Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 5 | Issue : 02 | April-June | 2015
Pages: 307-317

Physiochemical Evaluation of Olive Oil Extracted from Olive Fruits Treated by Gibberellic Acid

[1]Ali, H. E. and [2]EL Badry, N.

[1]Biochemistry Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt
[2]Food Science and Technology Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

ABSTRACT

The objective of this work was to determine the growth chemical composition and physiochemical characteristics as well as quality criteria of Manzanillo olive fruits and produced olive oil extracted from olive fruits treated by growth regulator (Gibberellic acid (GA_3) 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 Manzanillo olive fruit (moisture, oil, crude protein, ash, fiber and total carbohydrate) treated with (GA_3) which was applied, 10 days after fruit set as foliar application on the trees as follows: GA_3 at 50 and 75 ppm. As well as the physiochemical characteristics and most important quality criteria of Manzanillo olive oil (refractive index , color , K232 and K270 specific extinction coefficients, free fatty acid, peroxide value (meq. active O_2/kg), Iodine value, TBA value, unsaponifable 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 Manzanillo olive fruits treated with growth regulator and control sample in protein content, ash content, fiber and total carbohydrate, while there are significant differences in moisture and oil content. Likewise the oil extracted from Manzanillo olive fruits treated by (GA_3) 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: Manzanillo olive oil, Quality criteria, Storage stability, Gibberellic acid.

Introduction

The olive tree (*Olea europaea* L.) is known the oldest cultivated tree in the world (Ozbek, 1975). Olive is considered one of the important fruit crops in Egypt.

Olive tree (*Olea europaea,* L.) is an evergreen tree belongs to Oleaceae family has a high economic value to Egypt and to many countries in Mediterranean sea region since they use it for pickling, oil extraction or for both purposes Payvandi *et al.,* (2001). Olive oil has a unique position among edible oils due to its delicate flavor, stability and health benefits Vekiari *et al.,* (2007). The Spanish cv. Manzanillo is the most important commercial variety in the world Hartmann and Papaioannou, (1971) Manzanillo is early ripening cultivar, well for table olives and for oil production and a heavy bearer (Bailey, 1961 and El Khawaga 2007).

Virgin olive oil is obtained only by mechanic or physical procedure and it should not be exposed to any heat or refining procedure, not treated except for washing decantation, centrifugation and filtration. Virgin olive oil has a color changing from green to yellow and a distinctive taste and besides it can be consumed as a food in its natural oil form (Bozdogan- Konuskan and Didin, 2009).

Its antioxidant capacity is stable due to its high monounsaturated fatty acid content with low polyunsaturated fatty acid content and the presence of natural antioxidants such as phenols, tocopherols and carotenoids. The fatty acid composition, especially the monounsaturated fatty acid (MUFA) content, and the natural antioxidants provide advantages for health (Boskou, 1996 and Diraman and Dibeklioglu, 2009). These quality and uniqueness parameters of specific extra virgin olive oils are determined by different factors such as cultivar, environment and cultural practices (Cosio *et al.,* 2006)

Gibberellins are known for their ability to increase cell enlargement (Arteca, 1996, Davis, 2004 and Pharis and King, 1995), thus enhancing fruit growth in certain species such as citrus (Eman *et al.,* 2007 and El-Sese, 2005), litchi (Stern and Gazit, 2000), guava (El-Sharkawy and Mehaisen, 2005), and pear (Zhang *et al*., 2007). In all species so far studied, gibberellins had the potential for increasing fruit size.

Corresponding Author: EL Badry, N., Food Science and Technology Department, Faculty of Agriculture, Al-Azhar University, Cairo Egypt
E-mail: nasserelbadry70@yahoo.com

Therefore, this study was aimed to study the physiochemical characteristics and quality criteria of manzanillo olive fruits and produced olive oil extracted from manzanillo olive fruits treated by growth regulator (Gibberellic acid (GA_3) and their effect on storage stability of produced olive oil for 24 months corresponding to sample without any treatments (control).

Materials and Methods

Olive fruits:

Olive fruits of the manzanillo, cultivar untreated (control) or treated by Gibberellic acid (GA_3) were obtained from a private farm in Wadi El-Faregh, Behira Governorate, Egypt.

Manzanillo olive fruit handpicked harvested at ripening stage from trees grown in sandy soil .The treatments growth regulator (plant hormone) were applied, 10 days after fruit set as foliar application on the trees as follows:

1. Control (Without any treatment)
2. Gibberellic acid at 50 ppm. (GA_3 at 50 ppm.)
3. Gibberellic acid at 75 ppm. (GA_3 at 75 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 manzanillo 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:

Refractive index of olive oil extracted from Manzanillo olive fruits treated by gibberellic acid (GA_3) growth regulator estimated using Carl Zeiss Refractometer, and the obtained results expressed at 25°C. According to the method described by the A.O.A.C., (2005).

Color:

Color of all the tested samples was determined by a Lovibond tintometer using three color scales (yellow, red and blue) in 5.25 inch cell, according to the methods described by A.O.A.C., (2005).

Specific extinction coefficients:

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

Induction period (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 manzanillo 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).

Other Chemical properties:

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).

Total phenol compounds:

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 absorbance of the solution at 725 nm were measured. Values were given as mg of Gallic acid per kg of oil (Gutfinger, 1981and Vazquez *et al*., 1973).

Chlorophyll and Carotene contents:

Chlorophyll and carotenoid compounds (mg/ Kg) were determined at wave length of 670 nm and 472 nm, 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 composition of the tested 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

Table (1) summarized the chemical composition of manzanillo olive fruit (moisture, oil, protein, ash, dietary fiber and total carbohydrates g/100g dm) treated by gibberellic acid (GA_3) growth regulator (GA_3 at 50 ppm and GA_3 at 75 ppm).

Data in Table (1) showed that moisture content was slightly decreased on samples treated by GA_3 at 50 ppm and GA_3 at 75 ppm which was 65.82 and 64.88 respectively, in comparing with control which was 66.20.

Also, data in Table (1) appeared that oil content in control sample was slightly lower (61.66) than sample treated by GA_3 growth regulator and it ranges from 63.87 to 65.18

From the same table it could be also observed that protein, ash and fiber contents of Manzanillo olive fruit showed no significant difference between olive fruits treated with growth regulator and control samples (without treatment) while carbohydrate content had slight variation in control samples (31.30) compared with samples treated by growth regulator which was 28.06 and 29.33 respectively in Manzanillo olive fruit treated by GA_3 at 50 and 75 ppm. These results are in agreement with these obtained by 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 Manzanillo olive fruit treated by GA_3 growth regulator on dry weight basis

	Moisture %	Oil (%)	Protein (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
Control	66.20± 2.11^{a}	61.66±1.53^{c}	3.55±0.25^{a}	3.49±0.11^{a}	11.18±0.10^{a}	31.30±0.93^{a}
GA_3 at 50 ppm	65.82±1.97^{ab}	65.18±1.25^{a}	3.39±1.63^{a}	3.36±0.15^{a}	11.35±0.09^{a}	28.06±0.87^{a}
GA_3 at 75 ppm	64.88±1.59^{b}	63.87±1.40^{ab}	3.47±0.22^{a}	3.33±0.21^{a}	11.13±0.16^{a}	29.33±0.45^{a}

GA_3: Gibberellic acid

Physical and chemical properties:

There are many physical and chemical properties of the edible oils such as refractive index, color, free fatty acid %, peroxide value, iodine value… etc. which play an important role in assessing their quality, as well

as they are related with the healthy safe quality criteria of these fats and oils. The physical and chemical properties of olive oil are dependant on the degree of unsaturation, the carbon chain length, the isomeric fatty acid form and molecular configuration and processing variables (Zaidul *et al.,* 2007 and Institute of Shortening of Edible Oils 2006)

The physical quality characteristics of olive oil extracted from manzanillo olive fruits treated by gibberellic acid (GA_3) growth regulator were determined in comparison with the characteristics of olive oil untreated with growth regulators (control sample) and the results are shown in Table (2).

Data in table (2) showed the refractive index values at 25 ºC of olive oil extracted from Manzanillo olive fruits treated by GA_3 growth regulator and control sample (with no treatment) and it were 1.4703, 1.4704 and 1.4704, respectively for control and samples treated with GA_3 at 50 ppm and GA_3 at 75 ppm, respectively, and this means that the refractive index of control sample and that of samples treated with growth regulator had nearly the same values. This was in agreement with IOC standard for olive oils and Olive Pomace Oils (2011) and Ghanbari, *et al.,* (2012).

In relation to the color of tested manzanillo olive oil after immediate extraction of control, GA_3 at 50 ppm and GA_3 at 75 ppm, were as follow: yellow cells fixed at 35 and red cells were 7.7, 9 and 7.1, respectively and blue cells were 10, 11 and 8.8, respectively, this variation in color intensity may be due to the difference in natural pigment content which passes from oil bearing materials to olive oil extracted from different treatments during extraction process as well as due to the treatment conditions of bearing material. The results were found to be in agreement with Vanoss, (1975) and Swern, (1979).

Table 2: Physical and chemical properties of olive oil extracted from Manzanillo olive fruits treated by GA_3 growth regulators

Parameter \ Treatments		Control	GA_3 at 50 ppm	GA_3 at 75 ppm	LSD at 0.05
Refractive index at 25ºC		1.4703±0.0004^{a}	1.4704±0.0003^{a}	1.4704±0.0002^{a}	0.0003
Color at yellow 35	Red	7.7^{b}	9.0^{a}	7.1^{c}	0.251
	Blue	10.0^{c}	11.0^{a}	8.8^{e}	0.217
Conjugated Diene (K 232 nm)		0.15±0.002^{d}	0.16±0.001^{d}	0.17±0.002^{cd}	0.017
Conjugated Triene (K270 nm)		1.65±0.03^{d}	1.72±0.02^{cd}	1.75±0.02^{bc}	0.033
Free fatty acid (as oleic acid %)		0.18±0.01^{b}	0.21±0.02^{b}	0.21±0.03^{ab}	0.0718
Peroxide value (meq/kg oil)		2.14±0.09^{b}	3.58±0.08^{a}	3.65±0.05^{a}	0.487
Iodine value (Hanus)		84.55±1.11^{a}	83.51±0.98^{a}	83.69±1.21^{a}	4.816
TBA values		0.0104±0.0001^{a}	0.0104±0.0002^{a}	0.0104±0.0001^{a}	0.0018
Unsaponifiable matter %		1.22±0.05^{b}	1.16±0.06^{ab}	1.03±0.07^{a}	0.077
Total phenols content (ppm)		374.99±9.54^{a}	280.45±8.65^{b}	183.95±9.47^{c}	5.251
Stability period (hr)		48.70±1.1^{a}	39.50±0.98^{b}	28.30±0.62^{c}	4.184
Chlorophyll (mg/ kg)		7.85±0.98^{a}	6.84±0.78^{c}	7.65±0.81^{b}	0.419
Carotenoids (mg/ kg)		5.89±0.23^{a}	5.32±0.24^{b}	5.41±0.41^{b}	0.434

*GA_3: Gibberellic acid LSD: *Least Significant Difference at 0.05*

The chemical quality 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 (K232 and K270 specific extinction coefficients), fatty acid, total phenols, chlorophyll and carotenoids for olive oil extracted from manzanillo olive fruits treated by GA3 growth regulator were determined in comparison with oil extracted from fruit untreated with growth regulator (control sample) as shown in Table (2)

From the results appeared in Table (2) it could be indicated that the free fatty acid % (as oleic acid), peroxide value (meq active O_2 /kg oil) and TBA values were found to be (0.18,0.21 and 0.21), (2.14, 3.58 and 3.65) and (0.0104, 0.0104 and 0.0104) respectively, for control, GA3 at 50 ppm and GA3 at 75 ppm respectively.

From these results it could be observed that free fatty acid % and peroxide value of control samples are found to be lower than the samples extracted from manzanillo olive fruits exposed to GA_3 growth regulator indicating that there is significant difference between control and treated samples. As regard TBA values it was 0.0104 for all tested samples.

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 Egyptian Standard specifications, (2005) for olive oils.

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

Also, from results in Table (2) it could be indicated that iodine value was found in the range 83.51 to 84.55 in all tested samples and the higher amount of iodine value was recorded by control sample as compared with other treatment. Also, indicating that there is significant difference between control and treated samples. The results of all tested treatment were found in agreement with Vanoss, (1975); Yap *et al.,* (1989); Berger, (1996); EEC., (2003) and Codex Standard for Olive Oils and Oilve Pomace (2009).

In relation to the unsaponifable matter contents of olive oil extracted from Manzanillo olive fruits treated by GA_3 growth regulator, were determined in comparison with control samples and it ranged from 1.03 to 1.22, the highest level were determined in control sample. These results are within the limits of The Egyptian Standard Specification for olive oil (2005); EEC (2003) and Codex Standard for Pomace Olive Oil (2009).

Induction period (IP) (oxidative stability) has no official standard, but it is useful measurement for comparing the relative stability of different oils, and therefore considered to be a good tool for evaluating the resistant of olive oil to oxidation. (Kiritsakis *et al.,* 2002)

The induction period of olive oil extracted from the investigated olive fruits treated with GA3 growth regulator were determined and the results are shown in table (2). From the obtained data, it could be observed that the induction period of investigated oil samples treated by growth regulator were 39.50 and 28.30 hr. for GA_3 at 50 ppm and GA_3 at 75 ppm, respectively and it was 48.70 for control (without treatment), indicating that there is significant decrease of stability period of treated samples as comparing with control sample.

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 manzanillo olive fruits were 0.15. 0.16 and 0.17nm and 1.65, 1.72 and 1.75 respectively, for control, GA3 at 50 ppm and GA3 75 ppm respectively.

The highest value of specific extinction values at 232 and 270 nm for olive oil extracted from tested Manzanillo olive fruits recorded by sample treated with GA3 at 75 ppm for both 232 and 270 nm, while the control sample found to 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 oil treatments Samaniego-Sanchez *et al.,* (2012).

Polyphenols (PP) or phenolic compound is perhaps the most important of the minor components in olive oil, owing to their powerful antioxidant effect on the oil and 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 manzanillo fruits treated with GA_3 growth regulator were 280.45 and 183.95 ppm for GA_3 50 ppm and GA_3 at 75 ppm, respectively in comparison with control sample which was 374.99 showing that phenolic compounds in samples treated with growth regulators is greatly reduced in relation to control sample.

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

The color of olive oil is dependant on 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) pigment.

Data in Table (2) showed that chlorophyll and carotenoid contents were ranged from 6.84 to 7.85 and 5.32 to 5.89 mg /kg respectively. This indicates that there was significant difference between all treatments in both chlorophyll and carotenoid content.

Fatty acid profile:

The fatty acid profile (FAP) of 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 different fatty acids in the oil influence 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 allow 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. (Kiritaskis, 1998 and Wiessbein *et al.,* 2008).

Fatty acid composition of evaluated olive oil extracted from manzanillo olive fruits treated with GA_3 growth regulator found to be satisfactory in terms of international olive council (IOC) imposed rules as shown in table (3).

To simplify the analysis and discussion of the results, only the main fatty acids will be discussed the palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3).

Data in table (3) showed that palmitic acid (C16:0) ranged from 13.1 to 14.99, stearic acid (C18:0) from 1.63 to 1.75, oleic (C18:1) from 68.23 to 69.86, linoleic acid (C18:2) from 9.86 to 11.15 and linolenic acid (C18:3) from 0.87 to 0.89

In relation to palmitic acid the highest level was recorded by GA_3 at 75 ppm (14.99) while the lowest level was found in the control (13.1).While stearic acid the lowest level was showed in sample treated with GA_3 at 75 ppm (1.63) and the highest level was found in the control (1.75).

In addition oleic acid and linoleic acid the highest level was in the control (69.86 and 11.15), respectively, while linolenic acid showed the highest level in the tested sample treated by GA_3 at 50 ppm (0.98) while the lowest level of oleic acid was recorded in GA_3 at 50 ppm (68.23) and linoleic and linolenic acids were recorded in GA_3 at 75 ppm (9.86 and 0.87).As well as total saturated fatty acids content in Manzanillo olive fruits treated by GA_3 growth regulator as control sample (untreated), GA_3 50 ppm and GA_3 at 75 ppm, were 15.12, 15.65 and 16.88**%** respectively; total unsaturated fatty acids content were 82.79, 81.96, 81.52% respectively and 5.48, 5.24 and 4.83**%**, respectively for unsaturated / saturated fatty acids ratio. These results are in agreement with the Egyptian Standard of olive oil (2005) and Manai *et al.,* (2008).

Table 3: Fatty acid composition of virgin olive oil extracted from Manzanillo olive fruit treated by GA_3 growth regulator

Fatty acid % / Treatments	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	TS	TUS	*R
Control	13.1	0.89	0.15	1.75	69.86	11.15	0.89	0.12	15.12	82.79	5.48
GA_3 at 50 ppm	13.7	2.52	0.11	1.72	68.23	10.23	0.98	0.12	15.65	81.96	5.24
GA_3 at 75 ppm	14.99	1.11	0.15	1.63	69.68	9.86	0.87	0.11	16.88	81.52	4.83

*GA3: Gibberellic acid TS: Total saturated fatty acids TUS: Total Unsaturated fatty acids *R : Ratio of unsaturated to saturated fatty acids*

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

The changes in the refractive index of evaluated olive oil extracted from manzanillo olive fruits treated by GA_3 growth regulator and comparing them with control (without treatment) during storage up to 24 months at ambient temperature. The results are tabulated in table (4).

Data in table (4) showed that, the refractive index values range from 1.4703 at 0 month to 1.4689 at 24 months for control sample, from 1.4704 at 0 month to 1.4681 at 24 months for GA_3 at 50 ppm and from 1.4704 at 0 month to 1.4679 at 24 months for GA_3 at 75 ppm

These results indicate that there was a very slow decrease in the values of refractive index during storage periods. This decrease may be due to the hydrolysis and oxidation of fatty acids during storage periods. These results were found to be in agreement with Vanoss, (1975); Swern, (1979) and Hui (1996).

Table 4: Refractive index at 25°C of virgin olive oil extracted from fruit of manzanillo olive trees treated by GA_3 growth regulator during storage period for 24 months at the ambient temperature.

Treatment	Storage period(months)								
	0	3	6	9	12	15	18	21	24
Control	1.4703	1.4703	1.4701	1.4700	1.4697	1.4696	1.4693	1.4691	1.4689
GA_3 at 50 pm	1.4704	1.4702	1.4699	1.4697	1.4695	1.4693	1.4688	1.4685	1.4681
GA_3 at 75 pm	1.4704	1.4702	1.4700	1.4698	1.4696	1.4694	1.4690	1.4686	1.4679
*LSD at 0.05	For treatment = 0.0021 For storage period = 0.0025 For interaction = 0.0028								

*GA_3: Gibberellic acid *Least Significant Difference at 0.05*

Free fatty acids (FFA)

The free fatty acid (FFA) contents of a percentage of oleic acid of the examined virgin olive oil extracted from Manzanillo olive fruits treated by GA_3 stored for two years at ambient storage condition was estimated and the obtained results are shown in table (5).

From data in Table (5) it is possible to notice the free fatty acids of olive oil at 0 times were 0.18, 0.21 and 0.21 for control (without treatment), GA_3 at 50 ppm and GA_3 at 75 ppm respectively.

After 24 months of storage period, the free fatty acids reached to 0.69, 0.78 and 0.79 for control, GA_3 at 50 ppm and GA_3 at 75 ppm, respectively.

From these data, it could be observed that 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 samples treated by growth regulators as compared with control sample (without treatment).

The values of the initial acidity of olive oils studied are below the maximum levels of extra virgin olive oils established by regulations EEC/2568/91 and EEC/2472/47 of The European Union Commission; The Egyptian Standard 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 0.9 (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) and olive pomace oil 1.0 (max).

The higher value of free fatty acids at the end of storage (24 months) was found to be 0.79 at GA_3 at 75 ppm and lesser degree of free fatty acids was found in control sample (0.69), all treated samples reached to higher than the limits of extra-virgin olive oil (0.78) and lower than the limits of virgin olive oil (1%) except control sample, it was found to be under the limits of extra-virgin olive oil according to Wiesman (2009). The increase of FFAs during storage may be due to exposing the oil to lipase reaction, or to other types of hydrolytic activity, thus leading to broken triglycerides and also oxidation of double bonds during storage which increased FFAs concentration. These results are in agreement with Hui (1996); Spyros *et al.,* (2004) and Paradiso *et al.,* (2010).

Table 5: Free fatty acids (as oleic acid %) of virgin olive oil extracted from Manzanillo olive trees fruit treated by GA_3 during storage period for 24 months at the ambient temperature

Treatments	Storage periods (months)								
	0	3	6	9	12	15	18	21	24
Control	0.18	0.21	0.27	0.31	0.35	0.39	0.47	0.53	0.69
GA_3 at 50 ppm	0.21	0.25	0.29	0.34	0.36	0.41	0.45	0.54	0.78
GA_3 at 75 ppm	0.21	0.25	0.32	0.38	0.46	0.51	0.59	0.71	0.79
*LSD at 0.05	For treatment = 0.03 For storage period = 0.03 For interaction = 0.08								

*GA_3: Gibberellic acid *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 effects on the sensory quality of oil, as rancid odors are produced as a consequence of oxidation. The PV is due to hydro peroxides (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 specifies less than 20 meq of active oxygen / kg oil for extra-virgin olive oil. The changes of PV of olive oil tested samples were determined during storage period and the obtained results were shown in table (6).

Results in Table (6) showed that the PV of the control sample ranged from 2.14 at the beginning of the assay to 16.39 after 24 month, from 3.58 to 19.51 after 24 month for GA_3 at 50 ppm and from 3.65 to 21.63 after 24 month for GA_3 at 75 ppm. These data indicated that the peroxide value of virgin olive oil under investigation stored up to 24 months was increased with increasing the storage period. The change of PV during storage period may be due to vicinity of the double bond that is attached by oxygen and variation in proportion of unsaturated bonds of triglycerides that or more prone to autoxidation.

In all samples, the peroxide value didn't exceed the upper limit (20meq /kg) of extra-virgin olive oil (except GA_3 at 75 ppm sample which recorded 21.66 after 24 months) based on PV of International Olive Council (IOOC) (2003); Egyptian Standards of Olive Oil (2005) and Wiesman, (2009).

Table 6: Peroxide value (meq O_2 /kg oil) of virgin olive oil extracted from fruit of Manzanillo olive trees treated by GA_3 during storage period for 24 months at the ambient temperature.

Treatments	Storage period(months)								
	0	3	6	9	12	15	18	21	24
Control	2.14	3.34	5.51	6.78	8.59	10.42	12.91	14.82	16.39
GA_3 at 50 ppm	3.58	4.14	6.43	8.62	10.77	12.22	14.62	16.98	19.51
GA_3 at 75 ppm	3.65	4.81	7.61	9.54	11.65	13.36	15.44	17.45	21.63
*LSD at 0.05	For treatment = 0.115 For storage period = 0.140 For interaction = 0.345								

*GA_3: Gibberellic acid *Least Significant Difference at 0.05*

Iodine value (IV)

The iodine value which reflects the degree of unsaturation in the lipid, therefore iodine value of olive oil obtained from investigated olive fruits were determined and results are shown in Table (7).

Data in table (7) showed that the initial value of iodine number in samples ranged from 84.55 at zero time to 73.55 after storage for 24 month for the control, from 83.51 at zero time to 72.21 after storage for 24 month for GA_3 at 50 ppm and from 83.69 at zero time to 70.74 after 24 month for GA_3 at 75 ppm.

These data illustrated that slight decrease is observed in a similar manner in all tested samples however, the variation of this parameter is confirmed not only with respect to temperature and storage time (Tawfikk and Huyghebaert, 1997) but also with respect to the type of treatment. The sharper decrease in respect to the initial value was in sample treated by GA_3 at 75 ppm and the highest level was at the control sample.

The iodine value can be characterized by decrease in the total unsaturated contents of the oil and thus is looked up on as an important indicator of deterioration of the oil (Naze *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 acids during storage period. These results are in harmony with Mendez and Falque, (2007).

Table 7: Iodine value of virgin olive oil extracted from fruit of manzanillo olive trees treated by GA_3 growth regulator during storage period for 24 months at the ambient temperature

Treatment	Storage period(months)								
	0	3	6	9	12	15	18	21	24
Control	84.55	83.29	82.56	81.54	80.64	79.85	77.86	75.08	73.55
GA_3 at 50 ppm	83.51	82.91	82.11	81.21	80.11	78.65	76.42	74.58	72.21
GA_3 at 75 ppm	83.69	82.11	81.52	80.44	78.52	76.23	74.56	72.44	70.74
*LSD at 0.05	For treatment = 1.71 For storage period = 2.09 For interaction = 5.13								

GA_3: Gibberellic acid **Least Significant Difference at 0.05*

Thiobarbituric acid (TBA)

Data in table (8) showed thiobarbituric acid values of olive oil extracted from manzanillo olive fruit treated by GA_3 growth regulator during storage period for 24 months.

From data in table (8) it could be observed that the TBA value at the beginning of storage time was 0.0104, 0.0104 and 0.0104 for control sample, GA_3 at 50 ppm and GA_3 at 75 ppm, respectively then gradually increased as the storage period increased up to 24 months it reached to 0.1638, 0.1690 and 0.1742 for control sample, GA_3 at 50 ppm and GA_3 at 75 ppm, respectively.

From the same table it could be noticed that TBA value at the end of storage period for 24 months showed the highest value in samples treated by GA_3 at 75 ppm (0.1742) and the lowest value recorded with control sample (0.1638).

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 GA_3 which was considerable lower in polyphenolic compounds. The variation in TBA values could be attributed to differences in the decomposition of the peroxides and hydro-peroxides into aldehydes and ketones. These results are in agreement with Mc Bride and Richardson (1983), Hui (1996) and Calvano *et al*. (2012).

Table 8: Thiobarbituric acid (TBA) of virgin olive oil extracted from fruit of Manzanillo olive trees treated by GA_3 during storage period for 24 months at the ambient temperature.

Treatments	Storage period(months)								
	0	3	6	9	12	15	18	21	24
Control	0.0104	0.0156	0.0208	0.0260	0.0312	0.0520	0.1040	0.1248	0.1638
GA_3 at 50 ppm	0.0104	0.0130	0.0156	0.0208	0.0260	0.0624	0.1144	0.1300	0.1690
GA_3 at 75 ppm	0.0104	0.0156	0.0208	0.0260	0.0598	0.0858	0.1118	0.1378	0.1742
*LSD at 0.05	For treatment = 0.0022 For storage period = 0.0028 For interaction = 0.0037								

GA_3: Gibberellic acid **Least Significant Difference at 0.05*

In conclusion, from all the previous results in this study it can be concluded that the oil extracted from manzanillo olive fruits treated by GA_3 growth regulator 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, although, growth regulators usually used to regulate flowering and cropping of such trees and consequently advance or delay fruit maturation and or ripping, also improves the quality of olive fruits Southwick, *et al*. (1995) however, treatment of olive fruits with growth regulators leading to decreasing in physiochemical characteristics and quality criteria in addition to bad storage stability compared with control samples without any treatment which was found high quality.

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