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Physiochemical Characteristics and Quality Criteria of Olive Oil Extracted from Picual Olive Fruits Treated by Some Growth Regulators

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ABESTRACT

This research was carried out to threw the light on the growth chemical composition and physiochemical characteristics as well as quality criteria of picual olive fruits and produced olive oil extracted from olive fruits treated by growth regulators (Gibberellic acid (GA3) and 1- Naphthaleneacetic acid (NAA) either alone or in combination and their effect on fatty acids composition, total phenols content (ppm), chlorophyll (mg/ kg), carotenoids and the most important of quality criteria of produced olive oil and storage stability for 24 months corresponding to samples without any treatments (control samples). The growth chemical composition of picual olive fruit (moisture, oil, crude protein, ash, fiber and total carbohydrate) treated with Gibberellic acid (GA3) and 1-Naphthaleneacetic acid (NAA) which were applied, 10 days after fruit set as foliar application on the trees as follows: GA3 at 30 ppm ,GA3 at 60 ppm .,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm., NAA at 135 ppm. As well as the physiochemical characteristics and most important quality criteria of picual olive oil (refractive index , color , K232 and K270 specific extinction coefficients, Free fatty acid (F.F.A), Peroxide value (pv) (meq. active O2/kg), Iodine value (IV), TBA value, unsaponifiable matter %, total phenols content (ppm), stability period (hr), chlorophyll (mg/ kg) and carotenoids (mg/ kg) as compared to control samples The current results indicated that, no significant differences between olive fruits treated with growth regulators and control samples in total oil content , protein content and total carbohydrate while there are significant differences between olive fruits treated with growth regulators and control samples in moisture, ash, and fiber content. Likewise the oil extracted from Picual olive fruits treated by gibberellic acid (GA3) and naphthalene acetic acid (NAA) at different concentration and stored for 24 months at ambient storage conditions were found to be lower in ability for storage and stability corresponding to control sample, However, treatments of olive fruit with growth regulators leading to decreasing in physiochemical characteristics and quality criteria in addition bad storage stability compared with control samples without any treatment which was found high quality, more stable and distinctive in total phenols, stability period (hr), and carotenoids and other some quality criteria.

Key words: *olive oil,* Quality criteria, storage stability, gibberellic acid , naphthalene acetic acid

Introduction

Olive “Olea europaea, L.” is one of the most important fruit crops in Egypt since it cultivated in a big area and ranks the fourth place among the fruit crops. The Picual variety is one of the most important commercial olive varieties which can be used for pickling, oil extraction or for the double purposes. Under sandy soil conditions, olive plants gave low yield especially in the newly reclaimed areas such as sides of the desert roads, Sinai and the north western coast.

Virgin olive oil is the only edible oil of great production obtained by physical methods from the fruit it shows sensory characteristics and nutritional properties which are the main reasons for the increment of its consumption all over the world in the recent years (EEC., 2003 and Manai *et al.,* 2008). Olive oil is a staple food for the people of the countries surrounding the Mediterranean Sea, but its use is now expanding to other parts of the world due to its unique flavor, high content of healthy monounsaturated fatty acid, and the presence of biologically important minor constituents. In the specialty food arena, olive oil is a dominant species that continues to grow in popularity

Different grades of olive oil are available, with extra virgin oil, obtained from the first pressing and left unprocessed, being the most healthful type. The major vitamins in olive oil are vitamin K and vitamin E. furthermore, oil contains trace amounts of minerals, including calcium, iron and potassium. as well oil contains other antioxidant components called polyphenols, such as tyrosol, hydrotyrosol, protocatechuic acid and oleuropein. The compounds chlorophyll and carotenoids are other beneficial components found in olive oil.

Olive oil is obtained from the fruit of olive trees and is a genuine fruit juice with excellent nutritional, sensorial and functional properties. Today, its biological, nutritional and healthful effects are universally acknowledged (Servili *et al.,* 2004; Morello *et al.,* 2005).

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As a matter of fact, olive tree is naturally characterized with alternate bearing habit as it tends to gain a large crop in one year and a very little crop in the following year (Daood, 2002). Growth regulators substances either promoting i.e. gibberellins (Southwick *et al.*, 1995), auxins,(Eris and Barut, 1993) cytokinins or inhibiting (retarding) ones i.e. paclobutrazol (Daood, 2002), succinic acid 2,2-dimethylhydrazide Alar and cycocel (CCC) were usually used to regulate flowering and cropping of such trees and consequently advance or delay fruit maturation and or ripening.

The olive industry today requires new cultivars adapted to modern intensive mechanized orchards. Applications of plant growth regulators have been focused by many researches in areas of plant physiology and nutriology (Pan, and Li, 1999, Amarjit, 2000,). The regulators have been intensively and extensively applied for agriculture production, and played a vital role in the growth and development of plants. Along with the development of intensive cultivation of fruits, applications of regulators for controlling the growth of fruits have been progressively paid more attention (Ma, and Liu, 1998).

Plant growth regulators applied in production promoted growth through boosting cell division and increasing cell volume, which ascribed to comprehensive effects of many hormone He,*et. al.,* (2009)

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Growth regulators such as gibberellic acid (GA3) and naphthalene acetic acid (NAA) significantly increased fruit weight and size of some date cultivars (Mohammed and Shabana, 1980) and of several other fruit types (Faust, 1989; Westwood, 1993; Davis, 2004) possibly by increasing cell size and/or cell numbers.

Olive oil quality is influenced by a great number of factors among which the geographical production area (altitude, soil composition, latitude), the cultivar chosen, Growth regulators types and concentration , the harvest period and extraction procedure, as well as the climatic conditions prevalent in the year of production (Abaza *et al.,* 2005; Ben Temime *et al.,* 2006; Baccouri *et al.,* 2007). During the ripening, several metabolic processes take place in olives with subsequent variations on profiles of some compounds and effect on plant physiologic behavior and, consequently, on chemical characteristics of its oil (Aparicio *et al,.* 1994; Pannelli *et al,.* 1994; Moussa and Gerasopoulos 1996; Ryan *et al.,* 1998).

These changes are reflected on the quality grade, sensorial characteristics, oxidative stability and nutritional value of the obtained product.

Thus, olive oil has become a subject of special attention and a considerable amount of research has been conducted to ensure its purity, authenticity and quality (Montedoro 1993; Kiritsakis *et al.,* 1998; Ranalli *et al.,* 1998; Sacchi *et al.,* 1998; Ranalli *et al.,* 1999).

Therefore, the present study was conducted to study the physiochemical characteristics and quality criteria of picual olive fruits and produced olive oil extracted from picual olive fruits treated by growth regulators(Gibberellic acid(GA3)and 1-Naphthaleneacetic acid (NAA)) and their effect on produced olive oil storage stability for 24 months corresponding to samples without any treatments (control,).

Materials and Methods

Materials:

olive fruits:

Olive fruits of the Picual, cultivar untreated (control) or treated by Gibberellic acid(GA3)and1-Naphthaleneacetic acid (NAA) were obtained from private farm at Berkash, Giza Governorate on the side of Alexandria desert road 30 km from Cairo. Picual olive fruit hand picked harvested at ripening stage from trees grown in sandy soil .The treatments growth regulators (plant hormones) were applied, 10 days after fruit set as foliar application on the trees as follows: Control without treatment, GA3 at 30 ppm ,GA3 at 60 ppm .,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm.,GA3 at 0.0 ppm + NAA at 135 ppm. Each treatment was replicated five times with one tree per replicate and ten liters of applied solution were sprayed on each tree using a compression sprayer.

Oil extracting from olive fruits:

Olive oil was extracted from the Picual olive fruit treatments as follows:(1) cleaning and leaves removal; (2) washing; (3) milling of olive fruits were performed using manual experimental crusher mill to obtain a fine paste, the olive oil extracted in batch operation using the traditional press method and the resulting liquid phase was put in a separator funnel and allowed to settle for 50 min. The upper oil layer was decanted threw dried over

anhydrous sodium sulphate and then filtered through Whatman No.1 filter paper and kept in brown glass bottle (100 ml) at ambient temperature and carried out for analysis at 0,3,6,9,12,15,18,21and 24 months.

1- Physical properties:

Refractive index: of olive oil extracted from Picual olive fruits treated by gibberellic acid (GA3) and naphthalene acetic acid (NAA) growth regulators estimated using a Carl Zeiss Refractometer, and the obtained results expressed at 25°C. According to the method described by A.O.A.C (2005).

Color: of all the tested samples was determined by a Lovibond tintometer using three colour scals (yellow, red and blue) in 5.25 inch cell. These analytical methods were carried out according to the methods described by A.O.A.C (2005).

specific extinction coefficients:K232 and K270 specific extinction coefficients. Absorption at 232 and 270 nm (1 cm path length) of a 1% (wt/vol) solution of oil in cyclohexane was measured using a Beckman DU 640 UV spectrophotometer (Beckman, Fullerton, CA) (EEC 1995)

Induction period by Rancimat (stability test): The induction periods, as the oxidative stability index, of the tested samples were measured by an automated Rancimat (Metrohm Ltd. CH-9100 Herisau, Switzeland, model 679), comprises of the control unit and the wet section containing 6 reaction vessels, according to the method described by Mendez *et al*, (1996).

2- Chemical properties:

Chemical composition of olive fruits:

Moisture, Lipid, crude protein, fiber and ash were determined according to A.O.A.C. (2005), Total carbohydrates were calculated by difference.% Total carbohydrates = 100 – (% moisture + % crude protein + % fat + % ash).

Free fatty acid:

(F.F.A) (as % Oleic acid); Peroxide value (meq. active O2/kg); Iodine value (measured according to the procedure of Hannus method) ; TBA value (as mg malonaldehyde/kg) and unsabonfable matter (%) were determined according to the procedure of A.O.A.C (2005).

Unsabonfable matter (%) :were determined according to the procedure of A.O.A.C (2005).

Total phenol compounds were isolated:

by extraction of a solution of oil in hexane, three times, with a water/methanol mixture (60:40). Folin–Ciocalteau reagent and sodium molybdate, 5% in 50% ethanol (Merck), were added to a suitable aliquot of the combined extracts and the absorbances of the solution at 725 nm were measured. Values were given as mg of Gallic acid per kg of oil (Gutfinger, 1981; Vazquez, *et. al.*, 1973).

Chlorophyll and Carotene contents:

Chlorophyll and carotene contents of the tested samples was determined according to Lichtentaler and Wellburn (1985). The weighed samples, have been put separately in 95% diethyl ether (50 ml for each gram), were homogenized with the B-Brawn type homogenizer at 1000 rpm for one minute. The homogenate was filtered through Whatman No. 1 filter paper, and was centrifuged using the centrifuge (Nüve Füj 650 model) at 2500 rpm for ten minutes. The was separated and the absorbances were read at 400 to 700 nm on Schimadzu UV-260 spectrophotometer. It was recorded that Chlorophyll (A) showed the maximum absorbance at 662 nm, chlorophyll (B) at 646 nm and total carotene at 470 nm and the amount of these pigments was calculated according to the formula:-

$C_a = 10.05\ K_{662} - 0.766\ K_{644}$, $C_b = 16.37\ K_{644} - 3.140\ K_{662}$

$C_{x+c} = 1000\ K_{470} - 1.280\ C_a - 56.7\ C_b/230$.

Where: C_a= chlorophyll (A), C_b= chlorophyll (B), Cx_{+c}= carotene

Pigment content

Chlorophyll and carotenoid compounds (mg/ Kg) were determined at wave length of 670 nm and 472nm, respectively, in cyclohexane using the specific extinction values, by the method of Minguez Mosquera *et al.,* (1991).

Fatty acids composition of the oil:

The fatty acids of the analyzed oil samples were determined by gas liquid chromatography according to the method described by International Olive Oil Council IOOC (1996).

Statistical analysis:

Data were subjected to the statistical analysis according to Analysis of Variance (ANOVA) of Completely Randomized Design as described by Gomez and Gomez (1984) Treatment means were compared using the Least Significant Differences (LSD) at 0.05 levels of probability and Standard Error. Computations and statistical analysis of data were done using facilities of computer and statistical analysis system package Costat 6.31 (CoHort Software, Berkeley, CA).

Results and Discussion

Gross Chemical Composition of tested olive fruits:

The chemical composition of the examined olive fruits treated by gibberellic acid (GA3) and naphthalene acetic acid (NAA) growth regulators (Control without treatment, GA3 at 30 ppm ,GA3 at 60 ppm .,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm.,GA3 at 0.0 ppm + NAA at 135 ppm) is summarized in Table 1, namely moisture, oil, protein, ash, dietary fiber and total carbohydrates, (gm/100g DM).

From data in table(1) It could be concluded that moisture and oil contents in control sample was slightly lower than sample treated by gibberellic acid (GA3) and naphthalene acetic acid (NAA) growth regulators which ranged from 62.52 – 64.75 and 58.3 - 60.2 % respectively.

On the other hand protein content was 5.12, 3.70, 4.99, 5.36, 5.13 and 5.02 % in Control, GA3 at 30 ppm ,GA3 at 60 ppm .,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and GA3 at 0.0 ppm + NAA at 135 ppm respectively. Beside that the results in Table (1) showed that total ash, ranged between 3.2-3.91 , fiber 7.03 – 8.18 and total carbohydrate 31.95-33.04% in tested olive fruit .

From the same table, it could be also observed that no significant differences between olive fruits treated with growth regulators and control samples (without any treatment) in total oil content , protein content and total carbohydrate while there are significant differences between olive fruits treated with growth regulators and control samples in moisture, ash, and fiber content. the highest level of moisture content was recorded in sample treated by GA3 at 60 ppm and GA3 at 30 ppm + NAA at 135 ppm which was found to be 64.75 % compared with 62.52% in control samples.Also the highest level of ash and fiber were 3.91 and 8.18 % found in sample treated by GA3 at 60 ppm and GA3 at 30 ppm respectively, compared with control samples which found to be 3.50 % for ash and 7.36 % for fiber. These results are agreement with Yorulmaz, *et. al., (*2013), Boskou, (2006), El-Mahdy and Rashwan (1997), Salvador *et al.,* (2001) and Ghanbari *et. al.,* (2012)

Table 1: Chemical composition of Picual olive fruit Treated by GA3 and NAA growth regulators On dry weight basis

Treatments	Moisture %	Oil (%)	Protein (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
Control	62.52±1.90^{b}	58.34±1.13^{a}	5.12±0.34^{a}	3.50±0.18^{ab}	7.36±0.33^{cd}	33.04±0.67^{a}
GA_3 at 30 pm	63.55± 1.83^{ab}	60.20±1.36^{a}	3.70±0.17^{b}	3.59±0.12^{ab}	8.18±0.48^{a}	32.51±0.35^{a}
GA_3 at 60 pm	64.75±1.68^{a}	59.13±1.25^{a}	4.99±0.12^{a}	3.91±0.15^{a}	7.97±0.30^{ab}	31.96±0.16^{a}
GA_3 at 30 ppm+ NAA at 90 ppm	63.59±1.38^{ab}	59.38±1.30^{a}	5.36±0.14^{a}	3.32±0.20^{b}	7.55±0.48^{bc}	31.95±0.51^{a}
GA_3 at 30ppm + NAA at 135 pm.	64.75±1.98^{a}	59.31±1.21^{a}	5.13±0.29^{a}	3.46±0.23^{ab}	7.80±0.20^{abc}	32.09±0.90^{a}
NAA at 135 pm.	64.32±1.94^{a}	58.44±1.58^{a}	5.02±0.12^{a}	3.50±0.17^{ab}	7.03±0.20^{d}	33.04±0.53^{a}
* LSD at 0.05	1.54	1.87	0.66	0.55	0.44	1.75

*GA_3 gibberellic acid (GA_3) NAA I-naphthaleneacetic acid (NAA) * LSD: *Least Significant Difference at 0.05*

Physiochemical properties of oil extracted from picual olive fruits treated by gibberellic acid (GA3) and naphthalene acetic acid (NAA) growth regulators:

1- Physical properties:

There are many physical characteristics of the edible oils such as refractive index, and colour, which are played an important role in assessing their quality and palatability, as well as the consumer acceptability of these products. The physical characteristics of olive oil are dependent on the degree of unsaturation, the carbon chain length, the isomeric fatty acid forms, and molecular configuration, and processing variables. Zaidul *et al.,* (2007) and Institute of Shortening and Edible Oils (2006).

The physical quality characteristics of olive oil extracted from Picual olive fruits treated by gibberellic acid (GA3) and naphthalene acetic acid (NAA) growth regulators were determined, in comparison with the corresponding characteristics of olive oil untreated with growth regulators (control samples) , as shown in Table (2). From the obtained data, it could be observed that,

The refractive index value at 25°C of olive oil extracted from olive fruits treated by gibberellic acid (GA3) and naphthalene acetic acid (NAA) growth regulators (Control without treatment, GA3 at 30 ppm, GA3 at 60 ppm., GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm.,GA3 at 0.0 ppm + NAA at 135 ppm) was, 1.4706,1.4701, 1.4703, 1.4705, 1.4703and 1.4704 respectively. These means of refractive index value for olive oil extracted from olive fruits treated by GA3 and NAA growth regulators had nearly the same degree of the unsaturated fatty acids in virgin olive oil as reported by IOC standard for olive oils and olive pomace oils (2011) and Ghanbari, *et. al.,* (2012). Also from data in table (2) noticed that no significant deference between tested treatment compared with control sample.

Regarding the color of tested Picual olive oil after immediate extraction of tested treatment was as follows: yellow cells fixed at 35 and red cells were 7, 8, 5, 7, 7 and 7; respectively, and blue cells were 8.3, 10 , 6.3, 9, 8, and 8.2; respectively. This variation in color intensity may be due to the difference in natural pigment content, which passes from oil-bearing material into olive oil extracted from different treatments during extraction process as well as due to the treatment conditions of bearing material. These results were found to be in agreement with Van Oss (1975) and Swern (1979).

2- Chemical properties:

The chemical characteristics of edible fats and oils are play an important role in assessing their quality assurance, palatability and consumer acceptability, as well as they are related with the healthy safe quality criteria of these fats and oils by using them. Thereupon, the chemical quality assurance criteria, including the acidity (free fatty acid %), peroxide value, iodine value, thiobarbituric acid (TBA) value, unsaponifable matter %, oxidative stability (Induction period by Rancemat), conjugated diene and triene fatty acid, total phenols, chlorophyll and carotenoids for olive oil extracted from Picual olive fruits treated by GA3 and NAA growth regulators were determined, in comparison with the corresponding characteristics of olive oil extracted from Picual olive fruits untreated with growth regulators (control samples), as shown in table (2).

Table 2: Physicochemical properties of virgin olive oil extracted from fruit of Picual olive trees Treated by GA3 and NAA growth regulators

Parameters		Control	GA_3 at 30 pm	GA_3 at 60 pm	GA_3 at 30 ppm +NAA at 90 ppm.	GA_3 at 30 ppm +NAA at 135 pm.	NAA at 135 ppm	* LSD at 0.05
Refractive index at 25ºC		1.4706±0.0002^{a}	1.4701±0.0003^{a}	1.4703±0.0004^{a}	1.4705±0.0003^{a}	1.4703±0.0002^{a}	1.4704±0.0003^{a}	0.0003
Color at yellow 35	Red	7^{b}	8^{a}	5^{c}	7^{b}	7^{b}	7^{b}	0.21
	Blue	8.3^{c}	10^{a}	6.3^{e}	9c^{b}	8^{d}	8.2^{cd}	0.217
Conjugated Diene (232 nm)		0.11±0.002^{d}	0.12±0.002^{d}	0.13±0.003^{cd}	0.15±0.001^{bc}	0.18±0.002^{a}	0.16±0.001^{ab}	0.0217
Conjugated Triene (270 nm)		1.61±0.01^{d}	1.65±0.02^{cd}	1.71±0.03^{bc}	1.75±0.02^{b}	1.85±0.02^{a}	1.77±0.03^{b}	0.078
Free fatty acid (as oleic acid %)		0.15±0.02^{b}	0.17±0.03^{b}	0.19±0.01^{ab}	0.25±0.03^{a}	0.26±0.02^{a}	0.20±0.03^{ab}	0.0707
Peroxide value (meq/kg oil)		4.35±0.08^{b}	4.63±0.05^{ab}	4.78±0.07^{ab}	4.84±0.09^{ab}	4.98±0.08^{a}	4.95±0.09^{a}	0.589
Iodine value (Hanus)		85.55±1.21^{a}	84.51±1.35^{a}	84.69±0.65^{a}	82.89±1.11^{a}	81.92±0.98^{a}	82.95±1.24^{a}	5.33
TBA values		0.0078±0.0002^{b}	0.0104±0.0003^{ab}	0.0104±0.0001^{ab}	0.0104±0.0002^{ab}	0.0130±0.0002^{a}	0.0104±0.0001^{ab}	0.0031
Unsaponifiable matter %		1.32±0.08^{b}	136±0.06$^{a\,b}$	1.43±0.07^{a}	1.15±0.05^{c}	1.29±0.08^{b}	1.36±0.09^{ab}	0.107
Total phenols content (ppm)		458.16±8.54^{a}	322.05±10.84^{b}	217.75±7.25^{c}	173.88±7.59^{d}	92.40±8.54^{f}	104.43±6.74^{e}	4.99
Stability period (hr)		55.20±1.22^{a}	43.52±1.11^{b}	32.50±0.9^{c}	27.60±0.82^{d}	16.50±0.65^{e}	17.70±0.71^{1}	1.3
Chlorophyll (mg/ kg)		21.13±0.44^{bc}	22.36±0.91^{ab}	20.17±0.75^{cd}	23.81±0.78^{a}	18.95±0.92^{d}	2045±0.69^{bcd}	2.073
Carotenoids (mg/ kg)		13.14±0.32^{ab}	12.36±0.41^{bc}	13.89±0.35^{a}	13.12±0.62^{ab}	11.75±0.58^{c}	12.67±0.49^{b}	0.805

*GA3 gibberellic acid (GA3) NAA I-naphthaleneacetic acid (NAA) * LSD: *Least Significant Difference at 0.05*

As illustrated in the obtained results of table (2), it could be indicated that the free fatty acid % (as oleic acid), peroxide value (meq. active O2/Kg oil) , thiobarbituric acid (TBA) value (mg malonaldehyde/ Kg oil) and Iodine values were found in the range 0.015 to 26 %, 4.35 to 4.98 (meq/Kg), 0.0078 to 0.0130 (mg/Kg) and 81.92 to 85.55 in all tested samples, respectively. The present results are found to be much greatly lower than the maximum values (with in the permissible values) for human consumption as reported by the Egyptain Standard specifications (2005) for olive oils.

From the same table, it could be also observed that free fatty acid %, peroxide value and thiobarbituric acid of control samples are found to be lower than the samples extracted from Picual olive fruits exposure to GA3 and NAA growth regulators. Also, there are significant differences between control and treated samples (table 2)

Iodine value is a measure of the unsaturated linkages in fat and is expressed in terms of percentage if iodine absorbed. The decline in iodine value can be used to monitor lipid oxidation. The unsaturated fatty acid residues of the glycerides react with iodine, and thus the iodine value indicates the degree of unsaturation of the fatty acid residues of the glycerides. Furthermore the iodine value is often most useful in identifying the source of an oil. Generally, the higher iodine values indicate oils and the lower values fats.

Iodine value is useful determining degree of hardness, since high iodine value indicate high content of unsaturated fatty acid components which contribute to the softness in butter fat. (Chaiseri and Dimick, 1989).

As can be seen from the table 2 , it could be indicated that the iodine value was found in the range 81.92 to 85.55 in all tested samples and the higher amount of iodine values was recorded by control samples as compared with other treatment, also there are significant differences between control and treated sample. These results within the limits of the Egyptian Standard for Olive Oil (2005), and the results of all tested treatment were found to be in agreement with Van Oss (1975); Yap *et al.,* (1989); Berger (1996), EEC, (2003) and the Codex Standard for olive oils and olive pomace (2009)

As is the case for previous parameter unsaponifiable matter content of olive oils extracted from Picual olive fruits GA3 and NAA growth regulators were determined, in comparison with the corresponding characteristics of control samples were ranged to 1.15 and 1.43 %, the highest level was found in sample treated by GA3 at 60 ppm and the lowest was found in sample treated by GA3 at 30 ppm + NAA at 90 ppm.

As illustrated in the obtained results of Table (3), it could be indicated that there are significant differences between all samples under investigation, These results are in the limits of Egyptian standard for Olive Oil (2005), EEC, (2003). the codex standard for olive oils and olive pomace (2009).

Induction period (oxidative stability (IP) has no official standard, but it is a useful measurement for comparing the relative stability of different oils, and is therefore considered to be a good tool for evaluating the resistance of olive oil to oxidation. The induction period (IP) measured by Rancimat at 120°C, as a measure for the oxidative stability of oils. To do this, the sample is heated and exposed to oxygen to initiate oxidation, and the formation of hydroperoxide is measured, either by titration or electronically. (Kiritsakis *et al.,* 2002).

The induction period (oxidative stability) of olive oil extracted from the investigated olive fruits treated with GA3 and NAA growth regulators were determined, using Rancimat method at 100°C and the results are shown in table(2). From the obtained data, it could be observed that the induction period (oxidative stability) of olive oil under investigation were 55.2 ,43.5,32.54, 27.6,16.5 and 17.7 hour for Control without treatment, GA3 at 30 ppm ,GA3 at 60 ppm .,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm respectively. In addition to there is no doubt that , treatment of olive fruit by GA3 and NAA growth regulators leading to decreasing in stability of olive oil as compared with control samples which was found to be significant differences and much greatly higher than other samples treatment.

The decreasing and increasing of olive oil stability in relation to the nature content of polyphenol and tocopherol compounds as shown in table (2) in total phenols content as we will discuss later.

The relationship between oxidative stability and the concentration of polyphenols has also been well established (Aparicio and Luna, 2002). The redox properties of polyphenols allow them to act as hydrogen donors and singlet oxygen quenchers, hence their role as antioxidants According to Jesus Tovar *et al.,* (2001) and Kanavouras *et al.,* (2006).

The specific absorption coefficients (specific extinction) in the ultraviolet region is needed for estimating the oxidation stage of olive oil. The absorption at specified wavelengths at 232 and 270 nm in the ultra violet region is related to the formation of conjugated diene and triene in the olive oil system, due to oxidation or refining processes. Compounds of oxidation of the conjugated dienes contribute to K232 while compounds of secondary oxidation (aldehydes, ketones etc.) contribute to K270 Kiritsakis, *et al.,* (2002) and Wiesman, (2009).

The specific extinction values at 232 and 270 nm for olive oil extracted from tested Picual olive fruit treatments under investigation was ranged between 1.61 to 1.85 and 0.11 to 0.18 nm of specific extinction values at 232 and 270 nm respectively. The highest value of specific extinction values at 232 and 270 nm for olive oil extracted from tested Picual olive fruit treatments were recorded by sample treated with GA3 at 30 ppm + NAA at 90 ppm for both 232 and 270 nm while the control samples found to be record the lowest amount. These results indicated that the measurement of K232 and K270 coefficient was found to be within the permitted legal limits in all oils treatments Samaniego-Sa´nchez *et al.,* (2012). Statistically significant variations. took place, depending on both control and treatments.

Polyphenols (PP), or phenolic compounds, is perhaps the most important of the minor components in olive oil, owing to their powerful antioxidant effect on the oil and the resulting contribution to shelf-life stability. Polyphenol is a general term used to describe natural substances that contain a benzene ring with one or more hydroxyl groups containing functional derivatives that include esters, methyl esters and glycosides According to Tsimidou, (1998) and Harborne and Dey (1989),

The results in Table (2) showed that total phenolic compounds of olive oil extracted from the investigated olive fruits treated with GA3 and NAA growth regulators were determined, in comparison with the corresponding characteristics of olive oil extracted from Picual olive fruits untreated with growth regulators (control samples) , as shown in table (2).

From data in table (2) it could be noticed that, phenolic compounds content in Picual olive oil untreated by growth regulators were showed much greatly higher than other samples treated by growth regulators and found to be significant differences between control and other treatments. phenolic compounds content in Picual olive oil expound The relationship between oxidative stability and the concentration of polyphenols. As we discussed previously in The induction period (oxidative stability) of olive oil treatment. The redox properties of polyphenols allow them to act as hydrogen donors and singlet oxygen quenchers, hence their role as

antioxidants (Jesus Tovar *et al.,* 2001), and inhibition of the lipid oxidation of olive oil, Several publications concluded that the remarkable olive oil resistance to oxidation was closely linked to its total polyphenol content (Franconi *et al.,* 2006; Alonso-Salces *et al.,* 2010)

The phenolic compounds in olive oil depend on several factors such as the crop, origin,variety, ripeness, conservation of the olives, origin, climate, plantation process, technological processes used for oil extraction, olive oil transport, and the harvesting system (Covas *et al.,* 2006; De Jong *et al.,* 2009 and Ben Othman *et al.,* 2009)

The color of olive oil is dependent on the pigments (Chlorophyll and carotenoid contents) in the fruit from which it was extracted green olives give green oil because of the high chlorophyll content, and ripe olives give yellow oil because of the carotenoid (yellow red) pigments. In general, the color of virgin olive oil generally ranges from greenish-yellow to gold. The color of the oil is influenced by the exact combination and proportions of pigments. Color is an important attribute to consumers, who associate the green hues from the chlorophyll in the oil with freshness of product (Ryan *et al.,*1998).

Data presented in Table (2) showed that there are significant differences among the six treatments in Chlorophyll and carotenoid contents. Chlorophyll and carotenoid contents were ranged from 18.95 to 23.81 and 11.75 to 13.89 mg/kg respectively. There was a significant differences between all treatments in both Chlorophyll and carotenoid contents.

Fatty acid composition:

The fatty acid profile (FAP) of the oil is a measure of the proportions of individual fatty acids in the oil, and is therefore an important factor in oil quality. The ratio of the different fatty acids in the oil influences the stability of the oil, as well as determining its nutritional value. Some fatty acids are considered to be better than others; in the case of olive oil, oleic acid is more desirable than the others from the nutritional point of view. Oils that have high levels of monounsaturated oleic acid are considered to be of the highest nutritive value. The fatty acid profile of the oil is mostly influenced by the cultivar and the environment. Although the IOC allows a wide range of fatty acids in extra-virgin olive oil, most growers prefer cultivars that have higher levels of the more desirable fatty acids. (Kiritsakis, 1998;Wiessbein *et al.,* 2008),

Fatty acid composition of evaluated olive oil extracted from Picual olive fruits treated by GA3 and NAA growth regulators found to be satisfactory in terms of international olive oouncil (IOC) imposed rules (Table 3). To simplify the analysis and discussion of the results, only the main fatty acids will be discussed: palmitic (C16:0), Stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3).

As expected, a larger range of values was observed for control samples of Picual olive oil compared to other treatment. Particularly for palmitic acid content went from 12.19% (control samples) to 18.22% (NAA at 135 ppm), Stearic acid: from 1.69% (control samples) to 3.95 % (GA3 at 30 ppm + NAA at 135 ppm), oleic acid: from 62.27% (NAA at 135 ppm) to 70.25.78% (control samples.), linoleic acid: from 9.35.4% (GA3 at 30 ppm + NAA at 135 ppm) to 11.27% (control samples) and linolenic acid: from 0.81% (GA3 at 30) to 0.99 % (control samples).

Table 3: Fatty acid composition of virgin olive oil extracted from fruit of Picual olive trees treated by GA3 and NAA growth regulators.

Fatty acid %	Fatty acid composition %											
Treatment	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	TS	TUS	*R	TF
Control	12.19	1.73	0.18	1.69	70.25	11.27	1.12	0.18	14.24	84.37	5.92	98.61
GA_3 at 30 ppm	13.11	2.21	0.11	2.11	68.66	10.4	0.81	0.13	15.46	82.08	5.31	97.54
GA_3 at 60 ppm	15.13	1.4	0.12	2	68.29	10.35	0.99	0.11	17.36	81.03	4.67	98.39
GA3 at 30ppm + NAA at 90 ppm.	16.93	2.8	0.14	3.81	62.79	9.98	0.92	0.52	21.4	76.49	3.57	97.89
GA_3 at 30ppm + NAA at 135 pm.	17.95	2.9	0.22	3.95	62.64	9.35	0.89	0.42	22.54	75.78	3.36	98.32
NAA at 135 ppm	18.22	2.85	0.14	3.9	61.77	9.75	0.96	0.56	22.82	75.33	3.30	98.15

*GA_3 gibberellic acid NAA I-naphthaleneacetic acid TF: Total fatty acids TS : Total saturated (F.A) TUS : Total Unsaturated (F.A) *R : Ratio of unsaturated to saturated (F.A)*

As well as saturated, unsaturated and unsaturated / saturated fatty acids ratio of evaluated olive oil extracted from Picual olive fruits treated by GA3 and NAA growth regulators was 14.24, 84.17 and 5.92; 15.46,82.08 and 5.31;17.36, 81.03 and 4.67; 21.40,77.49 and 3.57; 22.54,75.78 and 3.36 and 22.82,75.83 and 3.30 for Control without treatment, GA3 at 30 ppm, GA3 at 60 ppm.,GA3 at 30 ppm + NAA at 90 ppm., GA3

at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm respectively, These results are in agreement with the Egyptian Standards of Olive Oil (2005) and Manai *et al.,* (2008)

From the obtained results it could be observed that palmitic acid ($C_{16:0}$) was the major saturated fatty acids which was found to be the highest amount in sample treated with NAA at 135 ppm as well as oleic acid the major unsaturated fatty acids which was found to be the highest amount in control samples.

Concerning linolenic acid ($C_{18:2}$) which was much more susceptible to oxidation than mono unsaturated fatty acid, the highest percentage were found in the control samples which was11.27 % as compared with treated samples.

For the other fatty acids palmitolic ($C_{16:1}$), stearic ($C_{18:0}$) and linolenic ($C_{18:3}$) were found in small amount. As regards control samples was the best treatment as relation to total saturated, unsaturated and unsaturated / saturated fatty acids also the treatment of Picual olive fruits by GA3 and NAA growth regulators leading to increasing of saturated fatty acids and decreasing of unsaturated fatty acids as shown in table (3) The increase in the total unsaturated fatty acids and decreasing in saturated fatty acids in control sample compared with other treatment reflected the decrease and increasing in both iodine value and refractive index. These results are in reasonable agreement with Yap *et al*. (1989) and IOOC (1998).

Effect of storage for 24 months on some quality criteria of olive oil:

Refractive index is one of the most important oil characteristics as it generally gives a good idea of the level of unsaturation as well as these iodine values of the tested oil.

Table 4 shows the changes in the refractive index (RI) of evaluated olive oil extracted from Picual olive fruits treated by GA3 and NAA growth regulators.

Samples subjected to ambient storage.The RI values of all the studied olive oils decreased very slowly under ambient conditions during the early storage period of 180 days. Later on, there was a more pronounced decreased in the RI of samples stored under ambient storage conditions and this change were noted to become more distinct during the ending of storage periods. However, the storage conditions did not exhibit any significant ($P > 0.05$) effect on the RI values of evaluated olive oil extracted from Picual olive fruits treated by gibberellic acid GA3 and NAA growth regulators. At the end of the storage period overall decrease in RI of all the studied olive oils stored under ambient conditions Control without treatment, GA3 at 30 ppm, GA3 at 60 ppm.,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm was 1.4690, 1.4684, 1.4681, 1.4685, 1.4682 and 1.4679 respectively.

From table (4), it could be observed that a very low decrease change in refractive index during storage period. This decrease in refractive index may be due to the hydrolysis and oxidation of fatty acids during storage periods. These results were found to be in agreement with Van oss (1975); Swern (1979) and Hui (1996).

Free fatty acids (FFA):

Table 5 reports the free fatty acids FFA contents as a percentage of oleic acid of the examined virgin olive oils extracted from Picual olive fruits treated by GA3 and NAA stored for two years at ambient storage conditions. The changes in FFA of virgin olive oil extracted from olive fruit under study was estimated and the obtained results are shown in Table (5)

Table 4: Refractive index at 25°C of virgin olive oil extracted from fruit of Picual olive trees treated by GA3 and NAA growth regulators during storage period for 24 months at the ambient temperature (25±5°C).

Treatment	Storage period(month)								
	0	3	6	9	12	15	18	21	24
Control	1.4705	1.4704	1.4702	1.4701	1.4699	1.4697	1.4694	1.4692	1.4690
GA_3 at 30 pm	1.4701	1.4701	1.4700	1.4698	1.4696	1.4694	1.4691	1.4689	1.4684
GA_3 at 60 pm	1.4703	1.4702	1.4701	1.4699	1.4697	1.4694	1.4692	1.4689	1.4681
GA3 at 30ppm + NAA 90 pm.	1.4702	1.4703	1.4702	1.4700	1.4697	1.4995	1.4693	1.4690	1.4685
GA3 at 30ppm + NAA at 135 ppm	1.4703	1.4701	1.4700	1.4698	1.4697	1.4694	1.4690	1.4688	1.4682
NAA at 135 ppm	1.4704	1.4702	1.4701	1.4698	1.4696	1.4694	1.4691	1.4689	1.4679
**LSD at 0.05*	*For treatment = 0.0022 For storage period = 0.0026 For interaction = 0.0029*								
*GA_3 gibberellic acid NAA I-naphthaleneacetic acid *Least Significant Difference at 0.05*									

From data in table (5) It is possible to notice the free fatty acids of olive oils at zero time was 0.15, 0.17, 0.19, 0.25, 0.26 and 0.20 for Control without treatment, GA3 at 30 ppm, GA3 at 60 ppm.,GA3 at 30 ppm +

NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm respectively. After 24 months of storage period, the free fatty acids reached to 0.69, 0.98, 1.11, 1.4, 1.7 and 1.35 for Control without treatment, GA3 at 30 ppm, GA3 at 60 ppm.,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm respectively.

In particular, oil produced from Picual olive fruits treated GA3 and NAA growth regulators lead to significantly more degraded than control samples Caponio, *et al*., 2001; Caponio, *et al*., 2004).

Also From data in table (5) It could be noticed the different hydrolytic degradation of the six oils treatments was increased during storage with being affected by the type of growth regulators, but the rates were different between the oils, and this is probably due to the different starting hydrolysis levels.

Additionally**,** the free fatty acids of all samples were gradually increased as the storage period increased up to 24 months. However, the increase was more pronounced in case of the samples treated by growth regulators as compared with Control without treatment samples.

The values of the initial acidity of olive oils studied are below the maximum levels of extra-virgin olive oils established by the Regulations EEC/2568/91 and EEC/2472/97 of the European Union Commission; the Egyptian Standards of Olive Oil (2005) and the International Olive Oil Council(IOOC) (2003). The IOOC has specified different limits for FFAs for different categories of olive oils as Extra-virgin olive oil 0.8 (max.) Virgin olive oil 2.0 (max.) Ordinary virgin olive oil 3.3 (max.) Lampante virgin olive oil 3.3 (max.) Refined olive oil 0.3 (max.) Olive oil **1.**0 (max.) Crude olive pomace oil No limit Refined olive pomace oil 0.3 (max.) Olive pomace oil 1.0 (max.).

Higher values of FFA at the end of storage (24 monhts) were found to be 1.7 % in samples treated by GA3 at 30ppm +NAA at 135 ppm followed by GA3 at 30ppm +NAA 90 pm. 1.4% , NAA at 135 ppm 1.35, GA_3 at 60 pm 1.11% , GA_3 at 30 pm 0.98 % and a lesser degree of FFA were found in control samples, all samples reached to higher than the limits of extra-virgin olive oil (0.8 %) and lower than the limits of virgin olive oil (2 %) except control sample was found to be under the limits of extra-virgin olive oil according to General classification of olive oil based on FFA of International Olive Oil Council (IOOC) (2003) and Egyptian Standards of Olive Oil (2005) Wiesman, (2009). The increase of FFA may be due to exposing the oil to lipase from within the cell, or to other types of hydrolytic activity, thus leading to broken triglycerides and also oxidation of double bonds during storage which was increased FFA concentration. These results may be in agreement with Hui (1996) ; Paradiso, *et al*., (2010) and Spyros *et al*., (2004).

Table 5: Free fatty acids (as oleic acid %) of virgin olive oil extracted from fruit of Picual olive trees treated by GA3 and NAA growth regulators during storage period for 24 months at the ambient temperature (25±5°C).

Treatment	Storage period(month)								
	0	3	6	9	12	15	18	21	24
Control	0.15	0.19	0.25	0.27	0.32	0.35	0.42	0.51	0.69
GA_3 at 30 pm	0.17	0.21	0.26	0.31	0.45	0.56	0.69	0.81	0.98
GA_3 at 60 pm	0.19	0.25	0.30	0.33	0.4	0.55	0.75	0.86	1.11
GA3 at 30ppm + NAA 90 pm.	0.25	0.38	0.47	0.59	0.69	0.82	0.95	1.2	1.4
GA3 at 30ppm + NAA at 135 ppm	0.26	0.30	0.37	0.50	0.72	0.91	1.12	1.43	1.7
NAA at 135 ppm	0.20	0.25	0.35	0.47	0.59	0.78	0.97	1.18	1.35
*LSD at 0.05	For treatment = 0.02 For storage period = 0.03 For interaction = 0.07								
GA_3 gibberellic acid (GA_3) NAA I-naphthaleneacetic acid (NAA) *Least Significant Difference at 0.05									

Peroxide value:(PV):

Oils generally become oxidized, or auto-oxidation occurs, when they are exposed to oxygen in the air. This is considered to be undesirable because it affects the sensory qualities of the oil, as rancid odors are produced as a consequence of oxidation. The PV is due to hydroperoxides (primary stage of oxidation). The oxidation may be either enzymatic or chemical. Therefore, PV is another important test that should be performed on every batch of oil. The IOC has standards for PV that specify less than 20 meq of active oxygen/kg oil for extra-virgin olive oil. The changes of peroxide value of the olive oil tested samples were determined during storage period and the obtained results are shown in Table (6).

The results in Table (6) showed that peroxide value at the beginning of the assay for olive oils extracted from Picual olive fruits treated by GA3 and NAAstored for 24 months at ambient storage conditions were at range 4.35 to 4.95 milliequivalents of active oxygen per kilogramme of oil (meq O2/kg),. In all samples, the peroxide value was lower than 4.95 meq/kg at zero time of the evaluation (Table 6). In none of the oils did it exceed the upper limit (20 meq/kg) established by European Regulation for tested olive oil (EVOO) and also did it exceed the upper limit (20 meq/kg). During the 21- months storage period studied, (12.25 – 19.58 meq/kg) except samples treated by GA3 at 30ppm +NAA at 135 ppm.(20.55 meq/kg). After 24 months all tested oils were reached to higher than the limits of extra-virgin olive oil (20 meq/kg) except control sample was found to

be under the limits of extra-virgin olive oil which was fond to be 16.95 meq/kg according to General classification of olive oil based on peroxide value of International Olive Oil Council (IOOC) (2003) , EEC Regulations and Egyptian Standards of Olive Oil (2005) and Wiesman, (2009).

the peroxide value of virgin olive oil under investigation stored up to 24 month was increased with increasing the storage period. The changes in the peroxide value during storage period may be due to vicinity of the double bound that is attached by oxygen and variation in proportion of unsaturated bonds of triglycerides that or more prone to auto oxidation.

The results showed that there was statistically significant differences between these samples treated by GA3 and NAA and control sample. Moreover, the evolution of PV during storage showed obvious correlation with the initial Rancimat oxidative stability of these oils , as also with their phenolic compound contents. For example, control samples found to be contained higher amountes of phenolic compound and more stable than treated samples.

The relationship between oxidative stability and the concentration of polyphenols has also been well established by (Aparicio and Luna, 2002). The redox properties of polyphenols allow them to act as hydrogen donors and singlet oxygen quenchers, hence their role as antioxidants which play roles in decreasing oxidation of oils According to Jesus Tovar *et al.,* (2001) and Kanavouras *et al*. (2006) .

Iodine value(IVs):

The iodine value (IVs)which reflects the degree of unsaturation in the lipid. Therefore, the iodine value of the olive oil obtained from the investigated olive fruit was determined, and the obtained results are shown in table (7).

Table 6: Peroxide value (meq O2 /kg oil) of virgin olive oil extracted from fruit of Picual olive trees treated by GA3 and NAA growth regulators during storage period for 24 months at the ambient temperature (25±5°C).

Treatment	Storage period(month)								
	0	3	6	9	12	15	18	21	24
Control	4.35	4.99	5.85	6.88	7.95	9.12	10.32	12.25	16.95
GA_3 at 30 pm	4.63	6.18	7.81	9.22	10.32	11.84	13.08	15.22	19.98
GA_3 at 60 pm	4.78	6.21	9.93	11.54	13.41	15.63	17.12	19.11	21.88
GA3 at 30ppm + NAA 90 pm.	4.84	5.45	7.23	9.12	10.47	13.31	15.39	18.25	22.85
GA3 at 30ppm + NAA at 135 ppm	4.98	6.11	7.46	8.33	11.15	14.62	17.98	20.55	24.45
NAA at 135 ppm	4.95	5.88	7.68	9.23	10.99	12.45	15.63	19.58	22.1
**LSD at 0.05*	*For treatment = 0.112 For storage period = 0.150 For interaction =0.368*								
*GA_3 gibberellic acid NAA I-naphthaleneacetic acid *Least Significant Difference at 0.05*									

Data in table (7) showed that, the initial value of the iodine index of the samples is very homogenous (Table 7), varying between 82.41 and 87.93 g I_2/100 g oil. A slight decrease is observed in a similar manner in all the tested treatment. However, the variation of this parameter is confirmed, not only with respect to the temperature and storage time (TawWk and Huyghebaert, 1997), but also with respect to the type of treatment, showing a sharper decrease with respect to the initial value in sample treated byGA3 at 30 ppm + NAA at 90 pm. followed by GA_3 at 60 pm, GA3 at 30 ppm + NAA at 135 ppm, GA_3 at 30 pm, NAA at 135 ppm and control is again the sample in which the virgin olive oil is least affected by degradation of the double bonds that could be a consequence of oxidation. After 24 months of storage period the iodine number was reached to 76.13, 72.78, 69.68, 68.00, 70.10 and 73.13 g I_2/100 g oil for olive oil extracted from Picual olive fruits treated by Control without treatment, GA3 at 30 ppm, GA3 at 60 ppm.,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm; respectively.

The IV can be characterized by a decrease in the total unsaturated contents of the oil and thus is looked upon as an important indicator of deterioration of the oils (Naz *et al.,* 2004). The decrease in iodine value may be due to the levels of saturated and unsaturated fatty acids which depending on the olive oil treatment and oxidation of fatty acid during storage period. These results are in harmony with Méndez , and Falqué (2007)

The TBA test Malondialdehyde (MDA), is usually one of the well-known secondary products, has been measured by the TBA method. The TBA test involves the reaction of 2-thiobarbituric acid (TBA) with MDA in edible oils to produce a chromogen which can then be determined spectrophotometrically at 532–535 nm.

TheTBA (mg. malonaldehyde / kg oil) test measures the secondary products formed from hydroperoxides. TBA as (mg. malonaldehyde / kg oil) was determined in the olive oil obtained from the investigated olive varieties stored for 24 months, and the obtained results are shown in Table (8).

Table 7: Iodine value of virgin olive oil extracted from fruit of Picual olive trees treated by GA3 and NAA growth regulators during storage period for 24 months at the ambient temperature (25±5°C).

Treatment	Storage period(month)								
	0	3	6	9	12	15	18	21	24
Control	84.51	83.76	82.91	82.11	81.21	80.11	78.65	76.42	73.13
GA_3 at 30 pm	84.69	83.31	82.11	80.44	79.22	77.55	76.12	74.69	72.78
GA_3 at 60 pm	82.89	81.66	80.99	79.12	77.44	75.43	73.54	71.85	69.68
GA3 at 30ppm + NAA 90 pm.	81.92	81.00	80.44	79.33	77.57	75.61	72.35	70.84	68.00
GA3 at 30ppm + NAA at 135 ppm	82.95	81.73	80.39	79.05	78.00	76.42	74.62	72.32	70.10
NAA at 135 ppm	84.51	83.76	82.91	82.11	81.21	80.11	78.65	76.42	73.13
**LSD at 0.05*	*For treatment = 1.89 For storage period = 2.31 For interaction = 5.67*								
*GA_3 , gibberellic acid NAA: I-naphthaleneacetic acid *Least Significant Difference at 0.05*									

From data in table (8), it could be observed that the TBA value at the initial time was 0.0078, 0.0104, 0.0104, 0.0104, 0.0130, and 0.0104 mg. malonaldehyde / kg oil for olive oil extracted from Picual olive fruits without treatment(control sample), GA3 at 30 ppm, GA3 at 60 ppm.,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm respectively. At the initial of zero time, the gradually increased as the storage period increased up to 24 months at the end of storage period, the TBA value was reached to 0.1560, 0.1690, 0.1742, 0.1716, 0.1898 and 0.1768 mg. malonaldehyde / kg oil for olive oil extracted from Picual olive fruits without treatment(control sample), GA3 at 30 ppm, GA3 at 60 ppm.,GA3 at 30 ppm + NAA at 90 ppm., GA3 at 30 ppm + NAA at 135 ppm. and NAA at 135 ppm; respectively.

Table 8: Thiobarbituric acid (TBA) of virgin olive oil extracted from fruit of Picual olive trees treated by GA3 and NAA growth regulators during storage period for 24 months at the ambient temperature (25±5°C).

Treatment	Storage period(month)								
	0	3	6	9	12	15	18	21	24
Control	0.0078	0.0130	0.0182	0.0260	0.0364	0.0884	0.1144	0.1404	0.1560
GA_3 at 30 pm	0.0104	0.0130	0.0156	0.0182	0.0234	0.0364	0.0780	0.1300	0.1690
GA_3 at 60 pm	0.0104	0.0130	0.0156	0.0182	0.0208	0.0312	0.0988	0.1508	0.1742
GA3 at 30ppm + NAA 90 pm.	0.0104	0.0130	0.0182	0.0234	0.0390	0.0650	0.0910	0.1326	0.1716
GA3 at 30ppm + NAA at 135 ppm	0.0130	0.0208	0.0260	0.0338	0.0546	0.1066	0.1326	0.1534	0.1898
NAA at 135 ppm	0.0104	0.0130	0.0156	0.0208	0.0286	0.0728	0.1014	0.1508	0.1768
**LSD at 0.05*	*For treatment = 0.0018 For storage period = 0.0023 For interaction = 0.032*								
*GA_3 gibberellic acid NAA I-naphthaleneacetic acid *Least Significant Difference at 0.05*									

From the same table (8) it could be noticed that TBA value of olive oil obtained from picual olive fruit treated with GA3 at 30 ppm + NAA at 135 ppm found to be higher than those found in the other investigated olive oil at the end of storage period, Although the same treatment at the initial time found to be recorded lesser TBA amounts except the control sample and the lower TBA value at the end of storage periods was recorded by control samples. These results could be mainly) due to higher content of polyphenolic compounds(which having the natural antioxidant properties) in control samples corresponding to samples treated by GA3 and NAAwhich was considerable lower in polyphenolic compounds. The variation in TBA values could be differences in the decomposition of the peroxides and hydroperoxides into aldehydes and ketones.These results are in agreement with Mc Bride and Richardson (1983) and Hui (1996) Calvano *et al.,* (2012).

In conclusion, from all the previous results in this study it can be concluded that the oil extracted from Picual olive fruits treated by GA3 and NAA at different concentration and stored for 24 months at ambient storage conditions were found to be lower in ability for storage and stability of oil extracted from Picual olive fruits treated by GA3and NAA corresponding to control sample, Although, growth regulators usually used to regulate flowering and cropping of such trees and consequently advance or delay fruit maturation and or ripening, also Improves the quality of olive fruit Southwick, *et. al.,* (1995) However, treatments of olive fruit with growth regulators leading to decreasing in physiochemical characteristics and quality criteria in addition bad storage stability compared with control samples without any treatment which was found high quality.

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