$ at different storage ages are given in Table 4.3.

Table (4. 3): The measured intensities of some emission wavelengths of olive oil samples of Yasid (S_3)

Substance		$\lambda_{em.}$ (nm)	Storage age (years)			
			3	4	5	8
			Intensity			
Vitamin E	α-Tocopherol	320	0.075	0.008	0.002	0.000
	β-, δ- , γ-tocopherol	322	0.070	0.004	0.001	0.000
	α-, β-tocopherol	323	0.081	0.004	0.002	0.000
	δ- , γ-tocopherol	324	0.091	0.005	0.003	0.000
Chlorophylls	Chlorophyll a	668	0.176	0.092	0.035	0.029
	Chlorophyll b	648	0.062	0.015	0.006	0.003
	Pheophytin a	673	0.251	0.129	0.06	0.043
	Pheophytin b	661	0.115	0.068	0.031	0.017
Phenolic Compounds	Oleuropein	310	0.116	0.076	0.078	0.079
	Vanillic acid	349	0.168	0.038	0.011	0.000
	Syringic acid	361	0.235	0.054	0.039	0.014
	Gallic acid	382	0.313	0.065	0.043	0.033
	p-Coumaric acid, Ferulic acid	416	0.409	0.084	0.065	0.064
	o-Coumaric acid	426	0.412	0.085	0.067	0.067
	Cinnamic acid, tyrosol	420	0.409	0.076	0.063	0.066
	Caffeic acid	457	0.335	0.085	0.068	0.065

The components of vitamin E, chlorophylls and phenolic compounds in olive oil samples of Yasid (S_3) decrease as the storage age increases.

The component of α-tocopherol in olive oil samples of Saida (S_2) as a function of storage age is shown in Fig. 4.4.

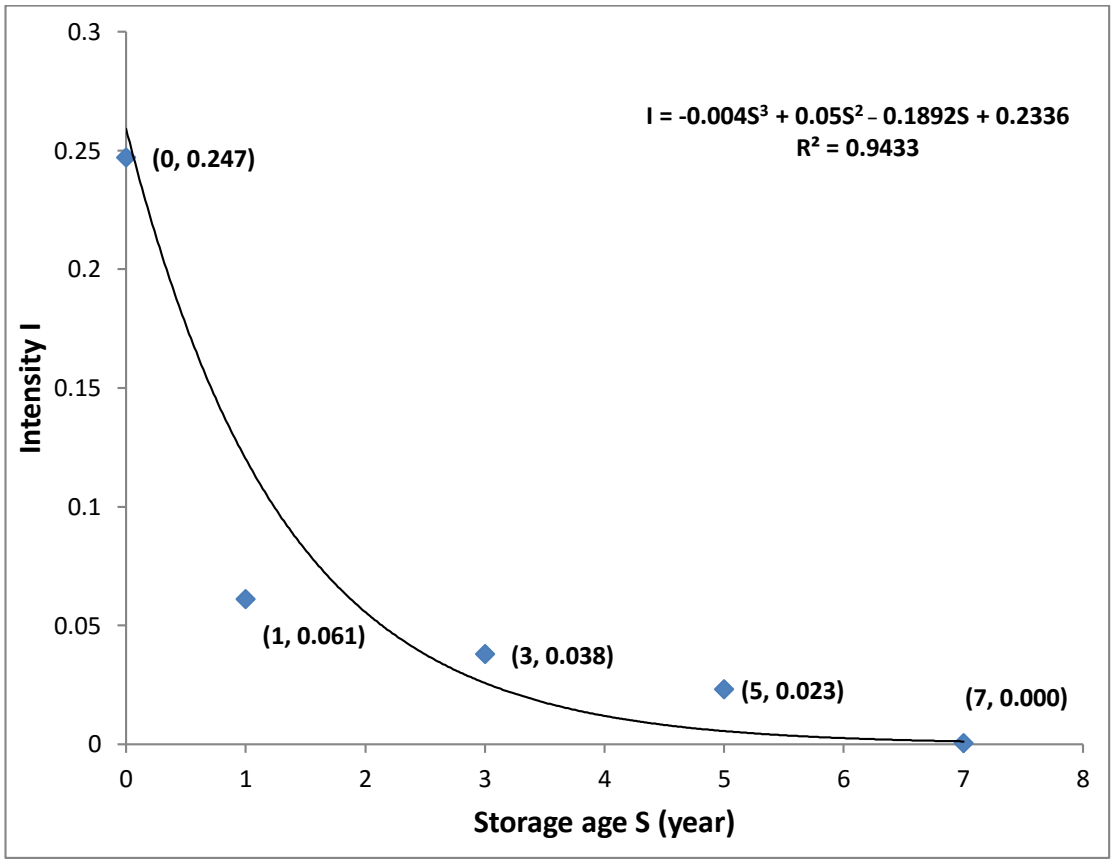

Figure (4. 4): The component of □-tocopherol in olive oil samples of Saida (S_2) as a function of storage age

The fitting equation of the decrease of α-tocopherol component in olive oil samples as a function of storage age is given by

$$I = -0.004S^3 + 0.05S^2 - 0.1892S + 0.2336 \quad (4.1)$$

The storage age of olive oil can be known from its intensity of α-tocopherol from equation (4.1). Olive oil sample contains 25 % of α-tocopherol after 1 year storage age from its original value, 15 % after 3 years storage age, 9 % after 5 years storage age and 0 % after 7 years storage age. α-tocopherol component in olive oil sample of 5 years storage age is accepted.

The emission spectra of olive oil samples of Allar (S_1), Saida (S_2) and Yasid (S_3) at room temperature of 3 years storage ages were plotted in Fig. 4.5.

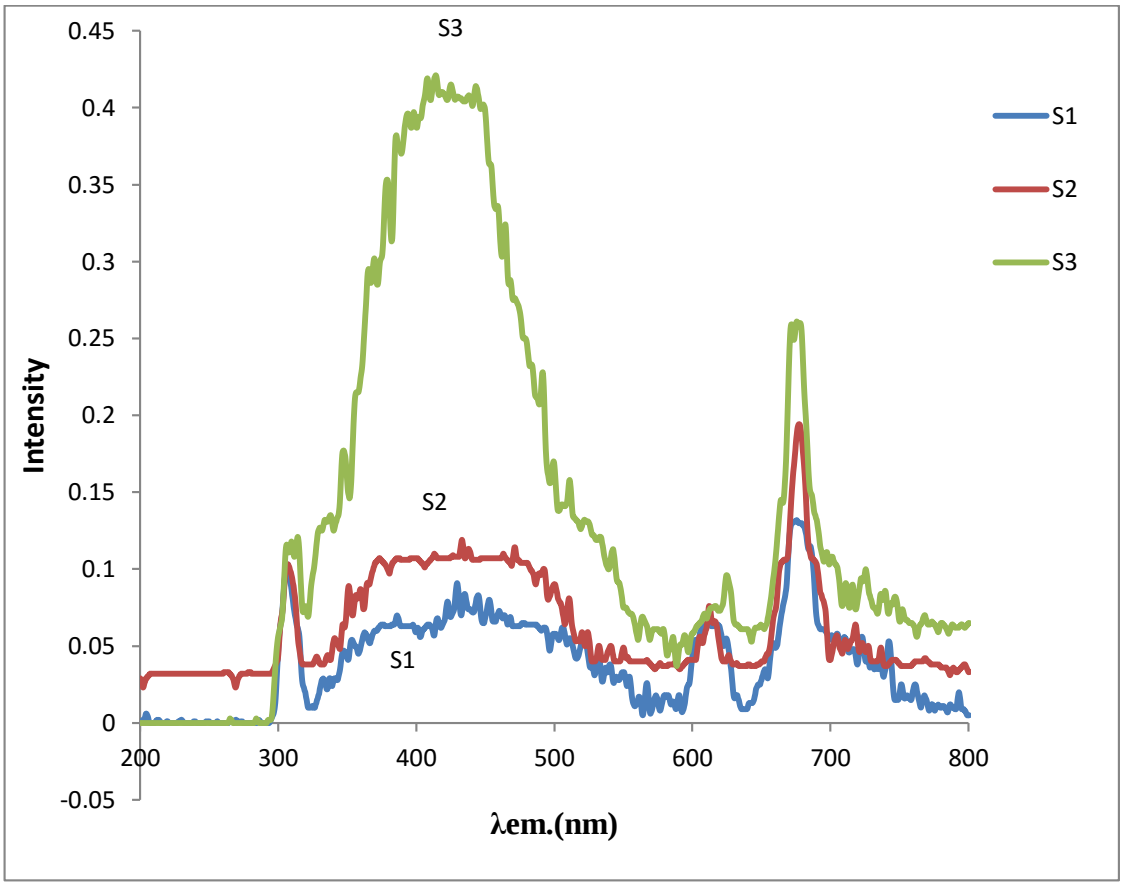

Figure (4. 5): The emission spectra of olive oil samples of Allar (S_1)**,** Saida (S_2) and Yasid (S_3) at room temperature of 3 years storage ages

The values of the intensities of some emission wavelengths of olive oil samples of Allar (S_1), Saida (S_2) and Yasid (S_3) of 3 years storage age are given in Table 4.4.

Table (4. 4): The measured intensities of some emission wavelengths of olive oil samples of Allar (S_1), Saida (S_2) and Yasid (S_3) of 3 years storage age

Substance		$\lambda_{em.}$ (nm)	Region		
			Allar (S_1)	Saida (S_2)	Yasid (S_3)
			Intensity		
Vitamin E	α-Tocopherol	320	0.017	0.038	0.075
	β-, δ- , γ-tocopherol	322	0.010	0.038	0.070
	α-, β-tocopherol	323	0.010	0.038	0.081
	δ- , γ-tocopherol	324	0.010	0.038	0.091
Chlorophylls	Chlorophyll a	668	0.087	0.106	0.176
	Chlorophyll b	648	0.025	0.038	0.062
	Pheophytin a	673	0.131	0.163	0.251
	Pheophytin b	661	0.050	0.079	0.115
Phenolic compounds	Oleuropein	310	0.083	0.094	0.116
	Vanillic acid	349	0.045	0.068	0.168
	Syringic acid	361	0.055	0.078	0.235
	Gallic acid	382	0.064	0.102	0.313
	p-Coumaric acid, Ferulic acid	416	0.066	0.107	0.409
	o-Coumaric acid	426	0.070	0.108	0.412
	Cinnamic acid, tyrosol	420	0.066	0.107	0.409
	Caffeic acid	457	0.066	0.107	0.335

Olive oil sample of Yasid (S_3) (3 years storage age) has the highest amount of all fluorescent components among the three regions at the same storage age. Saida (S_2) sample of olive oil (3 years storage age) has higher amount of fluorescent components than that in Allar (S_1) sample at the same storage age.

4.2 Absorption Wavelength Results

In this study, the absorption spectra of olive oil samples of Allar (S_1), Saida (S_2) and Yasid (S_3) were measured at different storage ages. The absorption spectra of olive oil samples of Allar (S_1) at 25°C at different storage ages (3 and 5 years) were plotted in Fig. 4.6.

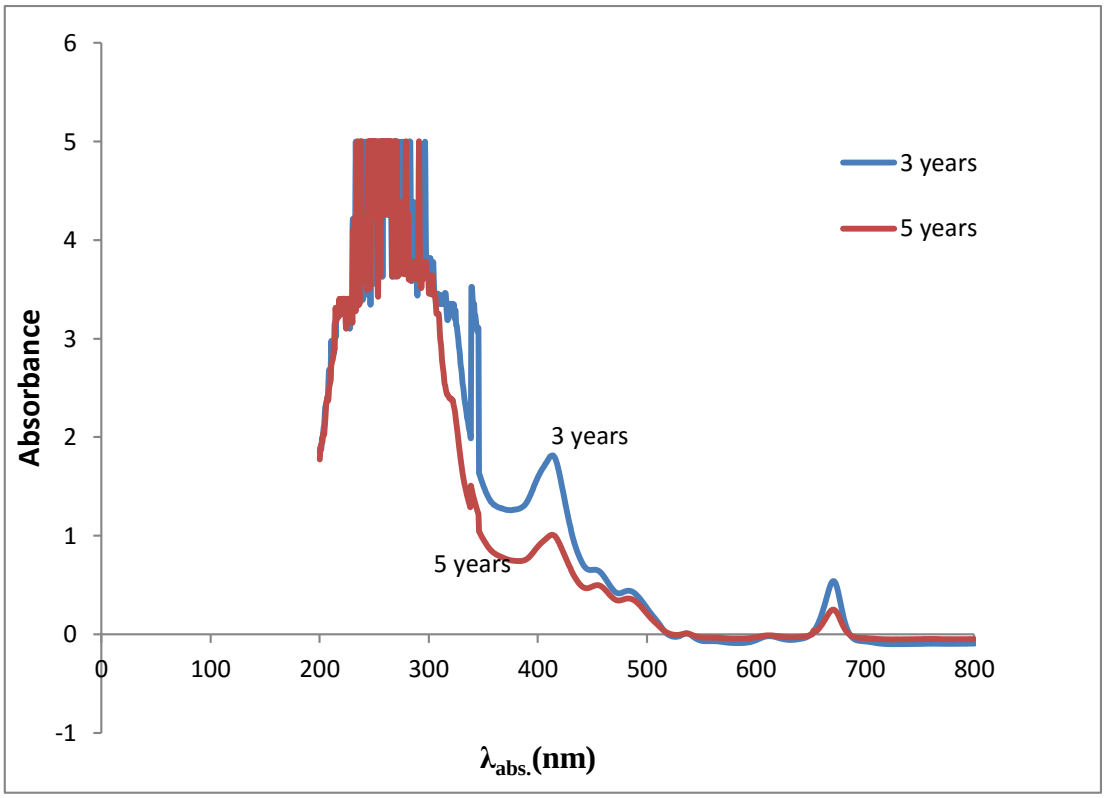

Figure (4. 6): The absorption spectra of olive oil samples of Allar (S_1) at 25°C at different storage ages (3 and 5 years)

The absorption wavelengths of some fluorescent components of olive oil are shown in Table 4.5.

Table (4. 5): Fluorescent components of olive oil with their absorption wavelengths

Substance	**$\lambda_{abs.}$ (nm)**	**Substance**	**$\lambda_{abs.}$(nm)**
Vitamin E		**Phenolic compounds**	
α-Tocopherol	292	Oleuropein	282
β- Tocopherol	296	Vanillic acid	260.2 – 291.4
δ- Tocopherol	298	Protocatechuic acid	259.0 – 293.0
γ- Tocopherol	298	P-hydroxyphenylacetic acid	274.4
α-Tocotrienol	292	Homovanillic acid	278.4
β- Tocotrienol	292	Syringic acid	273.4
δ- Tocotrienol	297	Gallic acid	270.6
γ- Tocotrienol	297	p-Coumaric acid	308.6
Chlorophylls		o-Coumaric	276.2 – 321.8
Chlorophyll a	430- 663	Cinnamic acid	
Chlorophyll b	455- 645	Tyrosol	275.2
Pheophytin a	409- 666	Caffeic acid	319.4
Pheophytin b	434-654	Ferulic acid	312.4
Pheophorbide a	409- 667		

The values of the absorbances of some absorption wavelengths of olive oil samples of Allar (S_1) at different storage ages are given in Table 4.6.

Table (4.6): The measured absorbances of some absorption wavelengths of olive oil samples of Allar (S_1)

Substance		$\lambda_{abs.}$ (nm)	Storage age (years)	
			3	5
			Absorbance	
Vitamin E	α-Tocopherol, α-and β-Tocotrienol	292	4.38	3.77
	β- Tocopherol	296	4.28	3.77
	δ- Tocopherol, γ-Tocopherol	298	3.77	3.61
	δ- Tocotrienol, γ-Tocotrienol	297	4.38	3.77
Chlorophylls	Chlorophyll a	430- 663	1.07- 0.30	0.66-0.10
	Chlorophyll b	455- 645	0.65- 0.04	0.50 -0.02
	Pheophytin a	409- 666	1.76 - 0.4	0.98-0.20
	Pheophytin b	434-654	0.90 -0.05	0.57-0.02
	Pheophorbide a	409- 667	1.77- 0.46	0.98-0.20
Phenolic compounds	Oleuropein	282	5.00	3.59
	Vanillic acid	260.2-291.4	5.00- 4.28	4.25-4.20
	Protocatechuic acid	259.0-293.0	5.00 -3.60	5.00-3.51
	P-hydroxyphenylacetic acid	274.4	3.77	3.65
	Homovanillic acid	278.4	3.66	3.65
	Syringic acid	273.4	5.00	3.77
	Gallic acid	270.6	4.25	4.25
	p-Coumaric acid	308.6	3.45	3.24
	o-Coumaric	276.2-21.8	5.00-3.35	3.77-2.37
	Tyrosol	275.2	4.26	3.65
	Caffeic acid	319.4	4.20	3.79
	Ferulic acid	312.4	4.48	4.36

The absorption spectra of olive oil samples of Saida (S_2) at different storage ages (3 and 5 years) were plotted in Fig. 4.7.

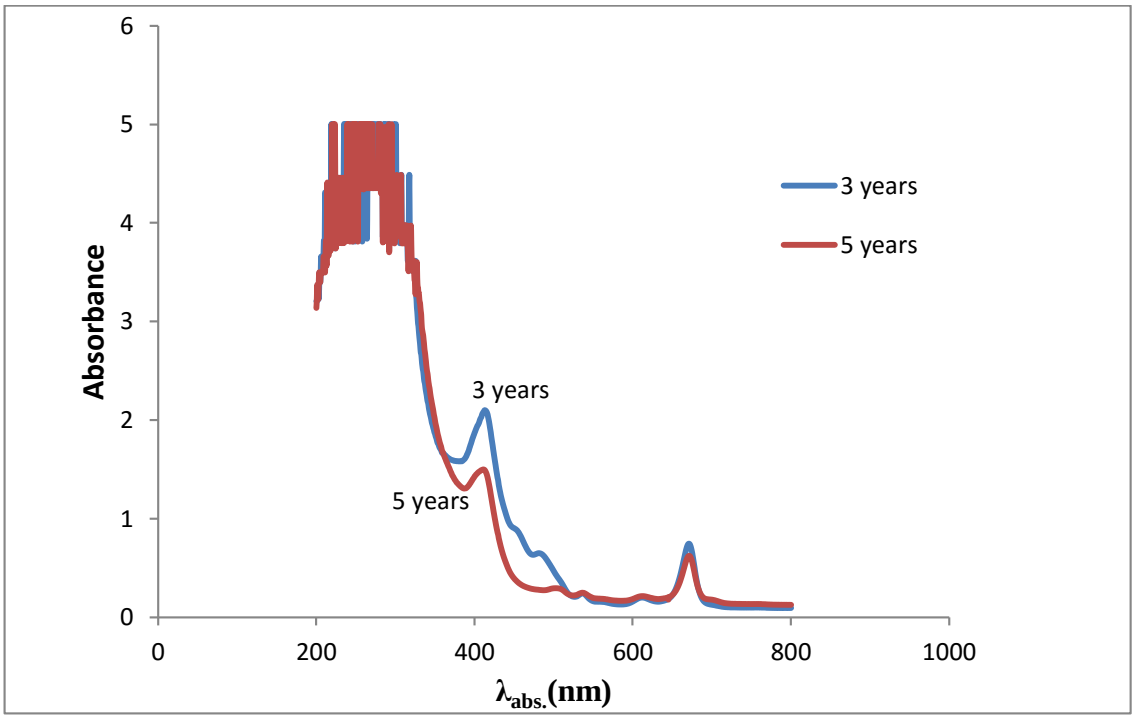

Figure (4. 7): The absorption spectra of olive oil samples of Saida (S_2) at 25°C at different storage ages (3 and 5 years)

The values of the absorbances of some absorption wavelengths of olive oil samples of Saida (S_2) at different storage ages are given in Table 4.7.

Table (4.7): The measured absorbances of some absorption wavelengths of olive oil samples of Saida (S_2)

Substance		$\lambda_{abs.}$ (nm)	Storage age (years)	
			3	5
			Absorbance	
Vitamin E	α-Tocopherol,α and β Tocotrienol	292	4.50	3.70
	β- Tocopherol	296	4.37	3.85
	δ-Tocopherol, γ- Tocopherol	298	4.49	3.97
	δ-Tocotrienol, γ- Tocotrienol	297	4.37	4.11
Chlorophylls	Chlorophyll a	430- 663	1.37-0.53	0.82-0.45
	Chlorophyll b	455- 645	0.87-0.19	0.350.18
	Pheophytin a	409- 666	2.04-0.28	1.49-0.26
	Pheophytin b	434-654	1.20-0.28	0.69-0.26

	Pheophorbide a	409- 667	2.04-0.67	1.49-0.56
Phenolic compounds	Oleuropein	282	4.35	4.30
	Vanillic acid	260.2-291.4	5.00-5.00	4.34-4.38
	Protocatechuic acid	259.0-293.0	4.46-5.00	4.35-4.37
	P-hydroxyphenylacetic acid	274.4	5.00	4.35
	Homovanillic acid	278.4	5.00	4.35
	Syringic acid	273.4	5.00	4.35
	Gallic acid	270.6	5.00	4.35
	p-Coumaric acid	308.6	3.97	3.79
	o-Coumaric	276.2-321.8	5.00-3.61	4.35-3.52
	Tyrosol	275.2	5.00	4.35
	Caffeic acid	319.4	3.97	3.61
	Ferulic acid	312.4	3.97	3.79

The absorption spectra of olive oil samples of Yasid (S_3) at different storage ages (3 and 5 years) were plotted in Fig. 4.8.

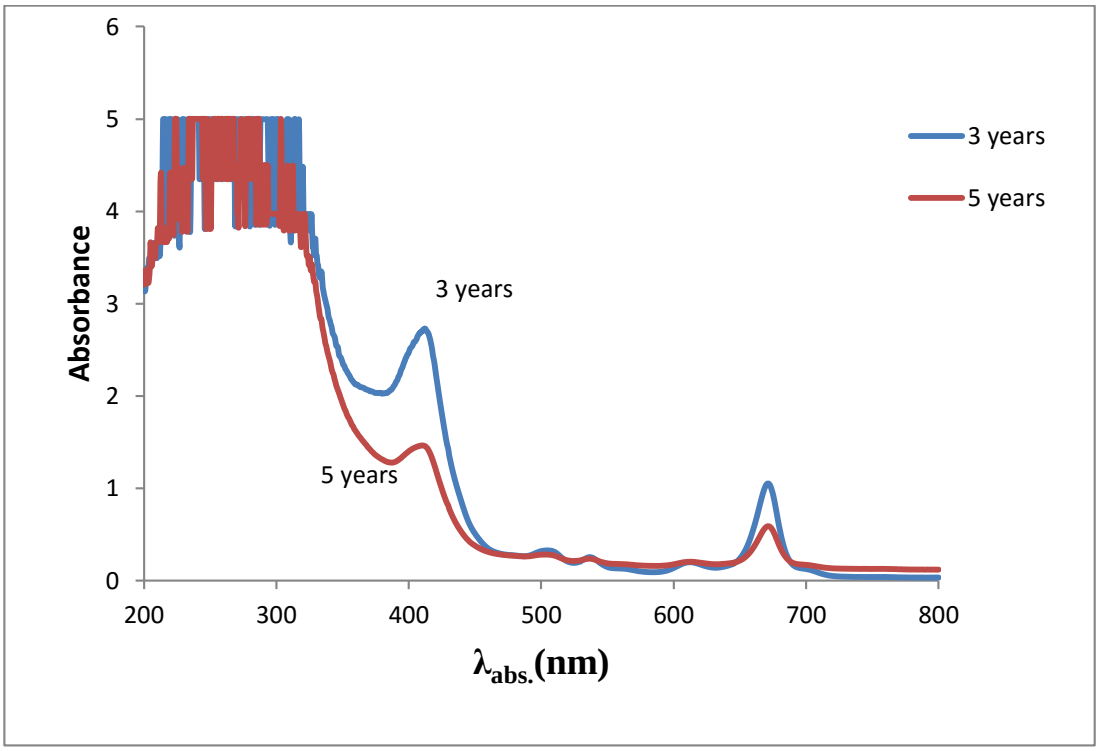

Figure (4. 8): The absorption spectra of olive oil samples of Yasid (S_3) at 25ºC at different storage ages (3and 5 years)

The values of the absorbances of some absorption wavelengths of olive oil samples of Yasid (S_3) at different storage ages are given in Table 4.8.

Table (4.8): The measured absorbances of some absorption wavelengths of olive oil samples of Yasid (S_3)

Substance		$\lambda_{abs.}$ (nm)	Storage age (years)	
			3	5
			Absorbance	
Vitamin E	α-Tocopherol, α-and β-Tocotrienol	292	5.00	4.48
	β- Tocopherol	296	5.00	3.97
	δ-Tocopherol,γ- Tocopherol	298	5.00	3.97
	δ-Tocotrienol,γ-Tocotrienol	297	4.36	3.97
Chlorophylls	Chlorophyll a	430- 663	1.40-0.70	0.80-0.43
	Chlorophyll b	455- 645	0.41-0.19	0.33-0.18
	Pheophytin a	409- 666	2.69-0.88	1.46-0.50

	Pheophytin b	434-654	1.13-0.33	0.66-0.25
	Pheophorbide a	409- 667	2.69-0.93	1.46-0.53
Phenolic compounds	Oleuropein	282	5.00	4.35
	Vanillic acid	260.2-291.4	5.00-5.00	4.35-4.35
	Protocatechuic acid	259.0-293.0	5.00-5.00	4.3514-4.35
	P-hydroxyphenylacetic acid	274.4	5.00	4.35
	Homovanillic acid	278.4	5.00	4.35
	Syringic acid	273.4	5.00	4.35
	Gallic acid	270.6	5.00	4.48
	p-Coumaric acid	308.6	5.00	4.36
	o-Coumaric	276.2-321.8	5.00-3.97	4.48-3.79
	Tyrosol	275.2	5.00	4.35
	Caffeic acid	319.4	4.20	3.79
	Ferulic acid	312.4	4.48	4.36

The amount of the fluorescent components of all the samples decreases as the storage age increases. The absorbance of olive oil samples of Yasid (S_3) (3 years storage age) is higher than that of Saida (S_2) and Allar (S_1) (3 years storage age), and the absorbance of Saida (S_2) is higher than that of Allar (S_1) (3 years storage age).

The absorption spectra of olive oil samples of Allar (S_1) (3 years storage age) were measured at different temperatures as shown in Fig. 9.

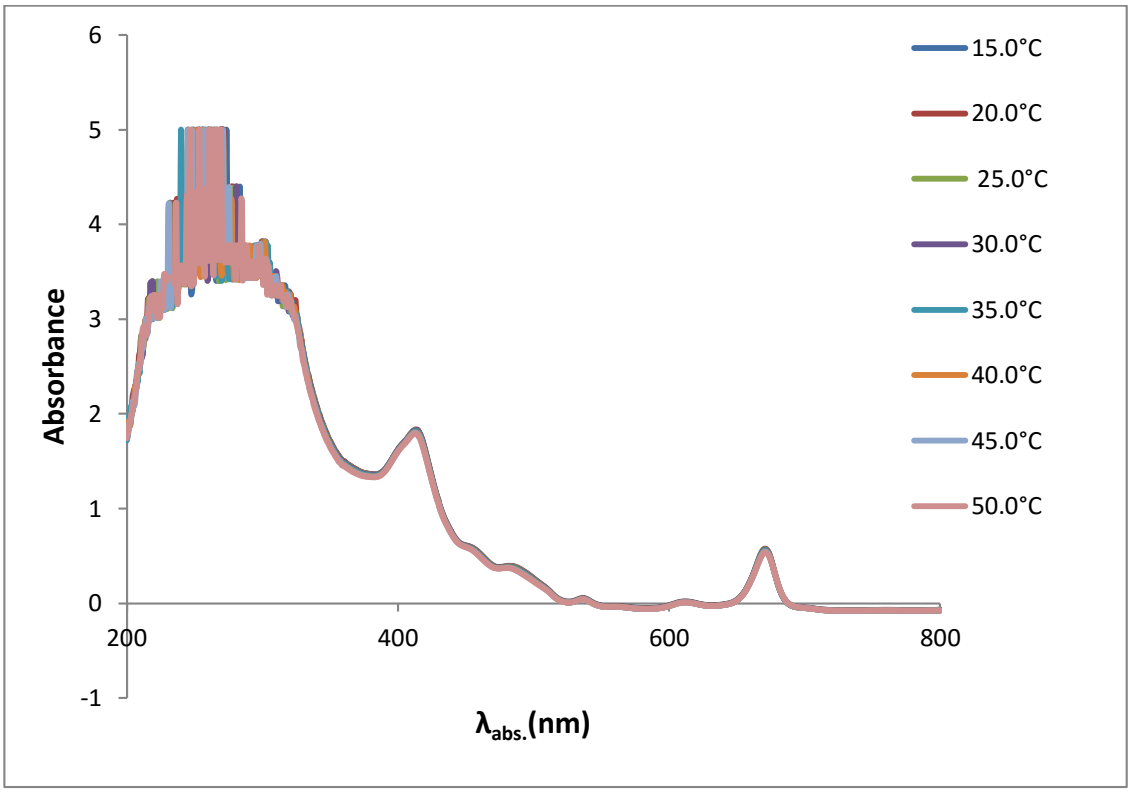

Figure (4. 9): The absorption spectra of olive oil samples of Allar (S_1) (3years storage age) at different temperatures

The temperature ranged from 15.0°C – 50.0°C does not affect the absorption spectra of olive oil.

4.3 Physical Properties

The results of measured physical properties are presented in this study; viscosity, refractive index, acidity and mass density.

4.3.1 Viscosity Results

The viscosity of olive oil samples at different storage ages were measured at 25°C as given in Table 4.9.

Table (4. 9): The measured viscosities of olive oil samples

Allar (S_1)		**Saida (S_2)**		**Yasid (S_3)**	
Storage age (years)	**$\eta(cP)$**	**Storage age (years)**	**$\eta(cP)$**	**Storage age (years)**	**$\eta(cP)$**
3	62.0	0	83.5	3	63.0
5	58.7	1	74.3	4	61.7
16	44.1	3	62.5	5	59.1
17	42.0	5	58.9	8	56.2
		7	50.4		

The viscosity of olive oil samples of Saida (S_2) at different storage ages were plotted as shown in Fig. 4.10

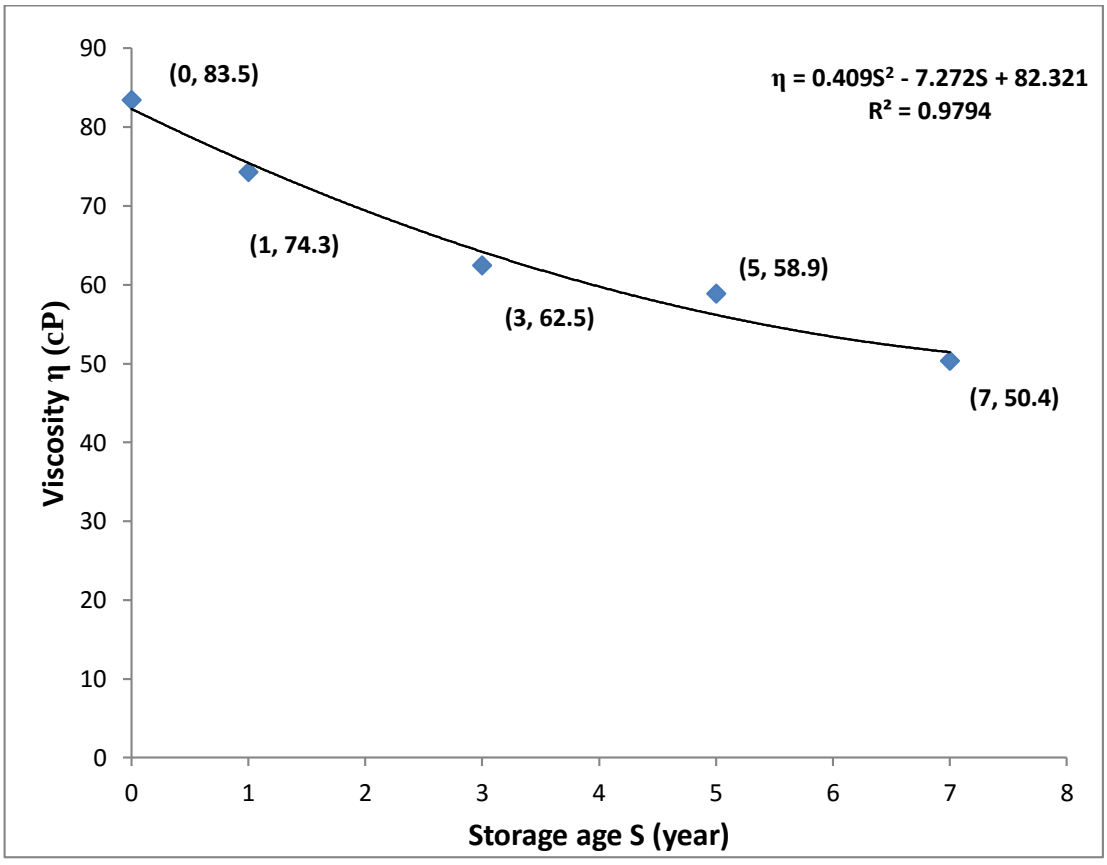

Figure (4. 10): The measured values of viscosity of olive oil samples of region S_2 at different storage ages at 25°C

The fitting equation of the viscosity of olive oil with the storage age is given by

$$\eta = 0.409S^2 - 7.272S + 82.321 \qquad (4.2)$$

The viscosity of olive oil samples is inversely proportional to the storage age. The storage age of olive oil can be known from its viscosity at 25°C from equation (4.2).

4.3.2 Refractive Index Results

The refractive index of olive oil samples at different storage ages were measured at 25°C as given in Table 4.10.

Table (4. 10): The measured refractive indices of olive oil samples

Allar (S_1)		**Saida (S_2)**		**Yasid (S_3)**	
Storage age (years)	**Refractive Index (n)**	**Storage age (years)**	**Refractive Index (n)**	**Storage age (years)**	**Refractive Index (n)**
3	1.4673	0	1.4681	3	1.4676
5	1.4671	1	1.4679	4	1.4672
16	1.4668	3	1.4674	5	1.4673
17	1.4666	5	1.4672	8	1.4670
		7	1.4670		

The refractive index of olive oil samples of Saida (S_2) at different storage age is plotted as a function of storage age as shown in Fig. 4.11.

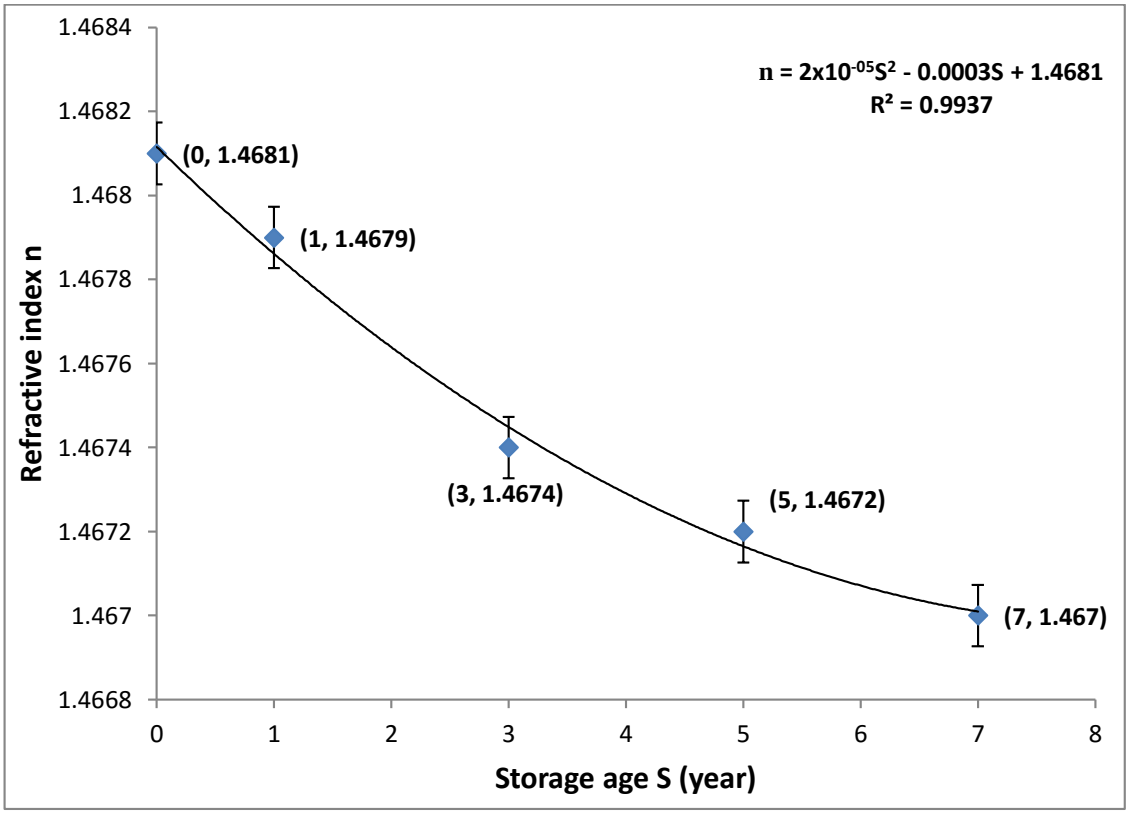

Figure (4. 11): The measured values of refractive index of olive oil samples of Saida (S_2) as a function of storage age at 25°C

The proposed refractive index of olive oil with the storage age is given by the relation

$$n = 2\times10^{-05}S^2 - 0.0003S + 1.4681 \qquad (4.3)$$

The refractive index of olive oil samples is inversely proportional to the storage age. The storage age of olive oil can be known from its refractive index at 25°C from equation (4.3).

4.3.3 Acidity Results

The values of acidity of olive oil samples at different storage ages were measured at room temperature are given in Table 4.11.

Table (4. 11): The measured acidities of olive oil samples

Allar (S_1)		Saida (S_2)		Yasid (S_3)	
Storage age (years)	**Acidity (FFA %)**	**Storage age (years)**	**Acidity (FFA %)**	**Storage age (years)**	**Acidity (FFA %)**
3	2.12	0	0.36	3	2.42
5	2.58	1	0.96	4	2.65
16	6.57	3	2.32	5	3.00
17	7.15	5	2.98	8	5.99
		7	4.87		
		16	10.32		
		17	11.48		
		18	12.03		

The acidity of olive oil samples increases as the storage age increases. According to Table 4.11, the acidity of olive oil samples of Yasid (S_3) is higher than the acidity of olive oil samples of Allar (S_1) and Saida (S_2) of the samples of 3 and 5 years storage age. The acidity of Yasid (S_3) is higher than that of Allar (S_1) at same years.

The acidity of olive oil samples of Saida (S_2) at different storage ages are plotted as a function of storage age in Fig. 4.12.

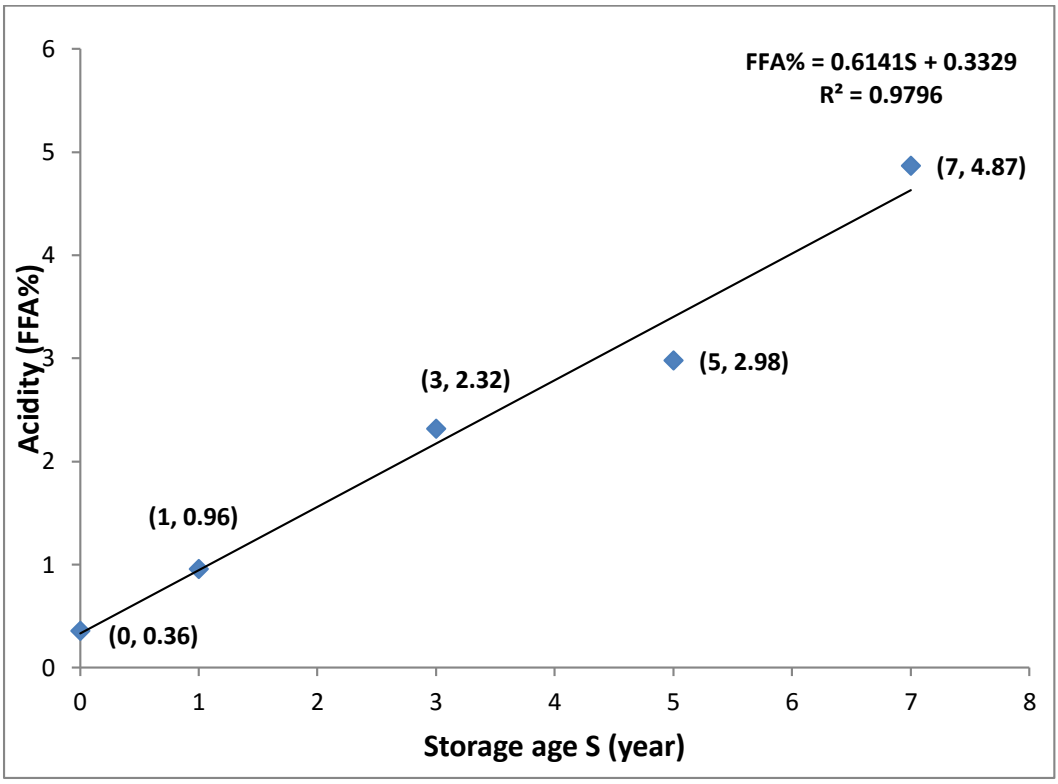

Figure (4. 12): The measured values of acidity of olive oil samples of Saida (S_2) at different storage ages at 25°C

The proposed fitting acidity of olive oil of Saida (S_2) percentage as a function of the storage age is given by the following relation

$$FFA\% = 0.6141S + 0.3329 \qquad (4.4)$$

The acidity of olive oil samples is directly proportional to the storage age. The storage age of olive oil can be known from its acidity at 25°C from equation (4.4).

4.3.4 Mass Density Results

The mass density of olive oil samples at different storage ages were measured at 25°C are given in Table 4.12.

Table (4. 12): The measured mass densities of olive oil samples

Allar (S_1)		**Saida (S_2)**		**Yasid (S_3)**	
Storage age (years)	**ρ(gm/cm^3)**	**Storage age (years)**	**ρ(gm/cm^3)**	**Storage age (years)**	**ρ(gm/cm^3)**
3	0.90946	0	0.9107	3	0.90949
5	0.90921	1	0.9103	4	0.90934
16	0.90531	3	0.90948	5	0.90924
17	0.90419	5	0.90922	8	0.90792
		7	0.90782		

The mass density of olive oil samples of Saida (S_2) are plotted as a function of storage age as shown in Fig. 4.13.

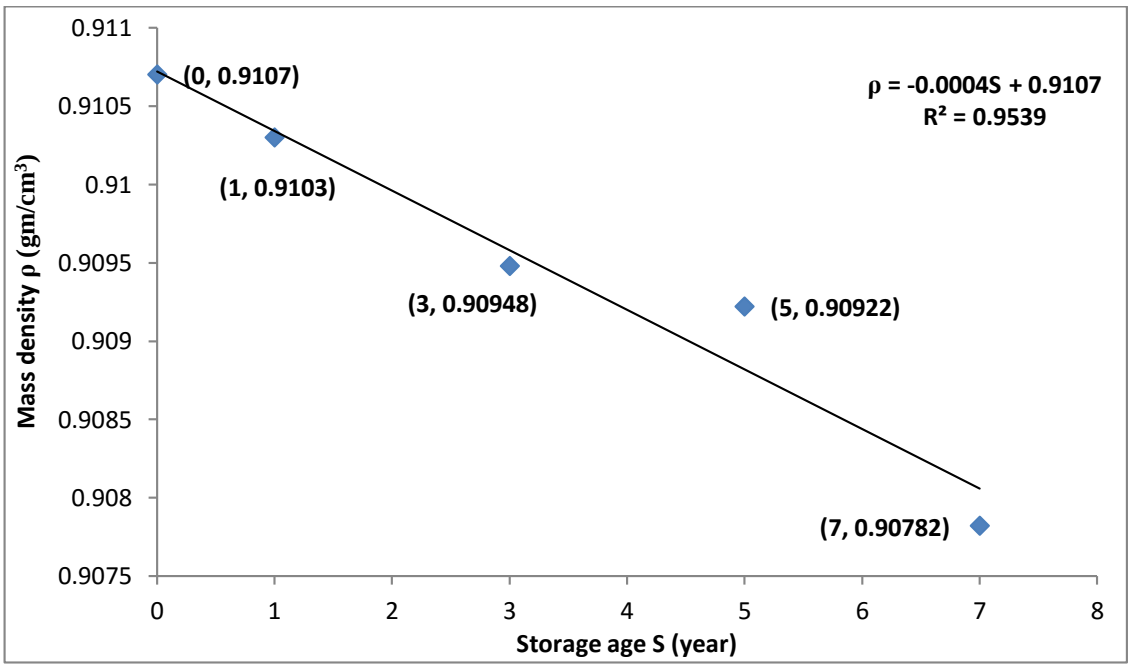

Figure (4. 13): The measured values of mass density of olive oil samples of Saida (S_2) as a function of storage age at 25^{o}C

The proposed fitting mass density of olive oil with the storage age is given by

$$\rho = - 0.0004S + 0.9107 \qquad (4.5)$$

The mass density of olive oil samples is inversely proportional to the storage age. The storage age of olive oil can be known from its mass density at 25^{o}C from equation (4.5).

Chapter Five

Discussion and Conclusion

5.1 Discussion and Conclusion

The results of the emission and absorption spectra of olive oil samples at different storage ages give indication about how the amounts of the fluorescent components of olive oil are affected by storage age. All vitamin E components (α-, β-, δ-, γ-tocopherol and α-, β-, δ-, γ-tocotrienol) decrease as the storage age increases. Chlorophyll a, b and pheophytin a, b decrease as the storage age increases. Phenolic compounds (oleuropein, vanillic acid, syringic acid, gallic acid, p-coumaric acid, o-coumaric, cinnamic acid, tyrosol and caffeic acid) decrease as the storage age increases. This result agrees with Mailer and his group study. They studied some Australian olive oil samples stored in different types of containers after 2, 4, 8, 16, 32 and 52 weeks. They found that phenols, chlorophylls and α-tocopherol decrease with storage age (Mailer *et al*., 2012).

Phenolic compounds, chlorophylls and vitamin E decrease in Australian olive oil as the storage age increase because they resist oxidation, they act as antioxidants and increase the shelf life of olive oil. The decrease of chlorophyll (green pigment in virgin olive oil) because after storing or heating it converts to yellow pigment (pheophtins) and brown pigment (pyropheophytins), this is a disadvantage (Mailer and Graham, 2009).

Australian olive oil of 1 and 3 years storage age 58% and 40 % of α-tocopherol from its orginal value (Mailer *et al*., 2012; Mailer and Graham,

2009) . Olive oil sample of Saida (S_2) contains 25 % and 15 % of α-tocopherol after 1and 3 year storage age from its original value. The loss of α-tocopherol in Australian Olive oil is less than that in the samples of this work.

Viscosity of the sample of Saida (S_2) of 0 years storage age at 25ºC is 83.5 cP agrees with the standard value of the International Olive Coucil for virgin olive oil which is 84 cP (IOC, 2015). Viscosity of the samples of 1, 3 and 5 years storage age at 25ºC are 74.3 cP, 62.5 cP and 58.9 cP agree with the measured values by Adnan and Robert which is 63.61 cP at 25ºC (Adnan *et al*; 2009, Robert *et al*; 1980). The viscosity of the samples of 7 years storage age at 25ºC is 50.4 cP. This value does not agree with Adnan and Robert study. The differences in the measured value of the viscosity of the samples of 7 years storage age of this study and Adnan and Robert study are due to the decrease of the fluorescent components by storage age. The viscosity was affected by the fatty acid and wax composition. The wax composition is influenced by cultivar, storage age and processing (Boskou, 2006).

Refractive index of the samples of Saida (S_2) of 0, 1, 3 and 5 years storage age are 1.4681, 1.4679, 1.4674 and 1.4672 agree with the standard values of Palestinian Standard. Palestinian Standard refractive index is 1.4677 – 1.4705 (PS188, 1997). The refractive index of the sample of 7 years storage age at 25ºC is 1.4670. This value do not agree with the standard value.

The results of this study show that the refractive index decreases as the storage age increases. The change is because of the fluorescent components

amount decreases with age olive oil becomes less dense and the speed of light in it increases so the refractive index decreases.

Acidity for the samples of Saida (S_2) of 0 and 1 year's storage age are 0.36% and 0.96%, according to Table 3.4 these two samples are considered as virgin olive oil. Acidity for the samples of 3 and 5 years storage age are 2.32% and 2.98% these two samples considered as ordinary olive oil (edible), their acidity values are in good agreement with the standard values of International Olive Council because they are below 3.3 %. The acidity of the sample of 7 years storage age is 4.87 % this sample is considered as lampante oil.

The results of this study showed that the acidity increases as the storage age increases, indicating that olive oil stored for more than 5 years becomes lampante oil and in good met with Nierat and Bahti studies (Nierat *et al*., 2014; Bahti *et al*., 2015).

Mass density of the samples of Saida (S_2) of 0, 1, 3 and 5 years storage age are 0.9107 g/cm^3, 0.9103 g/cm^3, 0.90948 g/cm^3 and 0.90922 g/cm^3 agree with the Palestinian Standard which is 0.910 g/cm^3 – 0.916 g/cm^3 (PS188, 1997). The mass density of the sample of 7 years storage age is 0.90782 g/cm^3. It does not agree with the standard values.

The results of this study showed that the mass density decreases as the storage age increases. This decrease because the fluorescent components amount decreases with age. The final result of this study shows that olive oil of storage age less than 5 years is considered as an edible olive oil.

Olive oil samples of Yasid (S_3) has the highest amount of fluorescent components than olive oil samples of Allar (S_1) and Saida (S_2) may because Yasid (S_3) altitude (698 m) is the highest among the three regions. Saida (S_2)

has higher amount of fluorescent components than Allar (S_1) may because Saida (S_2) altitude (374m) is higher than Allar (S_1) altitude (238 m) (http://www.Palweather, 2016). The fluorescent components amount in olive oil increases as the altitude of the region increases. Amount of annual rainfall of Yasid (S_3) of 2007 year (574.0 mm) is lower than that of Allar (S_1) and Saida (S_2) which is 581.9 mm. The amount of fluorescent components increases as the annual rainfall decreases (http://www.Pcbs., 2008).

5.2 Recommendations

Olive oil should be kept in bottles, and stored in dark place at room temperature, in order to minimize the loss of fluorescent components (vitamin E, chlorophylls and phenolic compounds). Olive oil of storage age less than 5 years is considered as an edible olive oil, if the storage conditions are ideal as the recommended in this work.

We suggest to build a central laboratory to test the olive oil quality using the fluorescence spectroscopy technique, according to the percentage of the fluorescent components of olive oil.

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كلية الدراسات العليا

تحليل زيت الزيتون الفلسطيني لأعمار التخزين المختلفة باستخدام تقنية مطيافية الوميض

اعداد
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قدمت هذه الاطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الفيزياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس – فلسطين
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الملخص

يركز هذا البحث على تأثير عمر التخزين لزيت الزيتون الفلسطيني على أطياف الانبعاث والامتصاص باستخدام تقنية مطيافية الوميض, بالإضافة إلى ذلك تأثير عمر زيت الزيتون على الخصائص الفيزيائية, حيث تم قياس اللزوجة و معامل الانكسار و الحموضة و الكثافة الكتلية.

نتائج طيف الانبعاث والامتصاص لعينات زيت الزيتون من أعمار تخزين مختلفة تعطي مؤشراً عن كيفية تأثر كمية المواد المفلورة بأعمار التخزين. لقد لوحظ ان جميع مكونات فيتامين هـ, مكونات الكلوروفيل والمركبات الفينولية في زيت الزيتون تنخفض بزيادة عمر التخزين.

اللزوجة لعينة زيت الزيتون (S_2) من عمر تخزين 5 سنوات على درجة حرارة 25°س هي 58.9cP, معامل الانكسار هو 1.4672, والحموضة هي 2.98% والكثافة الكتلية هي 0.90922 غم/سم^3, وهذه تنسجم مع المقياس الفلسطيني لمعيار زيت الزيتون.

اللزوجة و معامل الانكسار و الكثافة الكتلية تنخفض بزيادة عمر التخزين لعينات زيت الزيتون لأعمار تخزين مختلفة ، في حين أن الحموضة تزداد بزيادة عمر التخزين لزيت الزيتون لذا يعتبر زيت الزيتون من عمر تخزين أقل من 5 سنوات زيت زيتون صالح للأكل بناء" على المقياس الفلسطيني لمعيار زيت الزيتون.