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Effect of Treated Olive Fruits by Some Growth Regulators on Physiochemical properties of Extracted Olive Oil

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ABSTRACT

The present study aims to evaluate the physiochemical properties of Manzanillo olive fruits and olive oil extracted from it. The olive trees were treated by growth regulators (1- Naphthaleneacetic acid (NAA) and (Gibberellic acid (GA3) which was applied, 10 days after fruit set as foliar application on the trees as follows: NAA 75 ppm, GA3 75 + NAA 50 ppm and GA3 75 + NAA 75 ppm. Olive fruit samples were analyzed for its moisture, oil, protein, ash, fiber and carbohydrate content. Olive oil extracted from each olive fruits samples treated by growth regulators was also analyzed for its physical and chemical properties, oxidative stability and fatty acid profile. The effect of ambient temperature for 24 months storage on some physical and chemical properties of olive oil extracted was also evaluated and compare with sample without any treatments (control sample). The obtained results showed significant differences between Manzanillo olive fruits treated with growth regulators and control samples in moisture and oil content. Also, the oil extracted from Manzanillo olive fruits treated by (NAA) and (GA3) were found to be lower in stability and ability for storage, comparing to control sample, However, treatments of olive fruit with growth regulators led to decreasing in physiochemical properties and storage stability compared with control sample which was found of high quality, more stable and distinctive in total phenols and oxidative stability.

Key words: Physiochemical properties, Manzanillo olive oil, Naphthalene acetic acid, Gibberellic acid, Storage stability

Introduction

The olive tree (*Olea europaea* L.) is known as the oldest cultivated tree in the world (Ozbek, 1975). The olive tree grows in a subtropical climate as a traditional main crop in Mediterranean countries. It probably originates from Mesopotamia and has been cultivated from many centuries in southern European countries bordering the Mediterranean and in North Africa (Murkovic, *et al.* 2004). It is a plant that belongs to the Oleaceae family and is an evergreen tree. Olive 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 Manzanillo 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 EL-Badry (2012). Olive tree can thrive and produce in the new reclaimed areas where other crops can't grow. Besides, nutritional importance of olive fruits, either as table olive or for olive oil production. It is the problem of planted olives areas (productivity reduction). This habit causes severe loss for olive grower's income expressed in disturbances in yearly income of the orchard and poor fruit quality (Goldschmidt, 2005).

Environmental conditions play an important role in growth and productivity of olives kinds as productivity varies according to environmental and climate conditions (Lavee, 1989). Studies concerning environmental conditions influenced olive trees behavior (Lavee, 2007), especially its bearing habit, yield and fruit quality are still of need for further studies.

Using spray of some the growth 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). Growth regulators such as gibberellic acid (GA_3) 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) possibly by increasing cell size and/or cell numbers.

1-Naphthaleneacetic acid, commonly abbreviated (NAA) is an organic compound with the formula $C_{10}H_7CH_2CO_2H$. NAA is a plant hormone in the auxin family and is an ingredient in many commercial postharvest horticultural products; it is also a rooting agent and used for the vegetative propagation of plants from stem and leaf cutting (Dimitrios *et al*., 2008). On the other hand, another prominent phytohormone,

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Gibberellic acid (GA_3), has the potential control on growth and flowering process. In addition, GA_3 application increased petiole length, leaf area and delayed petal abscission and color fading (senescence) by the hydrolysis of starch and sucrose into fructose and glucose (Khan and Chaudhry, 2006; Emongor, 2004).

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)

Olive oil is of highly important due to presence of significant amount of mono-unsaturated Fatty Acid namely Oleic Acid and relatively low content of polyunsaturated fatty acids and natural antioxidants (Boskou, 1996, Garcia *et al.*, 2002; Cinquanta *et al.*, 2001; Okogeri and Tasioula-Margari, 2002). On the other hand, natural antioxidants, Phenolic compositions, tocopherol and carotene contribute to oil resistance against oxidation. There is a positive correlation between the presence of the said compound and Olive oil stability during storage (Baldioli *et al.,* 1996). Extra virgin olive oil (EVOO) is the highest grade of olive oil and is produced from fresh olives using mechanical extraction processes and without the use of excessive heat, chemical interference or blending with other edible oils.

It is known storage conditions are a major factor in the shelf life of olive oil, as well as the composition of the oil. Therefore the producer must make predictions based on the oils chemical composition and how the oil will be stored in order to determine what the shelf life for that oil will be and give the product a “use-by” or “best before” date. Predicting the shelf life of the olive oil is a complex process because of the influence of several factors such as temperature, light, oxygen availability, enzymes and microorganisms (Stefanoudaki, 2010).

Therefore, this study was aimed to study the physiochemical properties and quality criteria of Manzanillo olive fruits and produced olive oil extracted from Manzanillo olive fruits treated by growth regulators GA_3 and NAA and their effect on storage stability of produced olive oil for 24 months compared with sample without any treatments (control).

Material and Methods

Materials:

Olive fruits:

Olive fruits of the manzanillo, cultivar untreated (control) or treated by Gibberellic acid (GA_3) and 1-Naphthaleneacetic acid (NAA) 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 regulators (plant hormones) were applied, 10 days after fruit set as foliar application on the trees as follows:

1. Control (without any treatment)
2. 1-Naphthalene acetic acid at 75 ppm. (T1N)
3. Gibberellic acid at 75 ppm + Naphthalene acetic acid at 50 ppm. (T2GN)
4. Gibberellic acid at 75 ppm + Naphthalene acetic acid at 75 ppm. (T3GN)

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 then dried over anhydrous sodium sulphate and 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, 21 and 24 months.

Methods:

Physical properties:

Refractive index:

Refractive index of olive oil extracted from Manzanillo olive fruits treated by GA_3 and NAA growth regulators 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 oil tested samples was determined by a Lovibond tintometer using three color scales (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:

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).

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).

Chemical properties of olive oil:

Free fatty acid (F.F.A) (as % Oleic acid); Peroxide value (meq. active O_2/kg); Iodine value (g I_2/100 g oil) (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, 1981 and 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:

One-way analysis of variance using SPSS 16.0 for windows was performed on all experimental data sets. Post-hoc multiple comparisons were carried out by Duncan analysis to determine significant differences between sample means at 5% level.

Results and Discussion

Table (1) summarized the chemical composition of Manzanillo olive fruit (moisture, oil, protein, ash, dietary fiber and total carbohydrates g/100g on dry weight basis) treated by NAA and GA_3 growth regulators (T1N, T2GN and T3GN).Data in Table (1) showed that have no significant effect on moisture content for T1N and T3GN. It was 66.30 and 66.22%, respectively, 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 samples treated by NAA and GA_3 growth regulators and it ranges from 62.57 to 63.14%.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 regulators and control sample, while carbohydrate content had slight variation in control sample (31.30%) compared with samples treated by growth regulators which were 30.47, 30.72 and 30.41 % in manzanillo olive fruit treated by T1N, T2GN and T3GN, respectively. These results are in agreement with El-Mahdy and Rashwan, (1997); Salvador *et al.,* (2001); Boskou, (2006); Ghanbari *et al*., (2012) and Yorulmaz, *et al.,* (2013).

Table 1: Chemical composition of Manzanillo olive fruits treated by NAA and GA_3 growth regulators on dry weight basis.

Chemical composition (%)	Treatments			
	Control	T1N	T2GN	T3GN
Moisture	66.20± 2.11^{a}	66.30±1.58^{a}	65.43±1.26^{ab}	66.22±1.48^{a}
Oil	61.66±1.53^{b}	63.14±1.41^{a}	62.57±1.99^{a}	63.14±1.41^{a}
Protein	3.55±0.25^{a}	3.74±0.17^{a}	3.21±0.31^{a}	3.02±0.35^{a}
Ash	3.49±0.11^{a}	3.35±0.20^{a}	3.50±0.14^{a}	3.43±0.15^{a}
Fiber	11.18±0.10^{a}	11.04±0.14^{ab}	11.14±0.05^{a}	10.69±0.12^{b}
Carbohydrate	31.30±0.93^{a}	30.47±0.61^{a}	30.72±0.46^{a}	30.41±0.65^{a}

(M±S.D) = Mean ± Std. Deviation. Values with different small letters in the same row are significantly different (p<0.05).
(T1N): Naphthalene acetic acid at 75 ppm.
(T2GN): Gibberellic acid at 75 ppm + Naphthalene acetic acid at 50 ppm.
(T3GN): Gibberellic acid at 75 ppm + Naphthalene acetic acid at 75 ppm.

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 properties of olive oil extracted from manzanillo olive fruits treated by NAA and GA_3 growth regulators were determined in comparison with the properties of olive oil untreated with growth regulators (control sample) and the results are shown in Table (2).
Data in Table (2) showed that no significant deference between tested treatments compared with control sample. The refractive index values at 25 ºC of olive oil extracted from manzanillo olive fruits treated by NAA and GA_3 growth regulators and control sample were 1.4703, 1.4702, 1.4703 and 1.4702 for control, T1N, T2GN and T3GN, respectively, and this means that the refractive index of control sample and that of samples treated with growth regulators had nearly the same values. This was in agreement with International Olive Council (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 tested treatment control, T1N, T2GN and T3GN were as follow: yellow cells fixed at 35 and red cells were 7.7, 7.1, 7.0 and 8.8, respectively, and blue cells were 10.0, 8.5, 10.2, and 7.7 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).

The chemical quality criteria, including the acidity (free fatty acid %), peroxide value, iodine value, thiobarbituric acid (TBA) value, unsaponifable matter %, oxidative stability, 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 NAA and GA_3 growth regulators were determined in comparison with oil extracted from fruit untreated with growth regulators (control sample) as shown in Table (2).The results shown in Table (2) could be indicated that the free fatty acid % (as oleic acid), peroxide value (meq active O_2 /kg oil), thiobarbituric acid (TBA) value (mg malonaldehyde/kg oil) were found in the range of 0.18 to 0.29, 3.14 to 4.11 and 0.0078 to 0.0104, 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.Also, from these results it could be observed that free fatty acid %, peroxide value and thiobarbituric acid of control sample are found to be lower than the samples

extracted from manzanillo olive fruits exposed to NAA and GA_3 growth regulators indicating that there is significant difference between control and treated samples. Iodine value is useful indicator for the unsaturation degree of examination oil (Chaiseri and Dimick, 1989). From Table (2) it could be observed that iodine value was found in the range 80.22 to 84.55 in all tested samples and the higher amount of iodine value was recorded by control sample as compared with other treatment. The results of all tested treatments were found in agreement with Vanoss, (1975); Yap *et al.,* (1989); Beger, (1996); EEC, (2003) and Codex Standard for Olive Oils and Oilve Pomace (2009).Results of free fatty acid %, peroxide value, iodine value and thiobarbituric acid value (TBA) are found to be much lower than the maximum limits (within the permissible values) for human consumption as reported by the Egyptian Standard Specification for olive oil (2005).

In relation to the unsaponifable matter contents of olive oil extracted from manzanillo olive fruits treated by NAA and GA_3 growth regulators, were determined in comparison with control samples and it ranged from 1.22 to 1.39 the highest level were determined in sample treated by T1GN, and the lowest level was found 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 NAA and GA_3 growth regulators 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 regulators were 17.40, 21.50 and 15.20 hour for T1N, T2GN and T3GN, respectively, and it was 48.70 for control sample, indicating that there is significant decrease of stability period of treated samples as comparing with control sample.

The decreasing and increasing of olive oil stability in relation to the nature content of polyphenol and tocopherol compounds as shown in Table (2). The relationship between oxidative stability and the concentration of polyphenols has also been well established (Aparicio and Luna, 2002).

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 fruit treatments under investigation was ranged between 0.15 to 0.19 and 1.65 to 1.90 nm of specific extinction values at 232 and 270 nm, respectively. The highest values of specific extinction at 232 and 270 nm for olive oil extracted from tested manzanillo olive fruit treatments were recorded by sample treated with T2GN for both 232 and 270 nm, while, the control sample found to be record the lowest value. 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-Sanchez *et al.,* (2012). Statistically significant variations took place depending on both control and treatments.

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 NAA and GA_3 growth regulators were 93.96, 131.15 and 79.04 ppm for T1N, T2GN, and T3GN, respectively, in comparison with control samples which was 374.99 showing that phenolic compounds in samples treated with growth regulators is greatly reduced in relation to control samples.

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 and Lanari, (2009) and Benothman *et al.,* (2009). The results indicate that there is significant decrease of total phenolic compounds of treated samples as comparing with control sample.

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 there is significant difference among the four treatments in chlorophyll and carotenoid contents, which was found to be ranged from 6.50 to 8.54 and 4.57 to 6.54 mg/kg, respectively. This indicates that there was significant difference between all treatments in both chlorophyll and carotenoid content.

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

Physical and chemical properties		Treatments			
		Control	T1N	T2GN	T3GN
Refractive index at 25ºC		1.4703±0.0004[a]	1.4702±0.0003[a]	1.4703±0.0002[a]	1.4702±0.0003[a]
Color at yellow 35	Red	7.7±0.05[b]	7.1±0.06[c]	7.0±0.07[c]	8.8±0.01[a]
	Blue	10.0±0.02[a]	7.7±0.03[c]	8.5±0.04[b]	10.2±0.08[a]
Conjugated Diene (K 232 nm)		0.15±0.002[c]	0.17±0.003[b]	0.17±0.002[b]	0.19±0.001[a]
Conjugated Triene (K 270 nm)		1.65±0.03[c]	1.78±0.01[b]	1.82±0.02[b]	1.90±0.03[a]
Free fatty acids (as oleic acid %)		0.18±0.01[c]	0.22±0.03[b]	0.25±0.02[b]	0.29±0.03[a]
Peroxide value (meq/kg oil)		3.14±0.09[d]	3.95±0.08[b]	3.54±0.09[c]	4.11±0.07[a]
Iodine value (Hanus)		84.55±1.11[a]	81.94±0.65[a]	82.81±1.35[a]	80.22±1.15[a]
TBA values		0.0104±0.0001[a]	0.0104±0.0003[a]	0.0078±0.0002[b]	0.0104±0.0002[a]
Unsaponifiable matter %		1.22±0.05[c]	1.26±0.07[b]	1.39±0.05[a]	1.29±0.06[b]
Total phenols content (ppm)		374.99±9.54[a]	93.96±9.14[c]	131.15±7.69[b]	79.04±8.52[d]
Stability period (hr)		48.70±1.1[a]	17.40±0.47[c]	21.50±0.52[b]	15.20±0.43[d]
Chlorophyll (mg/ kg)		7.85±0.98[b]	7.11±0.85[bc]	8.54±0.92[a]	6.50±0.68[c]
Carotenoids (mg/ kg)		5.89±0.23[b]	4.57±0.35[d]	6.54±0.33[a]	4.98±0.28[c]

(M±S.D) = Mean ± Std. Deviation. Values with different small letters in the same row are significantly different (p<0.05).

Fatty acid profile:

The fatty acid profile (FA) 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 international olive council (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 NAA and GA_3 growth regulators found to be satisfactory in terms 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 13.10 to 15.22%, stearic acid (C18:0) from 1.54 to 2.89%, oleic (C18:1) from 67.14 to 69.86%, linoleic acid (C18:2) from 9.32 to 11.15% and linolenic acid (C18:3) from 0.84 to 0.89%. In relation to palmitic the highest level was recorded by T1N (15.50%), while, the lowest level was found in the control sample (13.10%).While stearic acid the lowest level was showed in sample treated with T2GN (1.54%) and the highest level was found in T3GN (2.89%).In addition, oleic acid and linoleic acid the highest level were in the control (69.86 and 11.15%), respectively, while linolenic acid showed the highest level in the tested sample treated by T1N and control (0.89%), while the lowest level was recorded in T2GN treatment. As well as total saturated fatty acids content in Manzanillo olive fruits treated by NAA and GA_3 growth regulators as control sample, T1N, T2GN and T3GN was 15.12, 18.47, 16.60 and 18.57%, respectively; total unsaturated fatty acids content was 82.79, 79.89, 81.30 and 79.80 %, respectively, and 5.48, 4.90, 4.30 and 4.33, 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 NAA and GA_3 growth regulators.

Fatty acid composition %	Treatments			
	Control	T1N	T2GN	T3GN
$C_{16:0}$	13.10	15.50	14.84	15.22
$C_{16:1}$	0.89	2.51	2.31	1.14
$C_{17:0}$	0.15	0.17	0.11	0.11
$C_{18:0}$	1.75	2.35	1.54	2.89
$C_{18:1}$	69.86	67.14	68.42	68.49
$C_{18:2}$	11.15	9.35	9.73	9.32
$C_{18:3}$	0.89	0.89	0.84	0.85
$C_{20:0}$	0.12	0.45	0.11	0.35
TSFAs	15.12	18.47	16.60	18.57
TUFAs	82.79	79.89	81.30	79.80
TFA	97.91	98.36	97.9	98.37
*R	5.48	4.33	4.90	4.30

*TSFAs: Total saturated fatty acids TUFAs: Total unsaturated fatty acids TFA: Total fatty acids *R: Ratio of unsaturated to saturated*

Effect of storage at the ambient temperature for 24 months on some physical and chemical properties of olive oil extracted from fruit of Manzanillo olive trees treated by NAA and GA_3 growth regulators:

Refractive index:

Table (4) illustrated the changes in the refractive index of evaluated olive oil extracted samples from Manzanillo olive fruits treated by NAA and GA_3 growth regulators and comparing them with control sample during storage up to 24 months at ambient temperature.

Data in Table (4) showed that, the refractive index values were ranged from 1.4703 to 1.4689, 1.4702 to 1.4671, 1.4703 to 1.4680 and 1.4702 to 1.4679 for control, T1N, T2GN and T3GN samples from the beginning of the storage period till 24 months of storage, respectively. Also, indicating that there is nonsignificant difference between control and treated samples.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 and variation in the length of hydrocarbon chain and number of double bonds. These results were found to be in agreement with Van Oss, (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 NAA and GA_3 growth regulators during storage period for 24 months at the ambient temperature.

Storage period (months)	Treatments			
	Control	T1N	T2GN	T3GN
0	1.4703±0.0004^a	1.4702±0.0003^a	1.4703±0.0002^a	1.4702±0.0003^a
3	1.4703±0.0002^a	1.4701±0.0001^a	1.4701±0.0000^a	1.4700±0.0005^a
6	1.4701±0.0004^a	1.4698±0.0007^a	1.4699±0.0004^a	1.4698±0.0001^a
9	1.4700±0.0006^a	1.4696±0.0000^a	1.4697±0.0002^a	1.4696±0.0004^a
12	1.4697±0.0003^a	1.4694±0.0004^a	1.4695±0.0002^a	1.4694±0.0003^a
15	1.4696±0.0004^a	1.4692±0.0003^a	1.4694±0.0005^a	1.4693±0.0004^a
18	1.4693±0.0000^a	1.4689±0.0003^a	1.4691±0.0001^a	1.4688±0.0002^a
21	1.4691±0.0006^a	1.4685±0.0002^a	1.4686±0.0006^a	1.4684±0.0004^a
24	1.4689±0.0001^a	1.4671±0.0005^a	1.4680±0.0004^a	1.4679±0.0001^a

(M±S.D) = Mean ± Std. Deviation. Values with different small letters in the same row are significantly different ($p<0.05$).

Free fatty acids (FFA)

The free fatty acid (FFA) content as a percentage of oleic acid of the examined virgin olive oil extracted from Manzanillo olive fruits treated by NAA and GA_3 and stored for two years at ambient temperature 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 (FFAs%) of olive oils at the beginning of the storage period were 0.18, 0.25, 0.29 and 0.24 and after 24 months of storage period the free fatty acids reached to 0.69, 0.88, 0.92 and 0.87 (as oleic acid %) for control, T1GN, T2GN and T3N samples, 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.The percentage of increasing of FFAs% between the beginning of the storage period and after 24 months of storage was 26.09, 28.41, 31.52 and 27.59% for control, T1GN, T2GN and T3N samples, respectively. 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).

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

Storage period (months)	Treatments			
	Control	T1N	T2GN	T3GN
0	0.18±0.01^c	0.24±0.03^b	0.25±0.02^b	0.29±0.03^a
3	0.21±0.02^d	0.28±0.01^c	0.36±0.03^a	0.33±0.03^b
6	0.27±0.04^c	0.37±0.03^b	0.46±0.05^a	0.37±0.04^b
9	0.31±0.01^c	0.45±0.03^b	0.59±0.01^a	0.45±0.03^b
12	0.35±0.03^c	0.52±0.02^b	0.64±0.05^a	0.54±0.03^b
15	0.39±0.02^c	0.61±0.02^b	0.71±0.04^a	0.65±0.01^b
18	0.47±0.04^c	0.73±0.04^b	0.79±0.05^a	0.72±0.04^b
21	0.53±0.01^b	0.80±0.06^a	0.82±0.07^a	0.81±0.05^a
24	0.69±0.05^b	0.87±0.05^a	0.88±0.05^a	0.92±0.06^a

(M±S.D) = Mean ± Std. Deviation. Values with different small letters in the same row are significantly different ($p<0.05$).

The higher value of free fatty acids at the end of storage (24 months) was found to sample treated with T3GN be (0.92) and the lesser degree of free fatty acids was found in control sample (0.69), all treated samples reached to higher level than the limits of extra-virgin olive oil (0.8) and lower than the limits of virgin olive oil (2%) except control sample, it was found to be under the limits of extra-virgin olive oil according to Egyptian Standards of Olive Oil (2005), 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 were in agreement with Hui (1996); Spyros *et al.,* (2004) and Paradiso *et al.,* (2010).

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 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 values were ranged from 3.14 to 15.50, 3.95 to 21.83, 3.54 to 19.75 and 4.11 to 22.35 for control, T1N, T2GN and T3GN samples at beginning of the storage period and after 24 months of storage, respectively.

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 percentage of increasing between the beginning of the storage period and after 24 months of storage was 79.74, 81.91, 82.08 and 81.61 % for control, T1N, T2GN and T3GN respectively. 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 difference between these samples treated by (NAA and GA_3) 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 amounts 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).

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

Storage period (months)	Treatments			
	Control	T1N	T2GN	T3GN
0	3.14±0.09^{d}	3.95±0.08^{b}	3.54±0.09^{c}	4.11±0.07^{a}
3	3.34±0.08^{c}	4.65±0.11^{b}	4.72±0.23^{b}	5.33±0.09^{a}
6	5.51±0.25^{c}	7.54±0.23^{b}	7.65±0.09b	8.49±0.55^{a}
9	6.78±0.18^{c}	9.99±0.31^{b}	9.73±0.42^{b}	10.53±0.49^{a}
12	8.59±0.31^{c}	11.45±0.17^{b}	11.21±0.22^{b}	12.23±0.09^{a}
15	10.42±0.22^{c}	13.12±0.15^{b}	13.35±0.35^{b}	15.21±0.33^{a}
18	12.91±0.10^{c}	16.11±0.19^{b}	15.44±0.21^{b}	17.79±0.41^{a}
21	14.82±0.81^{c}	19.77±0.16^{a}	17.75±0.52^{b}	19.45±0.22^{a}
24	15.50±0.22^{c}	21.83±0.27^{a}	19.75±0.40^{b}	22.35±0.32^{a}

(M±S.D) = Mean ± Std. Deviation. Values with different small letters in the same row are significantly different (p<0.05).

Iodine value (IV)

The iodine value 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).

From data in table (7) it could be observed that the iodine values at the beginning of storage were 84.55, 81.94, 82.81 and 80.22 g I_2/100 g oil, then gradually decreased during the storage period until reached to 73.55, 69.32, 68.45 and 68.24 g I_2/100 g oil after 24 months for control sample T1N, T2GN and T3GN, respectively. The variation in iodine number of all samples could be indicate the degree of unsaturation and the total number of double bonds (Hui, 1996).These data illustrated that clear 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 sharp decrease in respect to the initial value was in sample treated by T2GN and the lowest level was at the control sample.

From these results it could be noted that the rate of decrease in the number of iodine values during storage was higher in the samples treated by growth regulators as compared with control sample. The rate of decreasing between the beginning of the storage period and after 24 months of storage was 14.96, 18.21, 20.98 and 17.23 % for control, T1N, T2GN and T3GN, respectively.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 Mendaz and Falque, (2007).

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

Storage period (months)	Treatments			
	Control	T1N	T2GN	T3GN
0	84.55±1.11 [a]	81.94±0.65 [a]	82.81±1.35 [a]	80.22±1.15 [a]
3	83.29±2.00 [a]	81.23±1.09 [a]	82.11±1.49 [a]	79.19±0.99 [ab]
6	82.56±1.23 [a]	80.31±1.34 [a]	81.31±2.31 [a]	78.36±1.03 [ab]
9	81.54±1.94 [a]	79.15±0.89 [a]	80.22±1.00 [a]	77.22±1.20 [b]
12	80.64±1.21 [a]	77.92±1.55 [a]	78.85±1.92 [a]	75.98±2.09 [b]
15	79.85±1.01 [a]	76.34±2.03 [a]	76.42±2.50 [a]	74.22±1.42 [b]
18	77.86±1.13 [a]	74.88±1.38 [a]	74.39±1.99 [a]	72.55±1.56 [b]
21	75.08±2.23 [a]	72.51±0.42 [a]	71.41±2.11 [ab]	70.47±1.93 [b]
24	73.55±1.17 [a]	69.32±0.74 [b]	68.45±1.09 [b]	68.24±1.07 [b]

(M±S.D) = Mean ± Std. Deviation. Values with different small letters in the same row are significantly different (p<0.05).

Thiobarbituric acid (TBA)

Data in table (8) showed thiobarbituric acid values of olive oil extracted from manzanillo olive fruit treated by GA3 and NAA growth regulators during storage period for 24 months.

From data in Table (8) it could be observed that the TBA values at the beginning of the storage period were 0.0104, 0.0104, 0.0078 and 0.0104 mg. malonaldehyde / kg oil, then gradually increased as the storage period increased up to 24 months it were reached to 0.1638, 0.1742, 0.1924 and 0.1820 mg. malonaldehyde / kg oil for control sample, T1N, T2GN and T3GN, 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 sample treated by T3GN (0.1924) and the lowest value recorded with control sample (0.1638). The rate of increasing between the beginning of the storage period and after 24 months of storage was 93.65, 94.29, 95.52 and 94.59% for control, T1N, T2GN and T3GN, respectively.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 NAA and 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 NAA and GA_3 growth regulators during storage period for 24 months at the ambient temperature.

Storage period (months)	Treatments			
	Control	T1N	T2GN	T3GN
0	0.0104±±0.0001[a]	0.0104±0.0003[a]	0.0078±0.0002[b]	0.0104±0.0002[a]
3	0.0156±±0.0000[a]	0.0156±0.0001[a]	0.0130±0.0001[b]	0.0156±0.0001[a]
6	0.0208±0.0001[a]	0.0208±0.0002[a]	0.0208±0.0002[a]	0.0208±0.0002[a]
9	0.0260±0.0001[b]	0.0286±0.0003[a]	0.0260±0.0004[b]	0.0286±0.0006[a]
12	0.0312±0.0004[d]	0.0416±0.0011[b]	0.0338±0.0001[c]	0.0702±0.0011[a]
15	0.0520±0.0021[c]	0.0598±0.0001[d]	0.0598±0.0007[b]	0.1092±0.0011[a]
18	0.1040±0.0010[c]	0.1118±0.0050[d]	0.1118±0.0010[b]	0.1352±0.0023[a]
21	0.1248±0.0023[d]	0.1378±0.0024[c]	0.1482±0.0033[b]	0.1586±0.0062[a]
24	0.1638±0.0031[c]	0.1820±0.0071[b]	0.1742±0.0021[b]	0.1924±0.0054[a]

(M±S.D) = Mean ± Std. Deviation. Values with different small letters in the same row are significantly different (p<0.05).

Conclusion

From the obtained results in this study, by comparing the effect of means of treatments (T1N, T2GN and T3GN) on olive oil quality it was concluded that treatments of olive fruits with growth regulators leading to decreasing in quality of the oil, changing of physiochemical properties and storage stability for olive oil extracted compared with control sample which was found to be high quality, more stable and distinctive in total phenols and oxidative stability compared with other treatment.

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