An-Najah National University

Faculty of Graduate Studies

The effect of harvesting date on the oil chemical properties of Nabali Baladi olive cultivar

By

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Dedication

My great teacher and messenger, Mohammed (May Allah bless and grant him), who taught us the purpose of life.

Prisoners and martyrs hope for the Liberation of Palestine.

My parents whom continues to learn and develop, whose has been a source of encouragement and inspiration to me throughout my life.

My wife icon of sincerity, brothers, sister, sons and my daughter; the completion of this work was not possible without their support and help.

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Last but not least my thanks and gratitude to my friends and colleagues in my work for their help and support.

الإقرار

انا الموقع ادناه مقدم الرسالة التي تحمل العنوان:

The effect of harvesting date on the oil chemical properties of Nabali baladi olive cultivar

اقر بان ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد ، وان هذه الرسالة ككل ، أو أي جزء منها لم يقدم لنيل أية درجة أو لقب علمي أو بحثي 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.

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Date: التاريخ: ١٠/١١/٢٠١٦

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List of Abbreviations

Abbreviation	Full Name
CV	Cultivar
^{o}C	Centigrade
ΔK	Delta K, The variation of the specific extinction
FFA	Free Fatty acid
FAC	Fatty Acid Composition
FAME	Fatty Acid Methyl Esters
IOC	International Olive Oil Council
K_{232}	Extinction Coefficient for ultraviolet of 232 wavelengths
K_{270}	Extinction Coefficient for ultraviolet of 270 wavelengths
Meq	Milliequivalent, unit for the concentration of electrolytes
MI	Maturity Color Index
MUFAs	Monounsaturated Fatty Acids
Mm	Milli meter
NB	Nabali Baladi
OO	Olive Oil
OSI	Oxidative stability Index
PUFAs	Polyunsaturated Fatty Acids
PV	Peroxide Value
SFAs	Saturated Fatty Acids
TP	Total phenols, Total Polyphenol
USFs	Unsaturated Fatty Acids
UV	Ultra violet
VOO	Virgin Olive Oil
EVOO	Extra Virgin Olive Oil

The effect of harvesting date on the oil chemical properties of Nabali Baladi olive cultivar

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Abstract

Palestine is considered one of the original olive agricultural sites around the world. Harvesting date is one of the most important factors that contribute to oil quality. This study was conducted to investigate the effect of harvesting date of olives on oil chemical properties using Nabali Baladi (NB) cultivar. The study was conducted in a commercial olive orchards of NB planted in the Asira al Shamaliya village. Nine samples were taken starting from the September 20th 2014 of 10 days interval, each sample was tested for fruit characteristics including maturity index, moisture content ratio, fruit weight, length and width. The oil extracted by mechanical method was tested for acidity, peroxide, UV Light extinctions K, oil fatty acids composition, pigments, polyphenols and oxidative stability index. The oil content ratio was significantly increased during the harvesting dates, clear correlation was observed between the color index and each of the rate of fruit weight, oil content (85% and 98% correlation coefficient) respectively. Acidity, peroxide values were increased during harvesting dates. K_{232}, K_{272}, ΔK were within the limits of the International Oil Council specifications for extra virgin oil. Increasing of the linoleic

acid ratio from 7.38% to 14,38% was observed, however, stearic ratio was decreased from 6.53% to 4,47%. Oleic and palmitic ratio was ranged between 71%, 14.7% respectively. Both chlorophyll and carotenoid were decreased from (27.4, 10.9) mg/kg to (14.1,6.3) mg/kg respectively. Total polyphenols have no significant differences during the harvest dates. The lowest value was observed in the last sampling date (129 mg/kg) and the highest value in the 1/11 date (445 mg/kg). The results obtained could be useful for determining the proper harvesting date .

Chapter One
Introduction

The cultivated Olive (Olea europaea L.) is a long-lived evergreen tree native to the Mediterranean basin. [1] It is the most important fruit trees produced commercially in most of the Arab countries .

Palestine is a major producer of olives with an annual average production of 200,000 tons fruit and 20000 tons of oil , this qualifies Palestine to be the 12th in the world in olive oil production.[2] The total area of olive tree cultivated lands is about 100,000 hectare, which forms about 50% of total cultivated land, and about 80% of land cultivated in Fruit trees.[3] Olive cultivation is concentrated in the northern provinces, particularly Nablus area of 18000 hectares, that follow the climate of the Mediterranean sea, hot, dry summers, rainy and cold in winter and an annual rainfall of 660 mm according to the Palestinian Meteorological data. In addition, the Palestinian oil is considered to be of high quality among other olive oils in the world.[4] There are several cultivars of olive tree in Palestine but the Nabali Baladi olive cultivar is the most prevalent due to its ability to adapt to rain-fed agriculture and high oil content, and most of the NB cultivar yield is processed for oil.[5] Olive oil considered as essential consumptive food to the Palestinian people, with an estimated average annual per capita consumption of 3.6 liters.[6] Olive oil is rich in monounsaturated fatty acids, it is a major component of the Mediterranean diet, where it is attributed to protected from cardiovascular diseases compared to other parts of the world. Olive oil contains antioxidants such as pigments (chlorophyll

and carotenoids) in addition to vitamin E and phenolic compounds and is considered one of the most components that influence the quality of the oil.[5] The most important characteristic of olive cultivation in Palestine that its mainly organic farming and manual harvesting. Moreover modern olive presses extract olive oil, therefore, Palestine olive oil has a comparative advantage. Most of the research recently emphasizes on the importance of olive oil with good international standard.[2] Several factors affect oil quantity and quality, among these are cultivar, cultural practices , harvesting method, processing, handling and storage, and harvesting time. It is well known that oil quality is highly affected by the fruit maturity , oil quality determined by the chemical and physical specifications based on standards and tests adopted by the IOC.[2][4] In spite of increase in the global demand for olive oil with high standards of quality, and the excess of oil production in Palestine, the ability of Palestinian olive oil competition in the global market is low and more research are needed to optimize the oil quality. The manipulation of the factors affecting oil quality will improve oil production with high standards.[5][7] Since the harvest date highly affects the oil chemical properties The main determinant of the oil quality[8], therefore, the objectives of this research were :

1- To study the effect of different harvesting date on pomological properties of NB cultivar.

2- To study the effect of harvesting date on oil chemical properties of Nabali olive cultivar.

3 – To study the effect of antioxidants contents of olive oil on the stability of oxidation index of oil of NB CV during crop maturity.

Chapter Two
Literature Review

2.1. Olive tree, fruit and oil for (CV Nabali Baladi)

Olive trees CV NB characterized as medium strength and scattered twigs and moderate canopy structure, flowers bloom is at end of March to April onset and lasts 30 days, the average weight of the fruit during the ripening 2.5 grams and the average seed weight 0.389 grams. Fruit color becomes black at maturity complete, and the average length of 2.2 cm and the average diameter 1.46 cm. For rainfed agriculture, the percentage loss of fruits is 3% until the harvest. Vegetative growth began in the onset of February. The seed formation began in mid-June and discoloration of the fruit occur after mid-October, and the rate values oil properties in West Bank are phenols 380 (mg/kg oil), Delta K -0.003, the percentage of oleic acid is 66.2% and palmetic acid is15.5 %.[4] [5]

Comparing the total production between rainfed growing trees and irrigated trees, the production was reduced 35%, however with lower polyphenol content.[8] When studying the effect of altitude on the oil content of the CV NB, an inverse relationship was observed, the lower altitude of the farm, the more oil content, but under high altitude the harvesting date was delayed, but with better oil quality.[9]

When comparing the number of olive cultivars under the same conditions, NB CV showed the highest oil ratio as well the quality of the oil within the Extra Virgin Olive Oil criteria among different olive cultivars in

Jordan.[8][10] The fruit weight of NB was increased with advancing maturity up to 2.5 grams at harvest.[10] Also, the oil of CV NB showed criteria of Extra Virgin Olive when comparing different number of samples from different locations including (Nablus, Qalqilya, Salfit and Jenin Governorates, in the northern of West Bank). Samples from Nablus showed higher values up to 72% oleic acid and polyphenols content of 469 (mg/kg oil).[11] In another study, during 2013, of different oil samples taken from different locations in Palestine, results showed that 35% of the sample were with acidity < 0.8 and PV <12 meq O_2/kg, these oil specification was corresponded to special class Premium Ultra-Fine Extra Virgin Olive Oil (PUF-EVOO) which it is important for global competition.[12]

2.2. Olive oil criteria

The chemical characteristics of olive oil give a clear indication about the quality of the oil. Absorption spectroscopy at specific wavelengths shows cases of mixing different oils, while peroxide value determines the amount of the aldehydic and ketonic harmful free radicals formation with bad smell. The acidity indicator for the decomposition of hydro- glycerols and separation of fatty acids, health benefits and the stability of oxidative stress index depend on the ratio of phenols and antioxidant pigments in olive oil.[5][12]

The consumption of organic olive oil with a high specification has significant health benefits and prevents harmful oxidizing substances in the

human body.[13] Also, the appropriate degree of crop maturity produce oil with high specifications and high nutritional value.[8] Quality criteria of olive oil requirements of the Palestinian Institution for Standards had matched with the international Oil Council for the classification depending on the sensory and chemical characteristics to achieve fair competition in trade and ensure the nutritional value of essential part of the population diet.[2] [12] [13]

Table 2.1: Olive oil quality criteria (IOC). [14]

Test	EXTRA VIRGIN	VIRGIN	ORDINARY VIRGIN
Free Acidity %	**≥ 0.8**	**≥ 2**	**≥3.3**
Peroxide Value meq. O_2/kg	**≥ 20**		
K_{232}	**≤ 2.5**	**≤ 2.6**	**---**
K_{270}	**≤ 0.22**	**≤ 0.25**	**≤ 0.30**
Δ K	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**
H_2O and Volatiles %	**≤ 0.2**		
Insoluble Impurities %	**≤ 0.1**		
Palmitic acid % C 16:0	**7.5-20.0**		
Stearic acid % C 18:0	**0.5-5.0**		
Oleic acid % C 18:1	**55.0-83.0**		
Linoleic acid % C 18:2	**3.5-21.0**		
Phenols content	**Olive CV affect, The country may require a more specific ratio.**		

2.3. The effect of fruit maturity on the chemical properties of olive oil

2.3.1. Acidity and peroxide

Free Fat Acidity: This is a crude indicator of the quality. It is a measurement of hydrolytic breakdown of the fatty acid chains from triglycerides into diglycerides and monoglycerides, liberating free fatty acids. It is usually expressed as percent (%) of free fatty acids on the basis of the oleic acid, because that is the dominant fatty acid in olive oil.[12]

Peroxide Value: This is a crude indicator of the amount of primary oxidation that has occurred, forming peroxide compounds within the oil. It is expressed as a value in milequivalents of free oxygen per kilogram of oil (meq O_2/kg). [12][14]

The acidity values increases during the progress of maturity dates While peroxide decreases as shown in many studies, The maturity date was the main factor influencing free acidity and PV.[15][16][17][18] However, other study showed, that the acidity values decreases during the progress of maturity dates While peroxide increases,[19][20] both FFA and PV were increased during the progress of maturity dates of NB CV in Jordan.[10] Another study indicated no specific relationship in FFA and PV decreases during ripening.[21]

2.3.2. UV Light extinctions K

It measures the quantity of certain oxidized compounds that resonate at wavelengths of 232 and 270 nanometers (nm) in the ultraviolet spectrum in a spectrophotometer.[10]

K232 index of conjugated poly-unsaturated fatty acids and K270 index for aldehyde and ketone substances. Through maturity, the value of K232 increase and decreases the value of K270.[15] Another study showed K232 decreases and K270 increases during maturation.[19] While in other studies, K232 and K270 decreases by maturity, both indicators depends on the olive cultivar and the date of maturity.[18][20][21] In a study of Chemlal olive CV in Algeria, no impact was observed on K270 by maturity, while K232 were increases through maturity.[16]

2.3.3. Fatty acid composition

FAC is a carboxylic acid derived from triglycerides that can measured by gas chromatography and can help distinguish between varieties. It is divided into saturated and unsaturated fatty acids including monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs).[22] Conjugated fatty acids are a subset of PUFAs. The middle carbon of the triglyceride molecule in natural virgin olive oil always contains the non-saturated fatty acids such as oleic or linoleic.[23]

In various experiments to study the relationship of the progress of maturity on the components of the fatty acids of different varieties of olive showed decreased linoleic and oleic increase during ripening.[15][17][24] There was no change in the percentage of oleic during maturation with increasing

linoleic.[16][18][19] The ratio of MUFAs / PUFAs was decreased,[16][19][21] the ratio of USFs / SFAs was increased.[16][20] Chemlali CV oil in Tunisia showed lower oleic content through ripening (Average 62%).[18] While kalamata CV oil in Egypt showed higher oleic content through ripening (Average 75%).[20]

2.3.4. Pigments (chlorophyll and carotenoid)

The color of green olives changed from green to yellowish color and purple to black during maturation with concomitant decrease in the proportion of chlorophyll and carotenoids and increase the ratio of anthocyanins. The chlorophyll and carotenoids pigments are fat soluble and give the oil its nutritional value and desirable color.[23] About 20% chlorophyll and 50% carotenoids remain in the oil after extraction. This percentage depends on the cultivar.[25] All studies showed decrease in chlorophyll and carotenoids during progress maturity.[10][16][18][19][21] These studies Showed that the change in chlorophyll and carotenoids content depends on the olive CV and not on the site change.[10][18] In Turkey, in Adana area, the pigments content of Gemlik CV was higher than Adana CV during maturity,[17] but for the most common two CVs in Turkey, the pigments content of Memecik CV was higher than Edremit CV during maturity.[19] While in Jordan, Abo-shoka CV, contains higher content of pigments than the Nabali and Nabali Mohasen CV,s during maturity.[10]

2.3.5. Total phenols content

Phenolic compounds are minor components of olive oil. Four groups of polyphenols are present in VOO: simple phenolic acids, cinnamic acids,

oleuropein derivatives, and flavonoids, oleuropein is the most abundant in VOO. These substances stop VOO autooxidation and give its remarkable thermal stability , as well as give its preferred flavor and taste.[26] Olive oil polar phenol part, known as “polyphenols” differ in phenolic compounds content ranges (50-1000 mg/kg) but values are usually between 100 and 300 mg/kg in many studies, hammer crushers are advised to obtain a higher content of phenols during olive oil extraction.[23] Studies (human, animal, *in vivo* and *in vitro*) have confirmed that olive oil phenolic compounds have positive effects on certain physiological parameters, such as plasma lipoproteins, oxidative damage, inflammatory markers, antimicrobial activity and bone health.[27] Many studies have shown that the content of phenols increased during maturation until it reached a maximum and then appeared to decrease with advancing maturity.[15][17][19][21] Other studies have shown a decrease of olive oil polyphenols content during maturation process.[16][20] Chimlali CV in Tunisia has a few polyphenols content,[18] while a higher polyphenols content of Gemlik than Adana CV’s during progressing maturity[17]. kalmata CV in Egypt, contains polyphenols higher than manzanillo CV.[20] Memecik and Edremit CV’s have a high content of polyphenols and there was a clear correlation between polyphenols and antioxidant activity of the oil, ($r = 0.98$).[19] A study showed that Polyphenols synthesis continued in Gordal Sevillana olive CV fruits during maturation.[24]

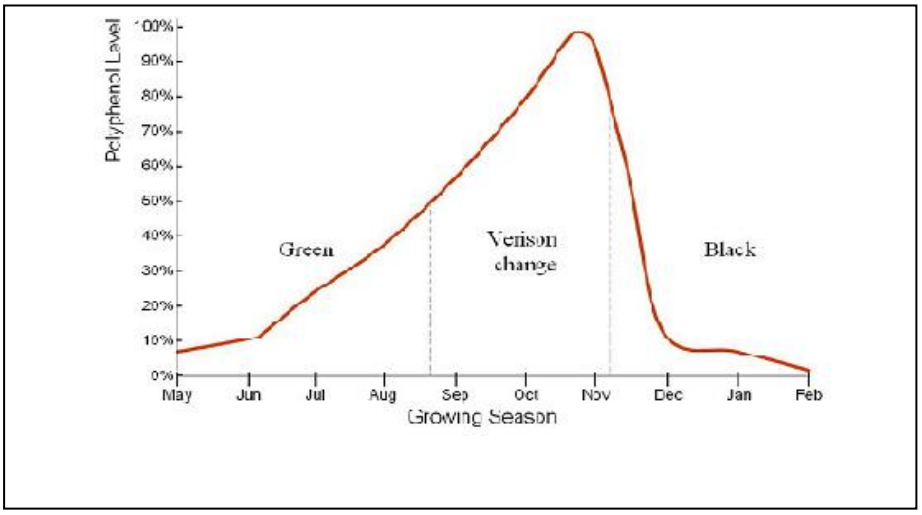


Figure 2-1: Olive fruit polyphenol level and color during growing season [12].

2.4. Oxidative stability

It is a measure of oil resistance to oxidation and all the oil constituents that change during the ripening and affect the quality of oil. It is important for oil storage and marketing for the longest time before the quality is deteriorating [17] .The most important components in olive oil, affecting the oxidative stability is PUFAs and the total phenols and pigments, but deterioration factors during storage including the exposure to oxygen, heat , light , the presence of enzymes, especially that run on oil deterioration in the lack of oxygen and production of free radicals instead of hydroperoxides.[23]

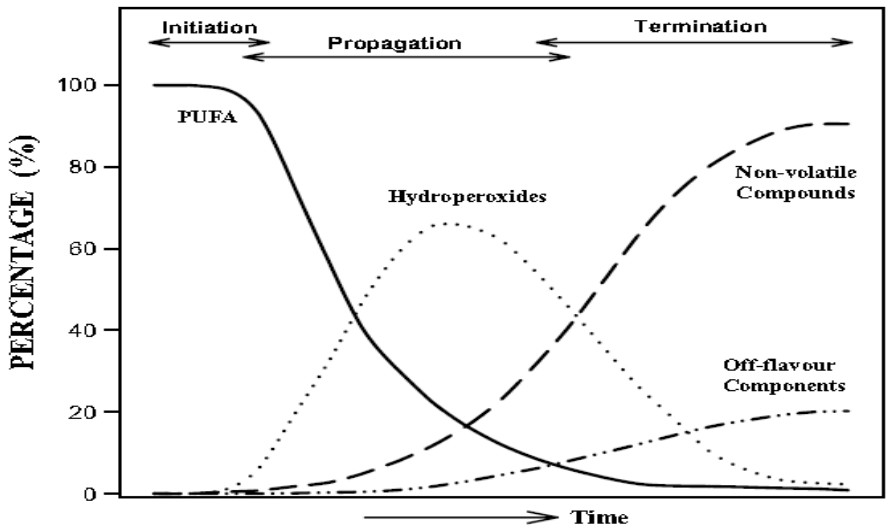


Figure 2-2: Oxidation of polyunsaturated fatty acids (*PUFA*) and formation of primary (hydro peroxides) and secondary (nonvolatile and off flavor component) products. [23]

- **PUFAs** affect the oxidative stability, because the rate of oxidation in oleic to linoleic and linoleic acid is 1:12:25. Olive oil with lower acidity is more oxidatively stable. [23]

- **Polyphenols** have antioxidants activity and works to remove free radicals in the case of oxidizing agents has increased and has a big role in shelf life.[17][28] Polar phenols are considered hydrophilic antioxidants and work through interface of oil-air and its impact oxidation resistance higher than lipophilic antioxidants.[26] .

- **Chlorophyll** works to stimulate oxidative stress in the light, while adversary in the dark, **however, carotenoids** protects the oil from photosensitized oxidation and works to quell oxidation reactions.[23][26]

- Thermal oxidation The thermal oxidation is much higher than autoxidation under the same conditions surrounding the degradation of oil , VOO have a low melting point lead to increase oil drain and reduce the amount of oil absorbed from fried food. [23][26]

During maturation of Chemlal CV in Algeria, EVOO oxidative stability was linked to polyphenols and the highest value for the oxidative stability were in November and then decrease over late maturity.[16] Several studies have shown that the oxidative stability increased at the beginning of maturity and then decreased for Adana, Gemlik' CV's , Memecik, and Edremit CV's in Turkey and Arbequina CV in Spain.[17][19][21] Another study in Egypt, indicated that the value of oxidative stability of Kalamata and Manzanillo CV's VOO was related to oil content of antioxidant during ripening. The highest value was in the early date of maturity of the olives.[20] A study to heat treatment of Picholine CV OO in Morocco showed oxidative stability of VOO was higher than of refined OO.[29]

Chapter Three
Methodology

3.1. Location and Plant Material

The study was conducted during the harvesting seasons of 2014 \ 2015 in a commercial olive orchards of NB CV, with an area of 2 donums. The orchard was selected as a representative for the areas in the northeast of Asira al Shamaliya village. The location is 600 m above sea level is just 1 km away from the village center, which is located north of Nablus province and seven kilometers away from the city center. Nablus is considered one of the northern provinces in the West Bank. It is bounded by Jenin in the north, Jericho in the east , Ramallah province in south and Qalqilya and Tulkarem in west.

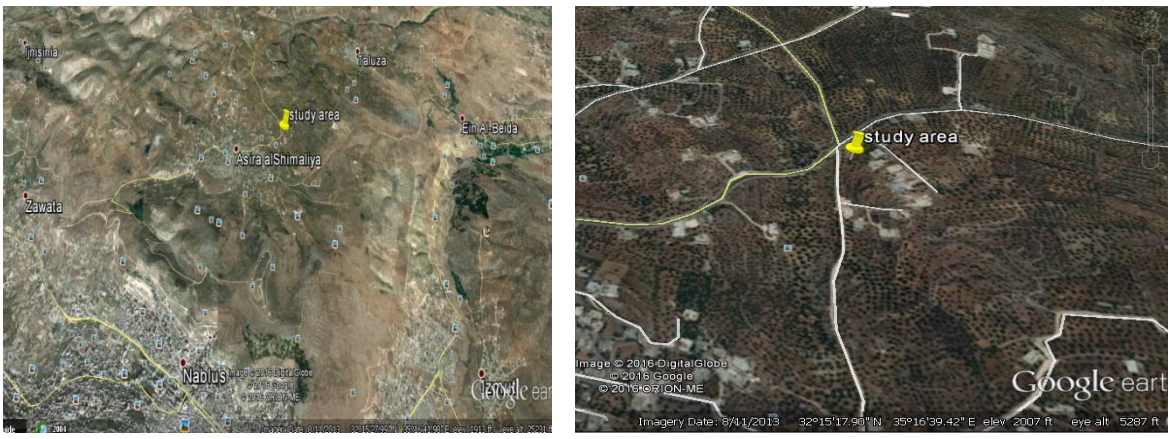


Figure 3-1: The location and the coordinates (32° 15' 16" N - 35° 16' 38" E) of study area (from the Google Earth site).

Trees planted by 10 meters spaces between the trees and 10 meters between rows as rainfed cultivation, however, the rain precipitation during the harvesting dates was 196.5 mm. The effective rainfall it was 182 mm in the

period from 1\11 to 20\12 . Cultivation procedures followed the common practice carried in the region of the plowing, pruning, and manual weeding. Two trees were selected randomly from the orchard represented them. Soil sample was tested showed it is clay loam soil and the content of organic matter was 3.5%.

Nine samples were taken starting from the 20\9\2014 to 20\12\2014 at subsequent harvest dates of 10 days apart. Fruit were picked manually from different parts, different heights, inside and outside of the tree canopy.

Figure 3-2: Trees and harvest method

The fruits were stored in a shaded dry area then were transferred to a laboratory of Agricultural Research Center (NARC) in Jenin province, where was physical examinations conducted on the fruits. The same olive fruit press was used to extract the oil by mechanical method used by local farmers, the weight of each sample was 10 kg (the least weight can be oil extraction by research olive pressing machine (TEM 3hp 220 v, Italy)).

Figure 3-3: Olive pressing machine

a knife crusher, with an horizontal malaxer with a 2 phase decanter of new generation was used. Extracted oil has been saved inside black bottles in order to conduct various chemical tests.

3.2. Physical analysis

3.2.1. Moisture content and oil content ratio in olive fruits

Materials

sample of 15 olive fruit, pestle grinder, drying oven (Raypa™ Spain), electronic balance has the limit measures of 0.1 g, Oil extraction (ankom ^{xt15}) device, Filter Bags (Fig 3-4) , Petroleum ether as solvent .

Method

The adopted method for determination the moisture content ratio[30] and oil content ratio based on the method of Rapid Determination of Oil Utilizing High Temperature Solvent Extraction[31], As follows:

Olive sample was crushed by pestle grinder then weighed with grinder and records the weight, it is placed in the oven at a temperature of 105 ° C for

24 hours then weighed the pestle grinder with dry weight of the sample after removing from the oven. Moisture content was calculated according to the following equation:

Figure 3-4: ankom ^{xt15}device

Moisture content ratio =

(Weight before entering oven - - weight after drying) / weight before entering the oven

Part of the dry weight of the sample between 1- 1.5 g was taken and was placed in a filter bags then weighed with the bag and then the bag was closed and was placed in the (ankom ^{xt15}) device to extract the oil by the solvent under heat process it took 15 minutes. After heat was finished, the bags were taken out and cooled under vacuum for 10 minutes. The filter bag was weighted and recorded after extraction .

Olive oil ratio Calculation

-Oil content ratio in the dry sample=

(Sample weight with the bag before extraction – Sample weight with the

bag after extraction) / dry weight of the sample

The dry weight ratio of the sample = 100 – Moisture content ratio

-Oil content ratio in the sample(fresh weight)= Oil content ratio in the dry sample * The dry weight ratio of the sample

3-2-2 Average weight, length and diameter of the olive fruit

The arithmetic average of the measurement of fifty olive fruits that randomly selected from harvested olive samples. Using millimeters graph paper. An electronic balance that has the limit measures of 0.1 g.

3.2.3. Olive maturity index

The World Oil Council method was used to find the olive maturity index depends on fruit colour change during ripening stages observed visually in olive cultivar . The skin usually turns from deep green to violet and black, the colour of the flesh also change during these ripening stages.[32] A hundred olive fruit were taken randomly from harvested sample were visually graded by classes as follow

Class 0: Skin color deep green.

Class1: Skin color yellow-green.

Class2: Skin color green with reddish spots on < half the fruit surface.

Class3: Skin color with > half the fruit surface turning reddish or purple.

Class4: skin color black with white flesh.

Class5: Skin color black with < half the surface turning purple.

Class6: Skin color black with not all the flesh purple to the stone.

Class7: Skin color black with all the flesh purple to the stone.

It used the following equation to find the maturity index

M.I = (**f**ruit **n**umber of **c**lass 0*0+ fnc1*1+ fnc2*2+ fn.c3*3+ fn.c4*4+ fnc5*4+ fnc5*5+ fnc6*6+ fnc7*7)/100

3-3. Chemical analysis

3.3.1. Main Instruments

3.3.1.1. (Jen Way Model 7305) spectrophotometer

Instrument Specification (look appendix)

Theory of spectrophotometer

The light is travelling through the sample, The Beer-Lambert

Law is used to relate the absorption of light to the properties

of the sample[33], That **A =ℰlc**

A is the absorbance,

ℰ is the molar absorption coefficient (l mol-1cm-1),

c is the concentration (mol l^{-1}) **l** is the path length (cm)

Absorbance is inversely related to transmittance.

3.3.1.2. (Thermo Finnegan Trace GC/MS ^{plus}) Gas Chromatographic Instrument Specification (look appendix)

Theory of GC/MS

The power of gas chromatography (GC) to separate compounds from a complex Mixture (Individual compounds travel along the long column at different rates, and as a result, emerge from the end of the column at different times. That is, they become separated on the column). GC is coupled with the ability of mass spectroscopy (MS) to identify those compounds (qualitative analysis), and accurately determine then amounts present (quantitative analysis) by (various techniques are used to ionize the emerging compounds then pass into an analyzer, that separates them and measures both their mass-to-charge ratio and intensity, generates a characteristic spectrum that enables identification). The chromatographic and mass spectral data is recorded by a computerized data system, which can also be used to analyze the information obtained and control the various instruments. [34]

3.3.2. Chemical analysis procedures

3.3.2.1. Acidity

Materials

Diethyl ether and 95 % (V/M **ethanol,** 1+1) mixture by volume, potassium hydroxide, standard, volumetric solution, (cKOH) = 0.1 mol/l , Phenolphthalein, 10 g/l solution in ethanol [95 % v/v)],sample of 10 g of oil, Usual laboratory apparatus.

Principle

Content of free fatty acids determined according to the procedure.[35] Acidity is expressed as a percentage by mass based on oleic acid. A test portion is dissolved in a mixed neutral solvent and titrated with an ethanol solution of potassium hydroxide.

Equation determine of acidity = **(V.C.M)/(10.m)**

V= is the volume, in millilitres, of the standard volumetric potassium hydroxide.

C= is the exact concentration, in moles per liter, of potassium hydroxide solution used= (0.1mol/l).

M= **Molar mass of oleic acid = 282 g/mol.**

m= is the mass, in grams, of the oil test sample.

3.3.2.2. Peroxide value

Material

Water, complying with grade 3 of ISO 3696, Glacial acetic acid, Isooctane, Acetic acid/isooctane solution [60:40 (by volume)], Potassium iodide solution (saturated) recently prepared and free from free iodine and iodates, Sodium thiosulfate solution c($Na_2S_2O_3$)= 0,01 mol/l, Starch solution 5 g/l, sample of 5 g of oil, Usual laboratory apparatus.

Principle

peroxide value = quantity of substances which oxidize potassium iodide in the sample according to the procedure,[36] expressed in terms of active oxygen, divided by the mass of the oil test sample. Usually expressed as milliequivalents O_2 per kilogram. An oil sample in solution in acetic acid and isooctane, is treated with a solution of potassium iodide. The liberated iodine is titrated with a standard volumetric sodium thiosulfate solution.

Equation determine of peroxide value = PV

Peroxide value expressed in mill equivalents of active oxygen per kilogram

$$\mathbf{P= 1000(V-V_0).C/m}$$

V= is the volume of sodium thiosulfate solution used for the determination, in milliliters

V_0= is the volume of sodium thiosulfate solution used for the blank determination, in milliliters, and it is ignored because it is small number.V_0= 0.

C= is the concentration of the sodium thiosulfate solution, in moles per litre = 0.01 mol/L

m = is the mass of the test portion, in grams.

3.3.2.3. K and ΔK analysis

Material

Jen Way spectrophotometer Model 7305, Rectangular quartz cuvettes, with covers, having an optical length of 1 cm, pure cyclohexane, distilled water, 1g oil sample.

Principle

The absorption at the wavelengths is due to the presence of conjugated diene and triene systems[12]. These absorptions are expressed as specific extinctions($^{1\%}E_{1cm}$) (the extinction of 1% solution of the fat in the specified solvent, in a thickness of 1 cm) conventionally indicated by K (also referred to as "extinction coefficient").by performing a spectrophotometric examination of fats in the ultraviolet at the specified wavelengths with reference to pure solvent[37].

Equation determine of K and ΔK value

$$K_{\lambda}=E_{\lambda}/(c.s)$$

$\mathbf{K_{\lambda}}$= specific extinction at wavelength ʎ

$\mathbf{E_{\lambda}}$ = extinction measured at wavelength ʎ . (ʎ=232 ,266,270,274)

C = concentration of the solution in g/100 ml

S= thickness of the cuvette in cm.(=1cm)

$\mathbf{\Delta K = K_{270} - (K_{266} + K_{274})/2}$

3.3.2.4. Chlorophyll and Carotenoid Content

Material

Jen Way spectrophotometer Model 7305, Rectangular quartz cuvettes, with covers, having an optical length of 1 cm, pure cyclohexane,7.5 g oil sample.

Principle

Oil color changes for different ripeness are directly related to pigment content , 7.5gm of the sample in 25ml of the cyclohexane.[38] The absorption maximum at 670 nm is usually considered to be related to the chlorophyll fraction, pheophytin being its major component. The dominant pigment in the carotenoid fraction is lutein, and the absorption maximum at 470 nm is related to lutein.[39]

Equation determine of Chlorophyll and Carotenoid Content:

chlorophyll (mg/kg) = (A670*1000000)/(613*100*d)

carotinoid(mg/kg) = (A470*1000000)/(2000*100*d)

Note: d is the spectrophotometer cell thickness (1 cm).

3.3.2.5. Total Polyphenol Content

Material

Jen Way spectrophotometer Model 7305, Folin – Ciocalteus phenol (Sigma-Aldrich) reagent solution 1:100 (V/V) in bidistilled water, Sodium Carbonate (Na_2CO_3) 20 % (W/V), standard solution of gallic acid 10 mmol/L methanol 96 % (V/V) (Faluka) , Bidistilled water, 10 g olive oil sample .

Principle

T.P.C was determined using spectrophotometric method [40]. With some modifications that include aliquot of methanolic solution of extract was added to Folin – Ciocalteus phenol reagent solution 1:100 (V/V) ml in Bidistilled, That requires multiply the output of standard curve of gallic acid Equation by 10 (dilution factor). The absorbance was determined using spectrophotometer at wavelength = 725 nm, blank was prepared in the same way, The same procedure was replicated for the standard solution of gallic acid and the calibration curve was constructed (figure 3-5) . Based on the measured absorbance the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/ kg of oil) or ppm of Gallic acid.

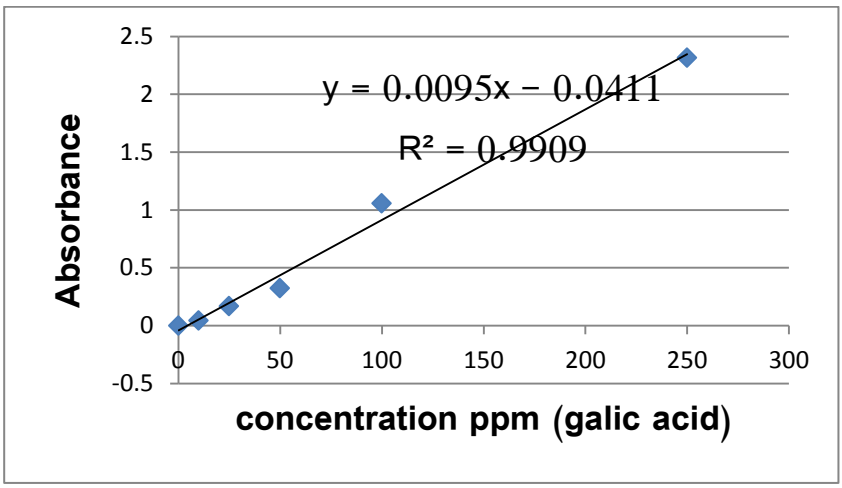


Figure 3-5: The calibration curve and equation Used to determine T.P.C relate to absorbance.

Equation determine for TP Content

The calibration curve equation was Y= 0.009X - 0.041

Y = reading absorption at 725 nm.

X = concentration of polyphenol in olives oil sample.

The total concentration of polyphenol in olives oil sample was determined as ppm of Gallic acid = X*10 modifications due to dilution factor.

3.3.2.6. Fatty acid composition

Material

Thermo Finnigan Trace GC/MS^{plus} splitless ,volume of injection 1µL, carrier gas He , solution of known concentration of four FAME's[oleic, palmetic, linoleic, stearic] Supelco USA ,KOH in 0.50 mol L^{-1} methanol. BF3 (14%) in methanol, isooctane, saturated sodium chloride, sample of 150 g of oil.

Principle

FAC was determined using Methylation of oils with boron trifluoride methanol method [41]. About 150 mg of oil, 6 mL of KOH in 0.50 mol L^{-1} methanol was added and agitated with heat for 5 minute. After that 5.0 mL BF3 (14%) in methanol was added with agitated under heat for 3 minute. Next 3.0 mL of isooctane and about 15.0 mL of saturated sodium chloride were added and strongly agitated for 15 seconds. After phase's separation, the upper layer, containing the methyl esters of fatty acids was collected.

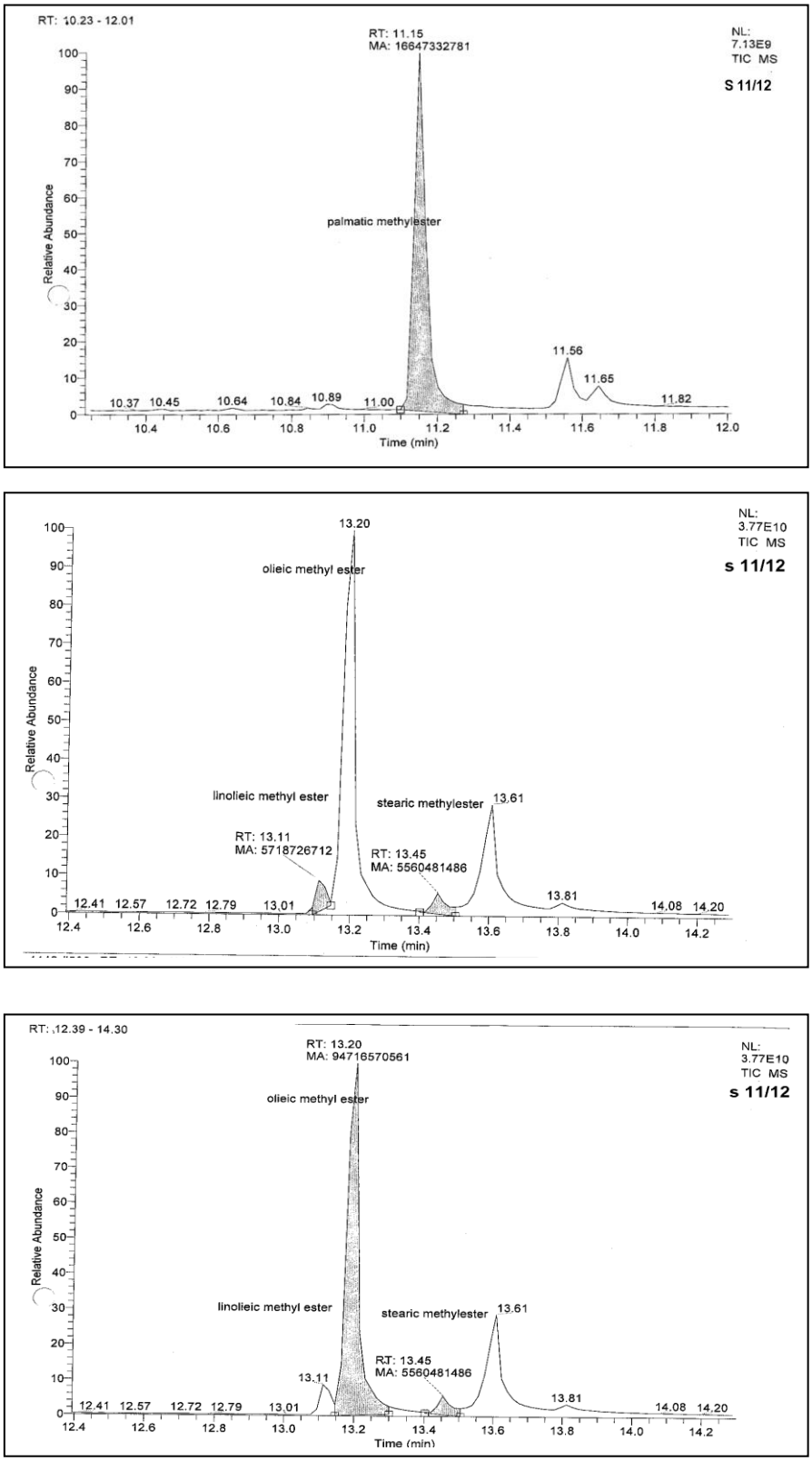


Figure 3-6: Chromatography of olive oil FAME and Area under curve for (oleic ME , palmetic ME , linoleic ME , stearic ME)

Samples were analyzed by gas chromatography which determines the proportion of each FAME of the four [oleic (C 18:1), palmetic (C 16:0), linoleic (C 18:20), stearic (C 18:0)]. That was measured after it is calibrated at the calibration solution of known concentration of four FAME's. Area under the curve determines the proportion of each FAME (Figure 3-6).

Equation determine for FAC

Total area under curve = oleic ME + palmetic ME + linoleic ME + stearic ME

FAC Percentage = Area under curve of FAME*100/ Total area .

3.3.2.7. Determination of Oxidative stability index

Materials

Oven (Raypa™), a sample of 50 g of oil was used to determine the OSI. The procedure was described previously .

Principle

OSI was determined according to a measure of an oil resistance to oxidation.[42] The oil samples maintained at 100 C^o in a conventional oven for 4 day. The samples were removed from the oven (three time intervals) to determine the PV.[35] The results data is displayed on a curve to find an exponential equation linking the time in hours and peroxide value, show figure 3-7, The results were showed as induction time, it is determined as

the number of **hours** required for the PV of the sample to be 70 meq O_2/kg oil.

Refer to the equation in figure 7, OSI= x when y = PV=70 milequa O2/ kg oil

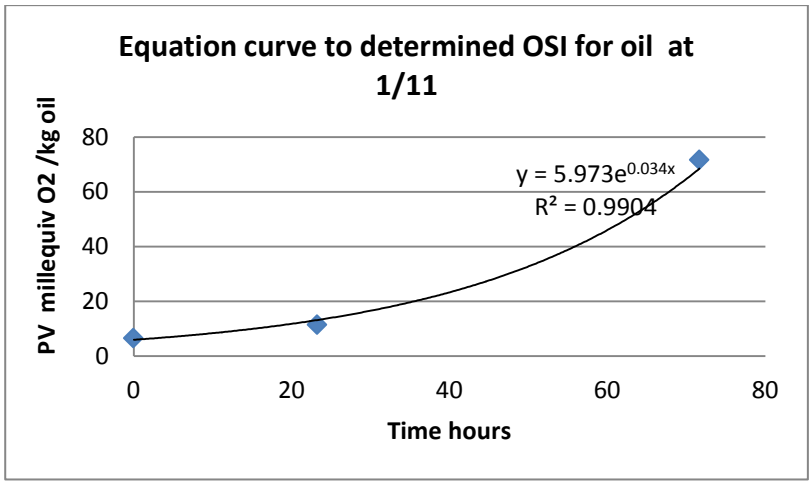


Figure 3-7: Exponential relationship curve between PV and time to determine OSI.

3.4. Statistical analysis method

Results were analyzed statistically using analysis of variance used the Least Significant Difference (SAS, 1994) ANOVA among the various transactions. Only chemical tests were repeated three times except fatty acids composition and oxidative stability index.

Chapter Four
Results

Results of olive fruits and olive oil tests were displayed on three concepts to facilitate discussion

1 The effect of harvest time on fruit characteristics include maturity index , moisture content ratio, fruit weight ,length and width.

2 The effect of harvest dates on the olive oil chemical characteristics related to oil quality include acidity and peroxide, UV Light extinctions K and oil fatty acids composition.

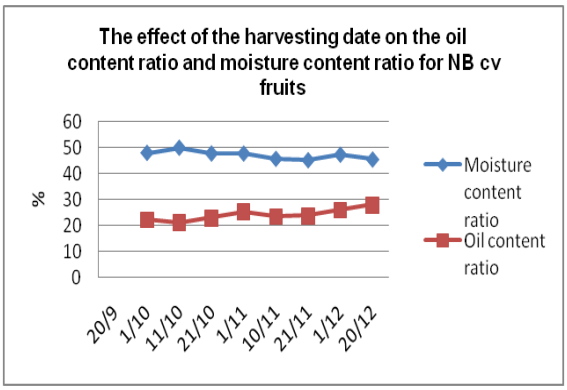
3 The effect of harvest time on olive oil antioxidants include pigments, polyphenols and Oxidative stability index.

4.1. The effect of harvest date on fruit characteristics

Table 4-1 shows all pomological values for olives during the harvest dates for the Nabali Baladi CV while the figure 4-1 illustrates the relationship between these indicators during the harvest dates from 20/9 to 20/12.

Table 4-1: Pomological parameters of NB CV during harvesting dates

Pomolgical parameter	Harvesting dates								
	20/9	1/10	11/10	21/10	1/11	10/11	21/11	1/12	20/12
Olive maturity index	0	0.17	0.75	2.4	3.97	4.78	5.24	6.33	6.5
Moisture content ratio		48	50	47.77	47.76	45.68	45.14	47.3	45.4
Oil content ratio (fresh weight)		22.22	21.15	23.02	25.29	23.55	23.78	26	28
Average fruit. Weight. g	2.18	2.25	2.2	2.48	2.84	2.84	2.89	3.17	3.24
Average Fruit length. cm	2.2	2.23	2.25	2.27	2.38	2.43	2.45	2.54	2.52
Average Fruit width. cm	1.45	1.5	1.5	1.57	1.61	1.63	1.64	1.76	1.76



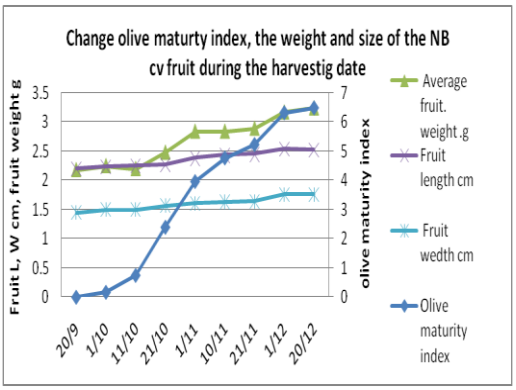


Figure 4.1: The effect of harvesting dates on pomologicl characteristics of NB olive cv

4.1.1. Maturity color Index

Results showed a significant increasing change of the indicator during the harvest dates from zero in the first harvesting date to 6.5 in the last of harvesting date, figure 4-2 shows the change in the color of the fruit and pulp during the harvest dates from 20/9 to 20/12.

Figure 4-2: Photos of fruits for NB CV during harvesting dates**.**

4.1.2. Moisture content of fruits

Results showed a decrease in moisture content during harvesting dates where the highest rate of moisture content of fruits 50% and the least was 45.14% (Table 4-1), the figure 4-1a illustrated a decrease of moisture content from 20/9 to 21/11 then its stable from 21/11 to 20/12, Also inverse correlation equal (-0.6) between moisture content ratio and oil content ratio .

4.1.3. Oil content ratio (fruit fresh weight basis)

Oil content ratio was increased during the harvesting dates of the harvest from 22.22% to 28% of 1/10 to 20/12 respectively, also the results showed clear increasing through Period from 21/11 to 20/12 (Figure 4-1a).

4.1.4. Average weight, length and width of fruit

Figure 4-1b illustrated clearly increased in weight ,length and width of the fruit, during harvesting dates starting from 20/9 until 1/12 , values rose from 2.18 gm, 2.2 cm, 1.45cm to 3.17 g, 2.54 cm, 1.76 cm respectively then values stabilized until 20/12. Table 4-4 showed correlation coefficient value between the average fruit weight and each of oil content, the fruit length, also between the color index and each of the rate of fruit weight, oil content .

4-2. The effect of harvest dates on the olive oil chemical characteristics related to oil quality

Chemical quality parameters specified by the IOC were studied during harvesting dates . Table 4-2 shows all chemical characteristics related to oil quality during the harvest dates for the Nabali Baladi CV olive oil.

Table 4-2: Chemical quality parameters of NB CV OO during harvesting dates

Chemical quality parameters	Harvesting dates								
	20/9	**1/10**	**11/10**	**21/10**	**1/11**	**10/11**	**21/11**	**1/12**	**20/12**
Acidity %	0.421^{E}	0.307^{E}	0.594^{E}	1.500$^{B\ C}$	1.071^{D}	1.227^{D}	1.622^{B}	1.272^{D}	3.664^{A}
Peroxide	3.239$^{A\ B}$	2.039^{B}	2.510^{A}	3.013$^{A\ B}$	3.041^{A}	3.715^{A}	2.584$^{A\ B}$	2.056^{B}	2.624^{A}
K 270 nm	0.1557^{A}	0.1432^{A}	0.2254^{A}	0.2183^{A}	0.0718^{B}	0.0538^{B}	0.1821^{A}	0.0618^{B}	0.1866^{A}
K 232 nm	1.5026^{A}	1.4855^{A}	1.5251^{A}	1.5033^{A}	1.1478^{A}	0.2058^{B}	1.6061^{A}	0.2295^{B}	1.8357^{A}
ΔK	-^{A}	0.00009^{A}	-^{A}	-^{A}	-^{A}	-^{A}	0.00250^{A}	-^{A}	-^{A}
Oliec FA	69.78	68.77	73.63	69.49	77.72	67.36	70.66	77.22	64.98
Palmetic FA ratio	16.29	13.99	15.11	15.13	12.01	14.09	15.54	13.57	16.16
Linoleic	7.38	11.96	7.08	7.94	5.15	9.62	7.88	4.66	14.38
Stearic FA	6.53	5.26	4.16	7.43	5.11	8.91	5.9	4.53	4.47
MUFAs / PUFAs	9.46	5.75	10.4	8.75	15.09	7	8.97	16.57	4.52
USFs / SFAs	3.38	4.19	4.19	3.43	4.84	3.34	3.66	4.52	3.85

Mean values with the same letter in the same line are not significantly different at $p > 0.05$.

4-2-1 Acidity

The Acidity (Percentage of free fatty acids) is shown in table 4-2. It shows the highest value for Acidity was 3.66% and least was 0.307%. Figure 4-3 illustrated significant increase in acidity from 0.6% at 20/9 to 1.5% at 21/10 then ranged about 1.3% from 21/10 to 1/12, finally showed a sharp increase from 1.2% at 1/12 to 3.6% at 20/12.

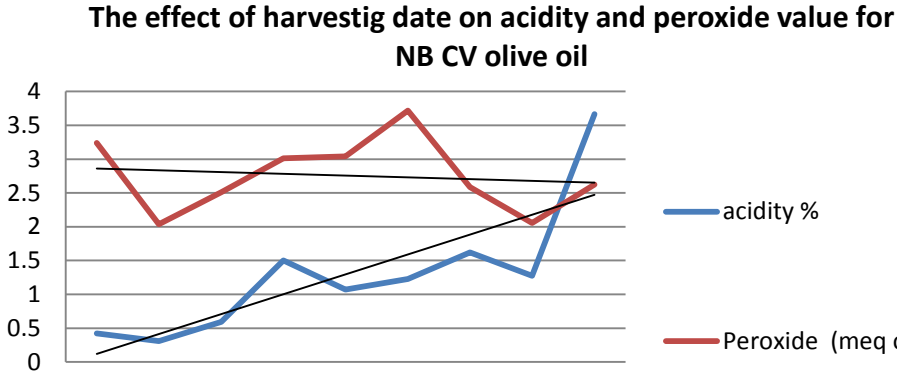


Figure 4-3: Alteration on acidity and peroxide value during harvesting date for NB CV olive oil

4.2.2. Peroxide value

The results of PV have no significant differences during the harvest dates except 10/11, the highest value for peroxide value was 3.71 and the least was 2.039 (meq O_2/kg oil)(Table 4-2). Figure 4-3 illustrated increasing PV slightly to top 3,7 at 10/11 date and then decreasing gradually until 10/12. The linear trend of the results indicate decreasing PV during harvesting dates.

4.2.3. UV Light extinctions K and ΔK

The tests of K and ΔK are indicator on the status and level of oxidation reactions in oil. The results in table 4-2 showed the highest and lowest value of K_{232}, K_{270}, ΔK during harvesting dates was (1.8, 0.2), (0.22, 0.06), (0.0025 ,-0.007) respectively. Also showed the results of ΔK did not show any significant change during the harvest dates and all results were negative except for 1/10 and 21/11, the average of ΔK during the harvest dates was (-0.0029).

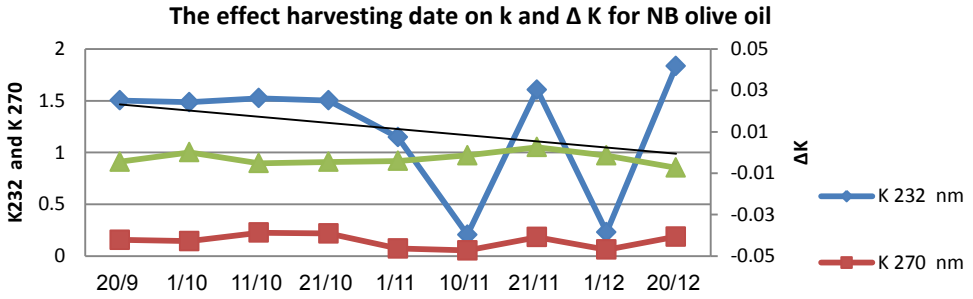


Figure 4-4: Change k and Δ K for NB olive oil during harvest dates

Figure 4-4 showed stability of ΔK values, fluctuated of K_{270}, The decreasing linear trend of K_{232} during harvesting dates.

4.2.4. Fatty acid composition

The content of fatty acid ratio (oleic, palmitic, linoleic and stearic) during harvesting dates is shown in table 4-2, the average content was 71%, 14.7%, 8.5%, 5.8% respectively. Figure 4-5A shows the effect of harvest dates on the four fatty acids ratio . The results show an increasing of the linoleic ratio from 7.38% to 14,38% , decreasing stearic ratio from 6.53%

to 4,47%, and stability levels of oleic and palmitic ratio (71%, 14.7), respectively, however, during the period from 20/9 to 1/11 an increase in oleic ratio from 69.78% to 77.72% was noted, decreasing in both palmitic and linoleic ratio from 16.29% , 7.38% to 12% , 5.15% respectively, the stearic acid was stable around the average(6%) . The result shows fluctuating change in proportions of MUFAs / PUFAs and USFs / SFAs during harvesting dates, the trend of the results for MUFAs / PUFAs and the USFs / SFAs proportions during harvesting dates showed an increasing then decreasing in the former and stability in the second, however, two peaks .

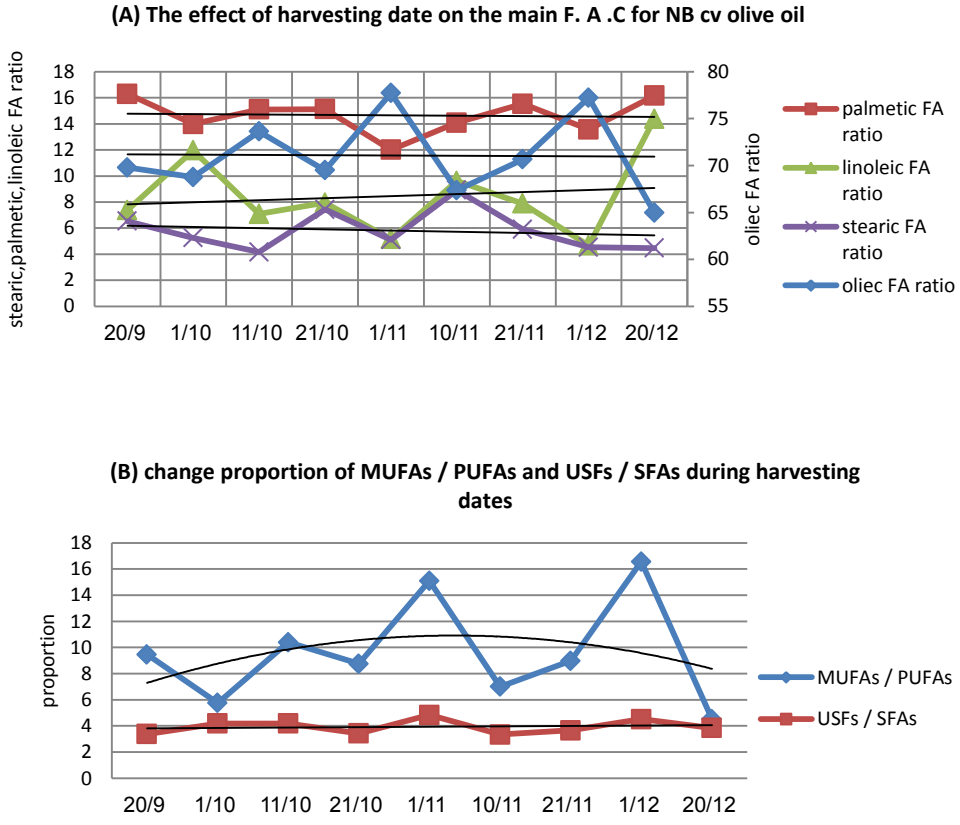


Figure 4-5: The effect of harvesting date on the main F. A .C for NB cv olive oil

values for both ratios (15, 4.84) at 1/11 and (16.5,4.52) at 1/12 were shown, respectively (Figure 4-5B). On the other hand, a sharp increase in linoleic ratio from 4.66 to 14.83 was observed during the period (1/12 to 20\12), however, oleic acid ratio was reduced from 77.2 to 64.98 as well as MUFAs / PUFAs from 16.57 to 4.52 during the same period .

4.3. The effect of harvest date on olive oil antioxidants include pigments, polyphenols and Oxidative stability index.

Antioxidants compounds preserve olive oil against oxidation and rancidity. Oxidative stability index expresses the shelf life. Table 4-3 illustrates Oxidative stability index related to olive oil antioxidant compounds during the harvest dates for the Nabali Baladi CV, it shows a significant effect of harvest time on the content of chlorophyll and carotenoids and stability oxidative index, while the date of the harvest did not significant affect the oil content of polyphenols except last harvesting date, It notes correlation coefficient between the content of chlorophyll and carotenoids content and the correlation between the content of phenols and stability of oxidative stress index (Table 4-4).

Table 4-3: Antioxidant and OSI during the harvesting dates for NB CV OO

Anti-oxidants	Harvesting dates								
	20/9	1/10	11/10	21/10	1/11	10/11	21/11	1/12	20/12
chlorophyll mg/kg	27.38^{B}	26.74^{B}	35.21^{A}	16.99$^{E\ D}$	18.80$^{C\ D}$	30.16^{B}	-	21.15^{C}	14.10^{E}
carotinoides mg/kg	10.86^{A}	10.35$^{A}_{B}$	9.84$^{A\ B\ C}$	7.18^{DE}	7.44$^{C\ D\ E}$	9.09$^{A\ B\ C\ D}$	-	8.24$^{B\ C\ D\ E}$	6.27^{E}
T.polyphenols (mg/kg oil)	322.63$_{A}$	317.094^{A}	302.216$^{A}_{B}$	433.52^{A}	445.806^{A}	326.43^{A}	358.96^{A}	364.84^{A}	129.90^{B}
Oxidative stability Index (hours)	66.576	70.002	72.389	72.717	79.9229	64.692	67.643	65.075	65.646

Mean values with the same letter in the same line are not significantly different at p > (0.05).

4.3.1. Pigments (chlorophyll and carotenoid)

Figure 4-6 shows linear trend of the results of pigments during harvesting dates

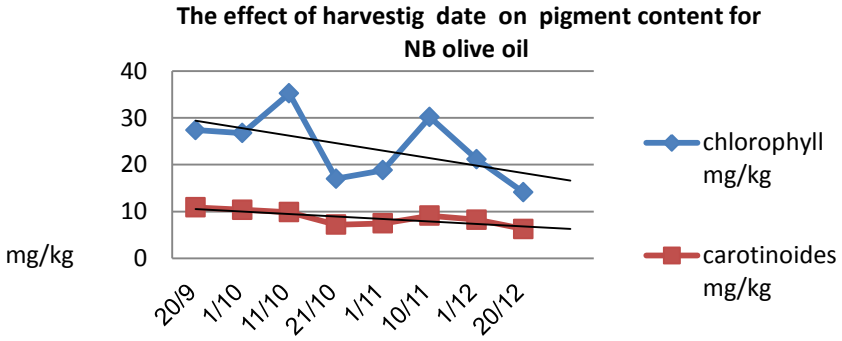


Figure 4-6: Change pigment content during harvesting dates

From 20/9 to 20/12 indicate decreasing of both chlorophyll and carotenoid from (27.4, 10.9) mg/kg to (14.1, 6.3) mg/kg respectively. It notes the lack values of the content of carotenoids chlorophyll in 21/11 because of a defect in the oil sample preparation.

4.3. 2. Total polyphenols

Results of polyphenols analysis show gradual increased from 322 to 445 (mg/kg oil) during the period from 20/9 to 1/11 then decreased gradually to 129 (mg/kg oil) until harvesting date 20/12 (Figure 4-7) .

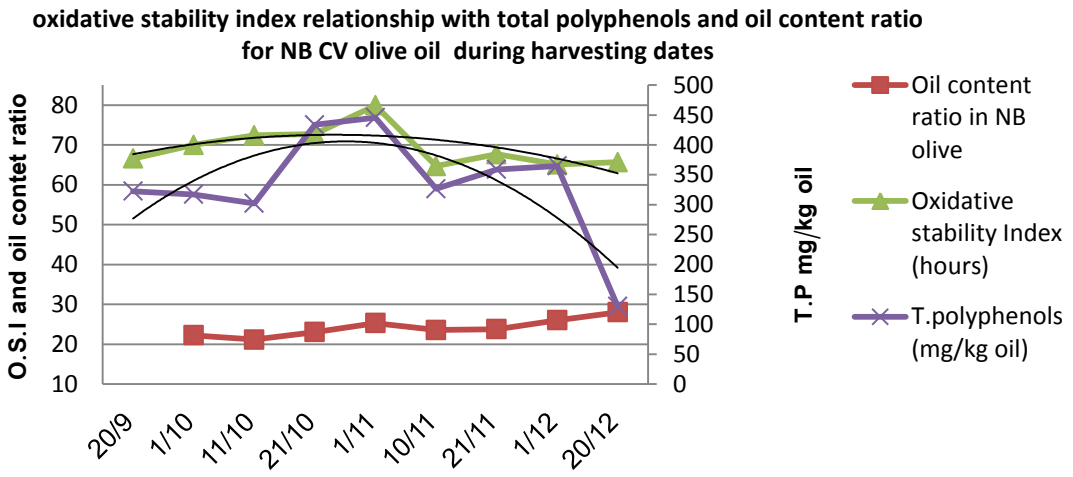


Figure 4-7: The effect of harvesting dates on OSI,TP and oil content ratio

after 1/12 it was shown increase in oil content ratio and a sharp decrease in polyphenols content with the stability of oxidative index.

4.3.3. Oxidative stability index

A measure of an oil resistance to oxidation, (Figure 4-7) showed increased OSI value gradually from 66 to 79.9 (hours) during the period from 20/9 to 1/11 then decreased gradually to 65,6 (hours) until harvesting date 20/12 , also showed there was the top value of each of polyphenol and oxidative stability index (445.9, 79.9) respectively in 1/11 harvesting date.

Table 4-4 Correlation coefficient values between results of parameters

Parameter 1	Parameter 2	correlation coefficient
Moisture content ratio	Oil content ratio	−0.60
Average fruit Weight	Oil content ratio	0.92
Average fruit. Weight	Fruit length	0.98
Average fruit. Weight	Fruit width	0.97
Maturity index	Average fruit Weight	0.85
Maturity index	Oil content ratio	0.98
Maturity index	Chlorophyll	−0.57
Maturity index	Carotinoides	−0.76
Oxidative stability Index	T.polyphenols	0.57
Oxidative stability Index	MUFAs / PUFAs	0.80

Chapter Five
Discussion

5.1. The characteristics of olive fruits

The results showed a significant effect of the harvesting dates on fruit weight, height, width, maturity index, oil content ratio and moisture content ratio. In spite of high correlation between fruit weight, length , width and oil content ratio , it could not considered reliable indicators for changing of chemical characteristics with harvesting dates, they were influenced mainly by climatic and agricultural practice. The maturity color index is attributable to the accumulation of anthocyanins through ripening. Although, it has been high correlation between both the oil content and the weight of the fruit, the maturity color index is preferred by farmers and researchers to link the chemical characteristics with harvesting date more over it is a proof of the oil quality . This is supported by the olive tree NB CV that is characterized by a rareness of fruit shedding during ripening and variance values of maturity color index from zero to 6.5 during the nine harvest dates. It is known that the moisture content of the fruit affect the proportion of oil extracted by mechanical means and is influenced by weather conditions.[28] The influential rainfall during the last three harvesting dates clearly impact on the increase in moisture content ratio (Fig 4-1a) and therefore, increasing the weight of the fruit until the last date of harvest , reaching 3.24 g, also it notes the increasing oil content ratio (fresh weight basis) up to 28%, may be explained that constantly anabolism and composition of the oil in the fruit until the last date for the

harvest. This matches the results of previous studies. [9][10] The decreasing in moisture content and increasing oil content was in agreement with previous studies,[15][20][21] and was contrary to the findings of by Bengana.,***et al*** (2013) that appeared there is no significant differences on the oil content of the Chemlal olive oil during the harvest dates.[16]

The results of average weight, length and width of the olive fruit during the nine harvesting dates was 2.76 g, 2.36 cm, 1.6 cm, respectively, and this agrees with the results by Omar,R.(2012) who studied fruit characteristics of NB CV in Palestine.[4]

5.2. The effect of harvest date on the olive oil chemical characteristics related to oil quality

Acidity results showed a significant effect of harvesting date on the Acidity , Figure 2 shows increasing acidity with harvesting date and this agrees to the results of the previous studies.[16][17][18][20] And contrary to with Yorulmaz.,***et al*** (2013) , whom showed a decrease in the acidity during ripening of the Turkish varieties (Memecik and Edremit).[19] The Results showed that the oil acidity during the first three harvesting dates matches the IOC specification of the extra virgin oil, during the period from 21/10 to 1/12 matches the IOC specification of the virgin oil, however, during the last harvesting date of the oil was not fit for marketing and consumption and classifies ordinary oil according to the IOC specification. The increasing in acidity of the oil after 21/10 may be explained by activity of enzymes when the skin of the fruit cells ruptured during maturity due to

mechanical injuries and obvious olive fruit fly infection which have been observed when taking the olive fruits samples that lead to olive damage in addition to increasing the activity of an internal lipase enzyme over ripening,[23] it is recommended to prevent this pest by using the available pheromone and color traps and avoid late harvesting olive after 1/12 which will lead to impaired florescence differentiation. Peroxide test is a second basic indicator to the quality of the oil and demonstrates the primary oxidation rate of the oil, results showed a significant effect of harvesting date on a peroxide value with a decreasing trend during harvesting date, this agrees with previous studies,[15][17][18][19][21] and contrary with Freihat,.***et al*** (2008), whom showed increasing peroxide value with the progress of the harvesting date for the NB CV in Jordan.[9] The decreasing in peroxide value may be explained due to decreasing lipoxygenase activity which produce peroxide material by linoleic-enzyme reaction, the slight increase in peroxide value from 21/10 to 10/11 may be explained by increasing phenolic content because peroxide produced by phenolic oxidation also. The results showed that all peroxide values were within IOC council specification for extra virgin oil. ΔK had no significant differences during the harvest dates were the results within IOC specification for extra virgin oil where as the negative values indicate the authenticity of NB CV oil Therefore this indicator is considered important for the detection of fraud in the oil, and this agree with a previous study.[12] The results of K_{232} came within IOC specification for extra virgin oil and the trend of the curve in Figure 4-4 is consistent with the trend of peroxide curve in Figure 4-3, also

the statistical analysis shows similar pattern between PV and K232 (Table 4-2) where reflect the primary oxidation of oil that agree with previous studies,[18][19][21] and contrary with Hamidoghli,.***et al*** (2008) who showed increase K_{232} while decrease PV for cultivars, Roghani, Zard and Lechino during harvesting dates.[15] The results of K_{270} detects the secondary oxidation, came within IOC specification for extra virgin oil and the results were fluctuated during the harvest dates on the average value of 0.144 and the results agree with previous studies results.[16] But contrary to previous studies,[15][18][21] there results show k_{270} decrease during harvest dates, and contrary with Abenozaa,.***et al*** (2015) whom results showed increased k_{270} during harvest dates for Arbequina CV in Spain.[21] Olive oil consists of a triglycerol 98% approx. The type and the proportion of fatty acids play a major role in the oil qualities, oxidative stability and nutritional value. The four fatty acids that have been studied oleic, palmitic, stearic, linoleic constitute more than 96% of the total oil fatty acids.[23] The Results showed that all the fatty acid ratios during the nine harvests have been below the upper limit of the specifications of the IOC for Extra Virgin Oil, exception stearic acid ratio increased on the upper limit for all harvesting dates except 11/10, 1/12, 20/12. The increased value of stearic acid over the maximum limit of IOC specifications may be explained due to an error in the calculation of area under the curve resulted from gas chromatography, in addition to the limitation of essential fatty acids use in study (oleic, linoleic, stearic and palmitic) interfering with values of other fatty acids that were not studied and may be due to other environmental factors that

need more research and study. Figure 4-5A shows linolic linear trend values increased and this agree with previous studies,[15][17] the low temperature during harvesting may be explain the promotion activity of oleate desaturase enzyme which convert oleic to linoleic,[17] also the linear trend of oleic acid from figure 4-5A showed a stable value around an average ratio of about 71%, this agrees with the previous study,[4] demonstrates the ratio of oleic acid in the NB CV in Palestine. The stable value of oleic may be explained by the equivalent of the produced amount of it to the converted amount to linoleic and this agree with the previous study,[8][18][19] and contrary with Hamidoghli,***.et al*** (2008) whom showed oleic decrease during ripening for Roghani, Zard and Lechino CV in Iran.[15] The changing in proportion of MUFAs / PUFAs and the proportion of USFs / SFAs are considered important indicators to the stability of oxidation and nutritional value of olive oil , Figure 4-5B showed increase then decrease in the first and stability in the second and this contrary to previous study,[16][21] which showed decrease in the first and increase in the second. Sharp increase noticed in the ratio of linoleic and sharp decrease in the ratio of oleic during the last harvest date cause the acute decrease in the proportion of MUFAs / PUFAs, may be explain the reduce OSI in this period.

5-3. The effect of harvest date on olive oil antioxidants

The results in Table 4-3 showed a significant effect of harvest date on the oil content of chlorophyll and carotenoids ($p < 0.5$), (Figure 4-6). This agree with the previous studies,[17][18][19][21] and contrary to previous study,[16] that showed no significant effect of harvesting date on carotenoids to Chemlal CV in Algeria. The decrease of chlorophyll and carotenoids during the harvesting date may be explained due to the biological reactions inside the fruit lead the transformation of chlorophyll and carotenoid to anthocyanins pigment that mostly extracted during mechanical extraction of the oil, with progress of harvesting date the color of olive oil turns from green to yellow or dark yellow, chlorophyll and carotenoids act as anti-oxidant and free radical quencher while keeping oil away from light in addition to the important value for the consumer who is looking for the green color of the high quality olive oil. Many cases of olive oil fraud depend on adding E 141 pigment derived from chlorophyll and adding E 160i derived from carotinoid from non-olive oil sources.[23] It also noticed that there is a negative correlation between the maturity color index and the chlorophyll and carotenoid -0.57 , -0.76 respectively, that was inagreement with Abenozaa,.***et al*** (2015) whom showed negative correlation between (carotenoid and chlorophyll) and maturity index for Arbequina CV OO in Spain.[21] The results showed that the average oil content of TP for all harvesting dates except the last harvesting was 360 mg/kg, agrees with previous study on TP content in NB CV.[4] The highest value in the 1/11 date up to 445 mg/kg, that was inagreement with Abu-Reidah,. **et al** (2013)

whom studied TP for NB CV in Nablus.[11] Figure 4-7 shows the change direction of polyphenols curve during the harvesting dates, where it is rising for maximum value in the period from 20/9 to 1/11 and then gradually decreases to 1/12 date, later at 20/12 decreases sharply to a lower value. The oxidative stability of the curve showed a great similarity in terms of the rising then decreasing during the harvesting dates and showing a maximum value at 1/11 harvesting date, and this agree with previous studies,[17][18][19][21] shows the effect of polyphenols resistant to oxidation. The importance of the two tests TP and OSI remains the most important to determine the optimal harvesting date for the TP importance as antioxidant material increasing shelf life in addition to preferred bitter taste to the consumer.[23] Except the odd result of proportion MUFAs / PUFAs at 1/12 the value of the correlation between the OSI of each of the TP content and the proportion of MUFAs / PUFAs is 0,57, 0.80 respectively, this is explain that the oxidation stability of NB CV depends on the TP content and the proportion MUFAs / PUFAs which agrees with Yorulmaz,.***et al*** (2013), who showed high correlation between the OSI values and TP for the Turkish varieties (Edremit and Memecik).[19] The correlation between the OSI values and TP in this study was 0.57 less than the correlation of the Turkish varieties (Edremit and Memecik) 0.95, 0.92 respectively.

Chapter six
Conclusions and Recommendations

6-1. Conclusions

1. The results of the study clarify the effect of harvesting dates on the physical properties of the olive fruit for NB CV. It showed a clear correlation between the maturity color index and (oil content, fruit weight). The negative correlation between percentage of moisture content and oil content.

2. In addition, the result indicated increasing acidity during harvesting dates.

3. Peroxide, ΔK and K results were indicating for the authenticity and quality of NB CV oil during the harvesting dates. It was within IOC specification for extra virgin olive oil.

4. The decreasing oil content of carotenoids and chlorophyll during the harvesting dates and high chlorophyll content, which lends to NB CV a distinctive green color. In addition it is being antioxidants in the dark.

5. A significant reduction of polyphenols result was showed in the last date of harvest.

6. A clear correlation between oxidative stability index and oil polyphenol content.

7. The results showed the high oleic percent with the increase in the linoleic percent during harvesting dates.

8. The stability oxidative index was reduced as the decreasing of oil antioxidants content.

6-2. Recommendations

1. It is strongly advised not to delay the harvesting date for olive NB CV after 1/12 due to deterioration of the oil quality and decreasing antioxidant content and oxidative stability index.

2. Monitoring and controlling olive fruit fly with different measurements to reduce the significant impact on the deterioration of the quality of the oil.

3. More research and studies are needed including different cultivars, locations and seasons.

4. The work should be extended to cover the sensory aspects of olive oil.

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Appendix

Main instruments specification

1 (Jen Way Model 7305) spectrophotometer

Wavelength Range 198 to 1000nm, Accuracy ± 2nm.Photometrics Transmittance(0 to 199.9%), Absorbance

(-0.300 to 2.500A) Accuracy ±1%T, ±0.01Abs at

1.000 Absorbance.

Light source Xenon lamp, Power 24V.

Figure A-1: Jen Way spectrophotometer 7305

2 (Thrmo Finnigan Trace GC/MS ^{plus}) Gas Chromatographic

Gas Chromatographic Options: Multiple-level temperature program with seven ramps and eight levels settable from 0.1°C to 120°C/min, Capillary split/splitless injectors with Digital Pressure and Flow Control (DPFC) including gas saver,Cold on-column injector for true on-column and large volume injections**.**

External Ion Source: Electron energy adjustable between 0 and 130 eV and emission current up to 850 µA, independently-controlled heating from 125°C to 300°C for stable operation and superior chromatographic integrity, GC interface temperature up to 350°C. **Quadrupole Ion Trap Mass Analyzer:** Unit mass resolution throughout the mass range of 10 – 1000 amu, Computer-controlled variable damping gas option for improved sensitivity.

Detection System: Pulsed Positive Ion/Negative Ion Chemical Ionization PPINICI option to acquire positive CI and negative CI spectra in alternating scans.

Vacuum System Options: 250 L/s turbomolecular pump allows greater flexibility in GC flow rate and column selection (recommended for CI).

Xcalibur™ Data System: optimization software for MS/MS experiments in either EI or CI mode.

Figure A-2: show Finnegan Trace GC/MS ^{plus}

جامعة النجاح الوطنية

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اشراف

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الإنتاج النباتي بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس- فلسطين.

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الملخص

تعتبر فلسطين احد المواطن الاصلية لزراعة الزيتون وموقعا لزراعة الزيتون العضوية حول العالم و يعتبر صنف زيتون النبالي البلدي الاكثر انتشارا بين الاصناف الاخرى المزروعة ، ويعتبر موعد الحصاد من اهم العوامل التي تؤثر في جودة الزيتون . ان الهدف الرئيسي لهذه الدراسة هو معرفة تاثير موعد الحصاد على الخصائص الكيمائية لزيت الزيتون صنف النبالي البلدي.تم جمع تسعة عينات من احد البساتين المزروعة بصنف النبالي البلدي في قرية عصيرة الشمالية شمال محافظة نابلس وكانت الفترة بين كل عينة والتي تليها عشرة أيام ابتداء من 20 ايلول 2014 ،خلالها تم جمع البيانات عن الخصائص الفيزيائية لثمرة الزيتون وخصوصا نسبة محتوى الزيت و نسبة المحتوى الرطوبي و المؤشر اللوني ، كما تم استخلاص الزيت من الثمار بطريقة ميكانيكية واجراء كل الفحوصات التي تحدد خصائص الزيت الكيمائية وتشمل الحموضة و البيروكسيد و قيمة الاخماد الضوئي K ومعامله ΔK ونسبة مكونات الزيت من الأحماض الدهنية و محتوى الزيت من الكلورفيل و الكاروتينويد و الفينولات الكلية و إيجاد مؤشر ثبات الأكسدة ، وتم تحليل البيانات باستخدام برنامج SAS الاحصائي. واظهرت النتائج زيادة نسبة الزيت خلال تقدم موعد الحصاد، وكان هناك ارتباط مابين مؤشر النضج وكل من وزن الثمرة ونسبة الزيت (0.85 , 0.98) على التوالي. و مع تقدم موعد الحصاد ازدادت قيمة الحموضة و البيركسيد، بينما كانت قيم كل من البيروكسيد و K و ΔK ضمن مواصفات مجلس الزيت العالمي لزيت زيتون إكسترا فيرجن . وكان هناك زيادة في نسبة حمض اللينوليك الدهني و تناقص في نسبة الستيريك وتذبذب في نسبة الاوليك و البالمتيك حول معدل (71%، 14.7%) على التوالي، وتناقص محتوى كل من الكلورفيل و الكاروتينويد من (10.9،27.4) الى (6.1،14.3) ملجم/كجم على التوالي. لم يكن

هناك أي تاثير معنوي لمواعيد الحصاد على محتوى الزيت الكلي من الفينولات باستثناء آخر موعد حصاد 12/20 بلغ 129 ملجم/كجم بينما اعلى قيمة بلغت 445 ملجم/كجم في موعد حصاد 11/1 ، كذلك أظهرت النتائج ارتباط مؤشر ثبات.