Current Science International
ISSN 2077-4435

Volume : 04 | Issue : 03| July-Sept.|2015
Pages: 370-386

Quality Attributes Of Naturally-Fermented Manzanillo Table Olives As Affected By Treating Of Olive Fruits with Some Plant Hormones (Plant Growth Regulators).

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ABSTRACT

The objective of this investigation was to evaluate the physico-chemical characteristics, and nutritional value of Manzanillo table olives treated by some growth regulators (Gibberellic acid (GA3) and Naphthalene acetic acid (NAA) during growth of olive fruit as compared with control samples before and after processing with untreated natural fermented style (natural fermented table olive) method immersion in brine without any preliminary treatments, the fermentations are left to develop ‘‘spontaneously’’ without adding any starter culture. Moreover, study the changes occurred in physico-chemical ,sensorial characteristics and determined the affecting and suitability for processing of table olives during storage as affected by treating with some growth regulators corresponding to control samples and their effect on produced natural fermented table olive (untreated natural fermented style). The obtained results showed that, the fermentation process of Manzanillo olive fruits treated by GA3 and /or NAA led to decreasing in moisture contents, protein content, total sugars, crude fiber and total carbohydrates on contrast dry matter, oil content and ash content was found to be increased in all tested samples. As regards, physical properties of Manzanillo olive fruit treated with GA3 either individually or in combination with NAA positively influenced fruit physical quality, i. e., fruit weight, flesh weight, stone weight, flesh /stone ratio and the percent of flesh. Also the results shown that decreasing in total phenols, total carotenoids and chlorophyll A and B in fresh treated samples when compared with fresh control sample as well as the samples treated by GA3 and NAA was showed more decreasing than the control sample in total phenols, total carotenoids and chlorophyll A and B as affected by fermentation process. Concerning sensory evaluation of tested Manzanillo olives showed no significant differences were found mainly between the control samples and other Manzanillo treatments in all sensory parameters (appearance, color, odor, Flavor and aroma) except Taste in sample T2 and T3 ,texture T6 and global appreciation T6 showed Significant differences**.** Based on the results obtained, sample T5 is highly appreciated by the consumers. This fact indicates that this treatment could be used for table olive production which was recorded high score in most sensory parameters, furthermore global appreciation**.** On the other hand, tested samples T6 was negatively evaluated in most the sensorial parameters and significant statistical differences were found between this table olive treatment and the others.

Key words: Fermented Manzanillo Table Olives, Natural fermentation style, Quality criteria of fermented olives, Total phenols, Total carotenoids, Chlorophyll A and B, Gibberellin acid , Naphthalene acetic acid

Introduction

Olive tree (*Olea Europaea* L.) is one of the most important fruit trees in the Mediterranean Basin and is widespread through the entire region (Schröder, 2007). Table olives are the most popular agro-fermented food product and are consumed and enjoyed throughout the entire world. Consumers perception of quality is improving and nowadays an increased seek for healthier products can be observed worldwide. Mainly composed by monounsaturated fatty acids, table olives consumption can prevent and reduce the risk of cardiovascular diseases (Kastorini *et al.,* 2010). In addition, other minor constituents like tocopherols and phenolic compounds are responsible for antioxidant and antimicrobial properties (Sousa *et al.,* 2008), protecting the organism from diseases in which free radicals and pathogenic microorganisms are involved, preventing also the body from certain kinds of cancer (Owen *et al.,* 2004) and arthrosclerosis (Armstrong *et al.,* 1997). The differences observed in the processes influence the chemical composition of the table olives by increasing the water content and salt levels due to NaCl penetration in the fruit (Gómez et al., 2006), reduction of carbohydrates in the fruit due to consumption by the microorganisms in order to obtain energy (Kailis and Harris, 2007), and the loss of minor compounds like phenolic compounds (Brenes *et al.,* 1995; Marsilio *et al*., 2001 and Romero *et al.,* 2004).

Table olives are the most important fermented food worldwide. In the 2010-2011 season, world table olive production reached 2,369,000 tons, the majority of which (48%) was produced in the European Union,

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particularly in Spain, Italy, Greece (809,000 tons) and Turkey (330,000 tons). Clearly, Turkey is one of the most important countries for table olive production. Other countries that produce significant amounts of olives include Argentina (10.5% of world production), Egypt (8.4%), the US (6.5%), Syria (5.9%) and Morocco (4.6%). World consumption has reached 2.205.000 tons, and the European Union is the major consumer (26%, 574,000 tons) followed by Turkey (11.8%, 260,000 tons), the USA (10.9%, 240,000 tons), Egypt (17%, 190,000 tons) and Algeria (5.8%, 129,000 tons) (IOOC, 2012). Currently, there are four main types of table olives processing: Spanish-style green olives, Gemlik-style or Greek-style naturally olives, Californian-style ripe olives and dry salted-style olives. Gemlik or the Greek-style olives are popular in Turkey, Greece and Northern African countries. Turkey is the largest producer of this type of olive (24-27%), followed by Greece (18-21%) (Piga *et al.,* 2001; Romero *et al.,* 2004 and Uylaser *et al.,* 2008).

Olive fruits are harvested at different stages of ripening depending on the cultivar and processing method, the most common being the Spanish-style (for green olives which ferment after a treatment with NaOH) or Greek-style (for black olives which ferment after immersion in brine without any preliminary treatment), both relying on a fermentation process which involves, in different ways, mixed populations of microorganisms, mainly represented by lactic acid bacteria (LAB) and yeasts. Currently, both green and black olives are fermented ‘‘naturally’’: in these preparations the fermentations are left to develop ‘‘spontaneously’’ without monitoring the microbiota during the process and without adding any starter culture (pyropoulou *et al.,* 2001; Rejano *et al.,* 2010 and Tofalo *et al.,* 2012).

The consumer and the processing industry request fruits with good size, proper shape, high pulp/stone ratio, good texture and color, and ease in releasing the pit. The nutritional and biological value of the fruit depends on the chemical composition of the pulp, with water and oil as the main components, and reducing sugars, polysaccharides, polyphenols, and minerals present in lower amounts. Together with their nutritional value, reducing sugars are also important because they are the raw material for fermentation during fruit processing (Garrido *et al.,* 1997). Cell wall structure seems to be harmed by the partial solubilization of polysaccharides, influencing fruit texture (Jimenez *et al.,* 1997). Phenolic compounds contribute significantly to table olive quality because of their recognized antioxidant capacity. They are also related, between others, to organoleptic characteristics such as bitterness, color of natural black olives and olives darkened by oxidation in alkaline medium (Vazquez and Janer, 1977) and to the palatability of processed fruits (Vinson *et al.,* 1998).

Olive tree has a high economic value and many countries such as Mediterranean countries and others worldwide use its oil and conserved fruits (Mitrakos *et al.,* 1991 and Payvandi *et al.,* 2001). Olive trees are very well adapted to the high temperature; tolerate dry weather, high soil salinity levels and infertile soil.

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). Although olive trees are well adapted to the region, alternate bearing is one of the major cultural problems and economic drawbacks (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). Alternate bearing causes severe economic losses in many of the important olive cultivars. Typical yield loss in commercial plantings in the "off' year is nearly 50% than that yield in commercial plantings in the "on' year (Daood, 2002 and Baktir *et al.*, 2004). The size of the fruit is important, not only because it is a component of productive yield, but also determines the acceptance by the consumer as conserved fruits. Gibberellins are known for their ability to increase cell enlargement (Pharis and King, 1995; Arteca, 1996 and Davis, 2004), thus enhancing fruit growth in certain species such as citrus (El-Sese, 2005 and Eman *et al.,* 2007), litchi (Stern and Gazit, 2000 and Chang and Lin, 2006), guava (El-Sharkawy *et al.,* 2005), and pear (Zhang *et al.,* 2007). In all species so far studied, gibberellins had the potential for increasing fruit size.

Applications of plant growth regulators have been focused by many researches in areas of plant physiology and nutriology (Pan and Li, 1999 and 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).

The beneficial effects of Gibberellic acid (GA3) sprays on yield and fruit quality of different fruit crops were mentioned by many investigators including Swietlik, (2002), reported that the use of GA3 as a growth regulator to promote size and to control fruit drop also was reported by Arteca, (1996).

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).

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 and Davis, 2004) possibly by increasing cell size and/or cell numbers.

Also Ramezani and Shekafandeh, (2009) studies the application of Gibberellic acid (GA3) on olive fruit and found that, 30 ppm GA3 along with 0.5% ZnSO4 at third stage of olive fruit growth stimulated cell enlargement in the mesocarp of Shengeh olive fruit, which in turn, caused a significant improvement in fruit size, weight and total yield.

Olive fruit 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 and the harvest period as well as the climatic conditions prevalent in the year of production (Abaza *et al.,* 2005; Ben Temime *et al.,* 2006 and 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 fruit (Aparicio *et al.,* 1994; Pannelli *et al.,* 1994; Mousa *et al.,* 1996 and Ryan *et al.,* 1998) and types of table olives processing. These changes are reflected on the chemical - physical quality, nutritional value and sensorial characteristics, of the obtained product natural fermented table olives.

Since few report has been published as to the effect of treating olive fruit with some growth regulators Gibberellic acid(GA3) and Naphthaleneacetic acid (NAA) and no report has been published as to the their effect on produced natural fermented table olive.

In particular, the aim of this study was to investigate the physico-chemical characteristics, and nutritional value of table olives treated by some growth regulators (Gibberellic acid (GA3) and Naphthalene acetic acid (NAA) during growth of olive fruit as compared with control sample before and after processed with untreated natural fermented– style (natural fermented table olive) method immersion in brine without any preliminary treatments, the fermentations are left to develop ‘‘spontaneously’’ without adding any starter culture. Moreover, study the changes occurred in physico-chemical ,sensorial characteristics and determined affecting and suitability for processing of table olives during storage as affected by treating with some growth regulators corresponding to control sample and their effect on produced natural fermented table olive (untreated natural fermented– style).

Materials and Methods

Olive fruits

Olive fruits of the Manzanillo cultivar untreated (control) or treated by Gibberellic acid (GA3) and Naphthaleneacetic acid (NAA) were obtained from private farm in Wadi El-Faregh, Behira Governorate, Egypt. Manzanillo olive fruit handpicked harvested at ripening stage from trees of 10 years old 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:

T_1-Control without treatment,
T_2- GA3 at 50 ppm.
T_3- GA3 at 75 ppm.
T_4- GA3 at 75 ppm + NAA at 50 ppm.
T_5- GA3 at 75 ppm + NAA at 75 ppm.
T_6- NAA 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.

Solvent, reagents and standard

All solvent were distilled before use, Folin Ciocalteau reagent was obtained from Gerbsaure Chemical Co. Ltd Germany and Standard phenolic compounds were obtained from Koch-light Laboratory. Ltd Colubrook, Buckingham, Shira, England.

Salt: Sodium chloride, food grade was purchased from El-Nasr Salt Company, Alexandria, Egypt

Olive fruit processing

Olives fruit of the Manzanillo cultivars were taken randomly from the six treatments and were hand-picked when they had a green-yellow surface color at their mature stage of ripening and normal large size and transported to the laboratory and was processed as are untreated green olives in brine (processed by a natural fermentation style).The collected samples on arrival, were washed under tap water to remove the dust, hand selected to remove defective drupes only olives free of blemishes, cuts and insect punctures were selected and placed directly into plastic container containing 3 kg of olives and 2.25 L of brine 10% w/v food-grade sodium chloride in potable water (3 plastic container for every treatment) where over time they take up salt and undergo a weak fermentation. A perforated cap was used to submerge olives in the brine and olive oil layer was added. Fermentation vessels were maintained at room temperature for an overall period of 12 month (Kailis and Harris, 2004). During the process, salt level was maintained constant at 10% by periodic additions of coarse salt in the brine. The process was allowed to evolve spontaneously by the indigenous microbiota of olives based on the traditional anaerobic method as described by (Balatsouras, 1980). During brining, process water-soluble oleuropein and other phenolic compounds, sugars, vitamins and minerals leach out of the olive flesh; the net result is debittering and fermentation of the olives. During fermentation sugars are converted to lactic and acetic

acid, alcohol and other substances which contribute to the taste of the olives. The salt in the brine equilibrates with that in the fruit and should be maintained at around 10 % during debittering and fermentation (Kailis and Harris, 2004). A month later the brine salt concentration was gradually increased up to 10 % (w /w). Under these conditions the olive flesh bitterness disappeared and lactic fermentation took place, to some extent. Brine solution was taken periodically at one month for the analyses of pH, total acidity and Nacl for 12 months.

Fruit physical and chemical characteristics

For each batch of tested olives, 30 olives fruits were weighed and destoned with a hand destoner. The stones were then cleaned of flesh with a sharp knife and wiped thoroughly with a paper towel to remove any flesh adhering to the stones. The pooled flesh and the thirty stones were then weighed and the flesh to stone ratio calculated according to the methods described by Kailis and Harris, (2004) and Arslan, (2012) as follows:
(1) Fruit weight was determined by weighing the samples (30 fruits) by electric balance with 0.0001g sensitivity and average weight per fruit was calculated.
(2) Pit weight was determined by weighing the sample (30 pit) and average weight of pit was calculated.
(3)Flesh / Pit ratio was calculated by dividing the weight of the flesh over the weight of the pit.

Flesh: Stone ratio = (weight of olives – weight of stones) ÷ weight of stones

pH value

The pH value of brine samples was measured using digital pH meter (JENWAY model 3505, UK) according to the procedure described in AOAC., (2005),The pH meter was calibrated using to standard buffers having pH 4.0 and 7.0, respectively.

Chemical composition of olive fruits

Moisture, Lipid, crude protein, fiber and ash were determined according to AOAC., (2005). Total sugar contents were determined using the colorimetric method described by Herbert *et al.* (1971). Total carbohydrates were calculated by difference.
% Total carbohydrates = 100- (% moisture + % crude protein + % fat + % ash).

Total phenol compounds

The measurement of total phenolics (TPs) content was conducted according to the modified Folin–Ciocalteu colorimetric method Singleton *et al.* (1999) at 760 nm using an UV/Visible spectrophotometer. gallic acid was used as a standard, and then the results were expressed as gallic acid equivalents (GAE) per 100 g dry matter

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 Brawn type homogenizer (Braun AG Frank, 40-60 Hz /400W, Tipe MX 32, No. 4142, German) at 1000 rpm for one minute. The homogenate was filtered through Whatman No. 1 filter paper, and centrifuged using the centrifuge at 2500 rpm for ten minutes. The sample was separated and the absorbance was read using an UV/Visible spectrophotometer (Spekol 11 No. 849101, Carl Zeiss JENA). 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) mg/kg , C_b= chlorophyll (B mg/kg), Cx+c= carotene mg/kg

Total phenols content

The amount of total phenolics in the fruit extracts was determined colorimetrically as described previously by Montedoro *et al.* (1992). In fact, 50 ml of methanol 80% was added to 30 g of the olive pulp. The mixture was homogenized then centrifuged at 5,000 rpm for10 min. This step was done twice, and supernatants were mixed together and concentrated with a rotary evaporator until a final volume of 10 ml. Then, 10 ml of Folin-Ciocalteu reagent (FCR) (previously diluted 10-fold with distilled water)and 8 ml of Na2CO3 (75 g/L) were added to a suitable aliquot of the combined extracts, and the absorbance of the solution at 765 nm was measured after 2 h by using an UV/Visible spectrophotometer (Spekol 11 No. 849101, Carl Zeiss JENA). Gallic acid was used as a standard, and then the results were expressed in milligram per kilogram as Gallic acid per kg of dry weight.

The HPLC analysis of Phenolic compounds (identification and quantification)

Extraction and measurement of phenolic compounds in fresh and fermented olive fruit were analyzed by HPLC according to the method of Goupy *et al.* (1999) as follows:

Weighed sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Hewlett-Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set as 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 35 0c. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1 ml/min. Phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of Phenolic compounds concentration. The Phenolics compounds were determined in Ministry of Agricultural and Land Reclamation, Agricultural Research Center, Food Technology Research Institute.

Free acidity

The acidity of brines was determined by volumetric analysis by titrating samples with a standardized solution of 0.1M sodium hydroxide solution using phenolphthalein indicator and expressed as %w/v of Lactic Acid according to Garrido-Fernandez *et al.* (1997).

Sodium chloride levels

Levels of sodium chloride were measured by volumetric analysis titrating samples with a standardized solution of silver nitrate (0.1M) with potassium chromate as the indicator and the results expressed as %w/v sodium chloride according to IOOC. (1990).

Mineral contents

Minerals (sodium, potassium, calcium, magnesium, and phosphorus) content of different Manzanillo olive treatments (before and after fermentation) were determined according to the standard method of AOAC. (2005) using Atomic Absorption Spectrophotometer (GBC, Model 932AA, Australia) at the Regional Center of Mycology and Biotechnology, Cairo, Egypt. All the analysis samples were performed in triplicate for each sample.

Organoleptic evaluation

Organoleptic evaluation of processed table olives treated by some growth regulators (Gibberellic acid (GA3) and Naphthalene acetic acid (NAA) as compared with control samples was investigated. The processed table olives samples were codified and presented simultaneously to 10 panelists of Food Science and Technology Department, Faculty of Agriculture Al Azhar University to sensorial evaluation. The panelists were asked to rank the samples on a hedonic scale of 1(very poor), 2-4 (poor), 5-6 (fair), 7-8 (good) and 9-10(excellent)to evaluate the appearance, color, taste, odor, flavor, aroma, texture and global appreciation) according to Ziena *et al.* (1997) and Malheiro *et al.* (2012).

Statistical analysis

All the determinations were carried out in triplicate and the results 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 and duncan's multiple range of probability and standard error (S.E.). 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

Effect of treating with growth regulators on chemical composition of Manzanillo table olive before and after fermentation process.

The consumer and the processing industry of olive request fruits with good size, proper shape, high pulp/stone ratio, good texture and color, and ease in releasing the pit. The nutritional and biological value of the fruit depends on the chemical composition of the pulp, with water and oil as the main components, and reducing sugars, polysaccharides, polyphenols, and minerals present in lower amounts. Together with their nutritional value, reducing sugars are also important because they are the raw material for fermentation during fruit processing (Garrido *et al.,* 1997 and Morales-Sillero *et al.,* 2008).

The chemical composition of raw and fermented Manzanillo table olive treated with gibberellic acid (GA3) and naphthalene acetic acid (NAA) growth regulators (Control without treatment (T_1), gibberellic acid (GA3) at 50 ppm (T_2),Gibberellic acid (GA3) at 75 ppm (T_3),Gibberellic acid (GA3) at 75 ppm +

naphthaleneacetic acid (NAA)at 50 ppm (T_4),Gibberellic acid (GA3) at 75 ppm + naphthaleneacetic acid (NAA) at 75 ppm (T_5) and naphthaleneacetic acid (NAA) at 75 ppm(T_6)) are recorded in Table (1). The composition of raw and fermented Manzanillo table olive in table 1 are found as moisture, dry matter, oil, crude protein, ash, total sugars, crude fiber and total carbohydrates. The main constituents of the raw or fermented olive flesh are water (65.88 – 67.30 %) for fresh olive and (63.97 – 65.12%) for fermented olives and the higher amounts of moisture was found in T6 (67.3) flowed by T5 and T1 in fresh samples ,and T5 was contained the highest level of moisture flowed by T1 and T6 of fermented olives. Also, data in table(1)showed that no significant differences between samples treated with GA3 and /or NAA in moisture content and control sample whether in fresh or fermented samples. Dry matter in raw and fermented Manzanillo table olive was at range 32.7 -34.12 % for fresh and 34.88 – 36.03 % for fermented samples, also dry matter was slight increase in fermented treatments than that found in fresh treatments the reduction of water in olive fruits and migration of soluble salts (NaCl) to inside of olives, this phenomena are known by osmotic pressure which was led to increasing of soled gain. These increasing in dry matter of fermented samples may be due to a flux of water out of the olive fruit to the outer media and of other solutes (NaCl) into the olive fruit develops due to the difference in osmotic pressure. The olive fruit thus loses water and gains solid from the external solution these results are in the harmony with reported by (Li and Ramaswamy, 2006 and Shi *et al.,* 2009).

The main constituents of the olive flesh raw and fermented are water and lipids, there is an inverse relationship between moisture content and lipid (oil) content, for the same treatments. That is mean increasing oil by decreasing the water in fresh and fermented olive fruit treatments. Oil content of fresh and fermented olives treated with GA3 and /or NAA was at range 18.53 - 20.98 and 19.14 – 21.38% respectively. Oil content in fresh sample T_2 was found to be the greatest amount flowed by T_3, T_1, T_4, T_6 and T_5, as well as there is no significant differences between fresh treatments compared with T_1(control sample) in oil content, also the same trained was found in fermented treatments**.**

protein content of fresh samples was at range 1.02 -1.26 % and 0.92- 1.06 % for fermented samples, as well as protein content of fermented treatments were slight lower than fresh in the same types of treatments and the highest amounts of proteins was found in T_6 (1.26%) followed by T_3 (1.22%), T_1 (1.22%), T_2 (1.16%), T_4 (1.11%) and T_5 (1.02%) for fresh samples while, the highest amounts in the fermented treatments were found in T_3 (1.06%) followed by T_2 (1.03%) , T_1 andT_6 (1.01%), T_4 (1.00%) and , T_5 (0.92%). The slight decreasing in protein content of fermented olives treated with growth regulators may be due to dissolved of soluble proteins in brine solution.

Also from data summarized in Table 1, it could observed that ash content of fresh samples was ranged between 1.11 and 1.21% and statistically was found to be no significantly differences between fresh olive fruit samples in ash contents. On the other hand, ash content of fermented table olive was drastically increased by using naturally fermentation process as compared with fresh samples. The treatment T_6 was recorded highest value of ash followed by treatment T_4, T5, T2, T3 and T_1, in addition statistically no significant differences between treatments T_4, T5, and T_6. On contrast, there are significant differences between treatment T_1 (control samples) and other treatments. The increasing of ash contents in table olive fruit as affected by fermentation process in brine solution (natural fermentation) may be resulted to soaking in sodium chloride solution and also as a result the migration of NaCl from brine solution to olive flesh during the fermentation process. These results are in agreement with the previous investigations about the compositional variation of olives during processing (Unal and Nergiz, 2003)

From the obtained results (Table 1), it could be also observed that the levels of total sugars contents in olive fruit flesh treated by GA3 and /or NAA was appears significant differences in treatments T_1 and T_2 (3.24 and 3.25 %) between T_3, T_4, T5 and T_6, which was found to be 3.74 , 3.85 , 4.12 and 4.15%; respectively, for fresh samples, while total sugars in fermented olive fruits flesh was sharply decreased. The decreasing in total sugars of fermented olive fruit flesh was accrued resulted to the microorganisms in the medium which using the sugars as an energy source which was consider as fermentable substrate in fermentation process (Kailis and Harris, 2007). The remained sugars after fermentation process was ranged at 0.09 – 0.17 % compared with 3.24 - 4.15% in fresh treatments.

Dietary fiber content in Manzanillo olive fruits treated by GA3 and /or NAA was at range of 3.61 – 3.91 and 2.47 – 3.1% for fresh and processed Manzanillo olive fruit; respectively. The dietary fiber content in fresh olive fruits treatments was found to be no significant differences between control sample (untreated) and other fresh treatments, on contrast, there are significant differences in dietary fiber content between control sample (untreated) and T_2, T_5 and T_6 while other treatments (T_3 and T_4) was found to be no significant differences with control samples in fermented treatments. Beside that, dietary fiber content in fresh Manzanillo olive fruits treatments was showed decreased in all processed Manzanillo olive fruits treatments as affected by natural fermentation process. The loss of dietary fiber may have been due to a loss of polysaccharides and other components, and also may have been due to a longer period in brine. This decrease would be caused by a more intense solubilization of polysaccharides into brine and also to higher modifications due to chemical conditions of storage (Jimenez *et al.,* 2000).

Table 1: Chemical composition of Manzanillo table olive treated with growth regulators (fresh and after fermentation) on wet weight bases (*M±SE).

Treatments	Attributes							
	Moisture		Dry matter		Oil		Protein	
	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented.	Fresh	Fermented
T_1	67.2±0.71^{a}	65.11±0.69^{a}	32.80±0.33^{a}	34.89±0.30^{a}	19.84±0.52^{a}	20.49±0.53^{a}	1.20±0.028^{a}	1.01±0.028^{ab}
T_2	66.82±0.75^{a}	64.54±0.65^{a}	33.18±0.24^{a}	35.46±0.35^{a}	20.98±0.51^{a}	21.38±0.52^{a}	1.16±0.040^{a}	1.03±0.017^{a}
T_3	65.88±0.57^{a}	63.97±0.66^{a}	34.12±0.35^{a}	36.03±0.25^{a}	20.43±0.53^{a}	20.99±0.53^{a}	1.22±0.034^{a}	1.06±0.034^{a}
T_4	66.43±0.65^{a}	64.57±0.72^{a}	33.57±0.32^{a}	35.43±0.36^{a}	19.23±0.5^{a}	20.01±0.52^{a}	1.11±0.034^{bc}	1.00±0.023^{ab}
T_5	67.22±0.72^{a}	65.12±0.69^{a}	32.78±0.31^{a}	34.88±0.31^{a}	18.53±0.51^{a}	19.14±0.51^{a}	1.02±0.023^{c}	0.92±0.011^{b}
T_6	67.3±0.75^{a}	65.1±0.77^{a}	32.70±0.40^{a}	34.90±0.27^{a}	18.73±0.53^{a}	19.27±0.50^{a}	1.26±0.028^{a}	1.01±0.017^{ab}
LSD at 0.05	2.15	2.16	1.02	0.96	1.60	1.61	0.09	0.07
	Ash		Sugars		Fiber		Carbohydrates	
	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented.	Fresh	Fermented
T_1	1.18±0.052^{a}	8.35±0.185^{C}	3.24±0.11^{b}	0.14±0.012^{b}	3.78±0.11^{a}	2.94±0.040^{b}	10.58±0.27^{a}	5.04±0.21^{a}
T_2	1.14±0.46^{a}	9.21±0.167^{b}	3.25±0.089^{b}	0.14±0.017^{b}	3.88±0.069^{a}	3.10±0.035^{a}	9.90±0.23^{a}	3.84±0.11^{a}
T_3	1.17±0.052^{a}	8.97±0.242^{bC}	3.74±0.081^{a}	0.09±0.006^{C}	3.91±0.064^{a}	2.88±0.040^{b}	11.30±0.22^{a}	5.01±0.13^{a}
T_4	1.21±0.029^{a}	10.24±0.248^{a}	3.85±0.087^{a}	0.21±0.012^{a}	3.85±0.087^{a}	2.97±0.029^{b}	12.02±0.21^{a}	4.18±0.09^{a}
T_5	1.16±0.012^{a}	9.98±0.237^{a}	4.12±0.11^{a}	0.15±0.017^{b}	3.61±0.069^{a}	2.47±0.023^{d}	12.07±0.22^{a}	4.84±0.07^{a}
T_6	1.11±0.035^{a}	10.27±0.219^{a}	4.15±0.104^{a}	0.17±0.006^{b}	3.72±0.11^{a}	2.75±0.035^{C}	11.60±0.24^{a}	4.35±0.08^{a}
LSD at 0.05	0.12	0.67	0.30	0.03	0.26	0.10	7.77	4.54

*T_1: Control without treatment, T_2: Gibberellic acid (GA3) at 50 ppm. T_3: Gibberellic acid (GA3) at 75 ppm. T_4: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 50 ppm. T_5: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 75 ppm. T_6: Naphthaleneacetic acid (NAA) at 75 ppm. LSD: Least Significant Difference at 0.05. *M±SE: Mean of triplicate determinations results ± standard error; the means, within the same column, having different superscripts are significantly.*

Data in table (1) also showed that total carbohydrates before and after natural fermentation process of Manzanillo olive fruit treated with GA3 and /or NAA. From data in table (1), it could be concluded that total carbohydrates before and after natural fermentation process of Manzanillo olive fruit was ranged between 9.9 - 12.07 % for fresh (unfermented) olive fruits and 3.84 – 5.04% for processed (fermented). Also, data in table (1) it could be noticed that, total carbohydrate contents of Manzanillo olive fruits was decreased by natural fermentation process in all tested samples under investigation. In addition, there is no significant difference between control samples (untreated) and other treatments in total carbohydrate contents before fermentation process, the same observation was concluded after fermentation process in total carbohydrates contents. The decreasing in total carbohydrates of fermented table olive fruits treatments may be due to as the results of decreasing in total sugars resulted to the microorganisms in the medium, which using the sugars as an energy source which was consider as fermentable substrate in fermentation process (Kailis and Harris, 2007) and also the decreasing in total carbohydrates of fermented table olive fruits treatments may be due to the decreasing in dietary fiber contents which was accrued resulted to a loss of polysaccharides and other components, and also may have been due to a longer period in brine. This decrease would be caused by a more intense solubilization of polysaccharides into brine and also to higher modifications due to chemical conditions of storage (Jimenez *et al.,* 2000). The previous results are in approximate with the previous investigation about the compositional variation of olives during processing (Unal and Nergiz, 2003).

Generally, from previous discussion it could be concluded that the fermentation process of Manzanillo olive fruits treated by GA3 and /or NAA led to decreasing in moisture contents, protein content, total sugars, crude fiber and total carbohydrates, on contrast dry matter, oil content and ash content was found to be increased in all tested samples.

Mineral content of raw and fermented olive fruit.

The levels of mineral contents (mg/ 100g) (sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and Phosphorus (P) in both raw and fermented Manzanillo olive fruits are presented in Table (2). From data in table (2), it could be noticed that fresh Manzanillo olive fruit was contained Na, K, Ca, Mg and P at range 15 – 21, 541 – 570, 299 – 360, 42 – 48, 29 – 37 and 37 – 45 mg /100gm, respectively, and olive fruits treated with GA3 and / or NAA after fermentation was 1847 – 2104, 299 – 360, 35- 40, 28 – 33 and 39 – 42 mg /100gm, respectively. Therefore, the mineral content of tested fermented Manzanillo olive fruit treatments was decreased in K, Ca, Mg and P contents of olive than that found in fresh samples except Na content, which was showed drastically increased in fermented samples as affected by fermentation process.

As explained previously described in Table 1 when discussing the ash contents, it can be clarified that the results from analysis of ash content in Manzanillo olive flesh before and after fermentation process showed that the contents increased at the end of fermentation, as compared with the initial value. This behavior was registered in all tested samples and is due mainly to the absorption of salt (sodium chloride) that passes from the brine to the flesh. This explains the significant increase of the sodium in the flesh fruits of the fermented olive.

Nutritionally, table olives are a good source of minerals. Raw olives contain reasonable amounts of potassium, phosphorus, calcium and magnesium. The levels of these minerals depend on the olive variety, maturation state and processing method. Some minerals, especially potassium, are lost when prolonged soaking methods are used during processing. Processing also increases the sodium content of olives when carried out in brine. Furthermore, minerals are lost when they diffuse out of the olive flesh into the processing brines where they can be utilized by fermentative organisms. The previous results are in agreement with the investigation about the compositional variation of olives during processing (Unal and Nergiz, 2003 and Kailis and Harris 2007)

Table 2: Minerals content of Manzanillo table olive (mg /100 gm) (fresh and after fermentation) treated with gibberellic acid (GA3) and naphthaleneacetic acid (NAA)

Treatments	Mineral contents (mg) (*Mean ± SE).									
	Na		K		Ca		Mg		P	
	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented
T_1	16±0.43^{cd}	1954±4.76^{c}	553±5.96^{ab}	345±4.76^{a}	47±0.70^{a}	39±0.70^{a}	29±0.78^{b}	32±0.71^{ab}	45±1.09^{a}	42±0.98^{a}
T_2	17±0.38^{bc}	1847±5.34^{d}	561±4.76^{ab}	324±5.34^{b}	46±0.65^{a}	38±0.82^{ab}	31±0.81^{b}	30±0.73^{abc}	42±0.87^{a}	40±1.05^{a}
T_3	18±0.41^{b}	1977±2.85^{b}	541±5.47^{b}	311b±5.47^{c}	45±0.70^{a}	38±0.78^{ab}	34±0.94^{a}	28±0.77^{c}	41±1.00^{a}	40±1.03^{a}
T_4	16±0.43^{cd}	2089±6.03^{a}	549±4.13^{ab}	304b±5.51^{c}	47±0.69^{a}	38±0.70^{ab}	30±0.80^{b}	29±0.73^{bc}	37±0.94^{b}	39±1.09^{a}
T_5	15±0.34^{d}	2104±5.51^{a}	564±4.99^{a}	360±4.13^{a}	48±0.64^{a}	40±0.69^{a}	35±0.82^{a}	32±0.82^{ab}	41±1.00^{a}	41±1.10^{a}
T_6	21±0.46^{a}	1988±6.66^{b}	570±4.36^{a}	299±6.66^{c}	42±0.64^{b}	35±0.70^{b}	37±0.94^{a}	33±0.82^{a}	42±1.08^{a}	39±0.90^{a}
LSD value at p > 0.05	1.26	16.42	15.35	16.53	2.06	2.26	2.61	2.35	3.08	3.16

*T_1: Control without treatment, T_2: Gibberellic acid (GA3) at 50 ppm. T_3: Gibberellic acid (GA3) at 75 ppm. T_4: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 50 ppm. T_5: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 75 ppm. T_6: Naphthaleneacetic acid (NAA) at 75 ppm. LSD: Least Significant Difference at 0.05. *M±SE: Mean of triplicate determinations results ± standard error; the means, within the same column, having different superscripts are significantly.*

Physical properties of Manzanillo olive fruit treating with growth regulators before and after fermentation process.

Fruit weight is a function of moisture and oil content, so that marked changes in these parameters will affect the weight of olives. Olive weight is important in table olives, because consumers show a preference for larger olives than smaller ones and also the olive fruit weight determined for recognizes the fruit weight within ranges expected for those varieties and within desirable olive weights. The stone weight provide useful information When used to calculate the flesh to stone ratio this latter parameter is a crude indicator of the nutritional quality of the olive and flesh available per olive eaten. The Flesh: Stone ratio is derived by dividing the weight of flesh by the weight of the stone. Ideally the Flesh: Stone ratio of table olives should be 5:1. With processed olives the Flesh: Stone ratio can be used as a crude indicator of the nutritional quality of the olives. An indication of the required processing time can be obtained knowing the weight of the olives and the Flesh: Stone ratio. Smaller olives with a low Flesh: Stone ratio process faster than larger olives with a high Flesh: Stone ratio especially by traditional methods. Large olives with high Flesh: Stone ratios should be slit, stoned, cracked or bruised to facilitate processing**.**

The fruit weight, flesh weight, stone weight, flesh /stone ratio and the percent of flesh in fresh and fermented Manzanillo olive fruit treated with GA3 either individually or in combination with NAA are listed in Table (3).

From data in table (3) it could be noticed that, the Physical quality attributes of Manzanillo olive fruit before and after natural fermentation process was affected by foliar application treatments with GA3 either individually or in combination with NAA and also had pronounced effect on improving Manzanillo fruit Physical quality attributes. The fruit weights, flesh weights, stone weight, flash \ stone ratios and flesh percent in raw and fermented Manzanillo olive fruit was increased as a result of treatments with GA3 either individually or in combination with NAA when compared to control samples which was recorded the lowest value in all tested physical quality attributes of Manzanillo olive fruit before and after natural fermentation. In addition to the T_3 samples was showed the best treatment either before or after fermentation process which found to be fresh fruit weight 6.55 gm , fresh flesh weight 5.84 gm, stone weight 0.71 gm , flash \ stone ratios 8.22 and flesh percent 89.15 %. Spraying Manzanillo olive trees 10 days after fruit set with GA3 either individually or in combination with NAA slightly increased fruit weight, flesh weight, flash \ stone ratios and flesh percent than those of control samples. This means that Spraying Manzanillo olive fruit with GA3 either individually or in combination with NAA positively influenced fruit physical quality, i. e., fruit weight, flesh weight, stone weight, flesh /stone ratio and the percent of flesh These results are in harmony with that obtained by Lavee (2006) and Crous (2012). In relation to the effect of natural fermentation process on most Physical quality attributes (fruit weight, flesh weight, stone weight, flesh /stone ratio and the percent of flesh) of Manzanillo olive fruit as affected by treating with GA3 individually or in combination with NAA the fermentation process led to decreasing in fruit weight, flesh weight, flesh /stone ratio and the percent of flesh except stone weight was found

to be slight increased (Table 3). The decreasing as a result of the incident to the fermentation process may be due to leaching of water-soluble oleuropein and other phenolic compounds, sugars, vitamins and minerals out of the olive flesh. Kailis and Harris (2004) and Therios (2009) stated that due to salinity effects the water-soluble oleuropein, phenolics and minerals leach out from the olive fruits. During this period fermentation occurs, with the sugars being transformed into lactic and acetic acids, and other substances. Statistically, there are significant differences between Manzanillo olive fruit treatments as affected by treating with GA3 either individually or in combination with NAA and the control sample in tested Physical quality attributes.

Table 3: Physical properties of Manzanillo table olive (fresh and after fermentation) treated with gibberellic acid (GA3) and naphthalene acetic acid (NAA) (*Mean ± SE).

	Fruit weight		Flesh weight		Stone weight		Flesh / stone ratio		% Flesh	
Treat.	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented	Fresh	Fermented
T_1	4.82±0.2^{d}	4.55±0.22^{d}	4.01±0.17^{d}	3.71±0.18^{d}	0.81±0.02^{a}	0.84±0.03^{a}	4.95±0.2^{e}	4.42±0.19^{d}	83.20±2.5^{b}	81.542.56^{b}
T_2	5.71±0.1^{c}	5.50±0.24^{c}	5.02±0.19^{c}	4.76±0.21^{c}	0.69±0.01^{a}	0.74±0.02^{a}	7.28±0.30^{c}	6.43±0.21^{b}	87.92±3.2^{a}	86.55±2.4^{a}
T_3	6.55±0.1^{a}	6.31±0.26^{a}	5.84±0.20^{a}	5.57±0.23^{a}	0.71±0.01^{a}	0.74±0.01^{a}	8.22±0.30^{a}	7.53±0.30^{a}	89.15±3.12^{a}	88.27±3.2^{a}
T_4	6.11±0.2^{b}	5.86±0.26^{b}	5.39±0.24^{b}	5.11±0.19^{b}	0.72±$^{0.02a}$	0.75±0.02^{a}	7.49± 0.24^{c}	6.81±0.22^{b}	88.22±2.31^{a}	87.20±2.6^{a}
T_5	6.43±0.2^{a}	6.23±0.25^{a}	5.69±0.22^{a}	5.43±0.21^{a}	0.74±0.01^{a}	0.80±0.03^{a}	7.69±0.15^{b}	6.79±0.19^{b}	88.49±2.57^{a}	87.16±2.7^{a}
T_6	5.80±0.2^{c}	5.55±0.24^{c}	5.05±0.21^{c}	4.75±0.22^{c}	0.75±0.02^{a}	0.80±0.03^{a}	6.73±0.32^{d}	5.94±0.17^{c}	87.06±2.36^{a}	85.59±2.5^{a}
LSD	0.18	0.21	0.26	0.21	0.15	0.18	0.21	0.35±	2.61	2.44

*T_1: Control without treatment, T_2: Gibberellic acid (GA3) at 50 ppm. T_3: Gibberellic acid (GA3) at 75 ppm. T_4: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 50 ppm. T_5: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 75 ppm. T_6: Naphthaleneacetic acid (NAA) at 75 ppm. LSD: Least Significant Difference at 0.05. *M±SE: Mean of triplicate determinations results ± standard error; the means, within the same column, having different superscripts are significantly.*

Effect of natural fermentation process on total phenols, total carotenoids and chlorophyll A and B of Manzanillo table olive treated with GA3 and NAA.

Phenolic compounds, also called polyphenols, are secondary metabolites present in all plant tissues and are involved in protective mechanisms against external stresses. They have antioxidant activity, so when consumed in the diet they have beneficial effects on health. Polyphenols in olives include oleuropein, hydroxytyrosol, caffeic acid and tyrosol. Phenolic compounds make up 2–3% w/w of olive flesh, with oleuropein (a bitter tasting compound) the most abundant polyphenol.

Olive flesh contains chlorophyll A and B (green), carotenoids and triterpenic hydrocarbons (yellow) and anthocyanins (purple-black). The green and yellow pigments are oil soluble, whereas anthocyanins are water-soluble. Initially chlorophyll is the major pigment in the olive fruit that plays an important role in photosynthesis. As the fruit matures and ripens chlorophyll levels decrease while other pigments, beta-carotenes and the purple-black anthocyanins increase. Hui et al. (2007) Kailis and Harris (2007)

Total phenolic compounds, total carotenoids and total chlorophyll A and B in fresh and fermented table olive fruit treated with GA3 and NAA as importance ingredients in olive fruit was determined. The data are listed in table (4)

Data listed in table (4) elucidate the effect of natural fermentation process as well as treating with GA3 and NAA on the total phenolic compounds, total carotenoids and total chlorophyll A and B in Manzanillo olive fruit compared by control sample (without treatments). Total phenolic compounds, total carotenoids and total chlorophyll A and B in tested samples of Manzanillo olive fruit (fresh and fermented) were at range from 405 (T_6) - 759 (T_1) for fresh and 265 (T_6) – 532 (T_1) for fermented; 8.45 (T_6) - 11.51 (T_1) for fresh and 6.31 (T_5) – 9.20 (T_6) for fermented; 30.629(T_5) - 40.91(T_4) for fresh and 26.26(T_5)- 33.18(T_6) for fermented and 15.47 (T_5) – 18.24 (T_1) for fresh and 12.20 (T_6) – 15.11(T_4) ppm for fermented Manzanillo table olive; respectively.

From the obtained results Table (4), it is clear that fresh and fermented control sample of Manzanillo olive fruit was recorded the highest amounts in determined total phenolic compounds, total carotenoids and total chlorophyll A and B except in fresh sample chlorophyll A and chlorophyll B in fermented sample which were the highest value was recorded in sample T_4.

Statistically, there are significant differences between control sample (T1) and other treatments in total phenols, total carotenoids and chlorophyll A and B except sample T_4 in total carotenoids and chlorophyll A and B which was recorded near values (no significant differences) as shown in table (4).

From above discussion and data in table (4) it could be noticed that the treating of Manzanillo olive fruit with GA3 and NAA as described previously was resulted to decreasing in total phenols, total carotenoids and chlorophyll A and B in fresh treated samples when compared with fresh control samples as well as samples treated by GA3 and NAA was showed more decreasing than the control sample in total phenols, total carotenoids and chlorophyll A and B as affected by fermentation process.

During processing of table olives there are important changes in phenolic quality there are generally due to several mechanisms that occur during the fermentation .One of the phenomena is an osmotic exchange between fruit and brine mainly affecting the soluble sugars, Nacl and phenolic compounds. During fermentation the total phenolic content in the olive fruit is reduced due to diffusion of phenolic compounds into the brine and

it is closely related to the permeability of the olive skin and the type of fermentation. (Poiana and Romero, 2006; Ben Othman *et al.,* 2009), chlorophylls present in the fresh, green fruit disappear completely in the process giving rise to a mixture of their corresponding derivatives pheophytins and pheophorbides. This takes place by two different mechanisms, during the first fermentation phase, chlorophyll a gives rise pheophytin A, chlorophyllide A and pheophorbide a due to enzymatic hydrolysis with the help of a medium with optimum pH and not in a chemical way. At the end of the second period, when the pH of the medium falls to 4.5, chlorophyll b content falls, giving rise to pheophytin B and A mixture of chlorophyllide B and pheophorbide B in an important transformation. After seven months, neither chlorophylls nor chlorophyllides appear in the fruit, and the levels or pheophytins and pheophorbides remain practically constant during this time. According to Minguez-Mosquera, *et al.* (1989).The pigments (chlorophylls, pheophytins, xanthophylls and carotenes) are mostly present in the olive fruit at harvest time (Dıraman, 2010). When green olives are processed parts of chlorophyll A and B dissolve in water. This leads to some chlorophyll loss while the remaining parts are transformed into the corresponding pheophytins. At the end of treatment, green olives are straw-yellow colored, due to an enhancement of the carotenoid pigments (Bianchi, 2003 and Oloo, *et al.,* 2014)

Table 4: Total phenols, total carotenoids, chlorophyll A and B of Manzanillo table olive (fresh and after fermentation) treated with gibberellic acid (GA3) and naphthaleneacetic acid (NAA).

	Total phenols content (ppm)		Carotenoids (mg/ kg)	
treatments	Fresh	Fermented	Fresh	Fermented
T_1	759.65±3.26^{a}	532.65±4.12^{a}	11.51±0.20^{a}	9.2±0.18^{a}
T_2	497.45±3.10^{d}	310.94±4.97^{c}	10.94±0.24^{ab}	8.28±0.14^{b}
T_3	517.83±3.91^{c}	309.94±2.49^{c}	10.58±0.27^{b}	8.49±0.17^{b}
T_4	624.84±2.57^{b}	402.65±3.80^{b}	11.44±0.17^{a}	9.1±0.18^{a}
T_5	458.3±4.394^{e}	321.69±3.46^{c}	9.78±0.18^{c}	6.31±0.12^{d}
T_6	405.74±3.07^{f}	265.97±4.94^{d}	8.45±0.20^{d}	6.99±0.14^{c}
LSD value at p > 0.05	10.59	12.49	0.65	0.48
	Chlorophyll A (mg/ kg)		Chlorophyll B (mg/ kg)	
	Fresh	Fermented	Fresh	Fermented
T_1	38.45±0.82^{a}	33.18±0.78^{a}	18.24±0.38^{a}	14.97±0.29^{a}
T_2	32.54^{c}±0.84	27.97±0.69^{bc}	17.37±0.28^{ab}	13.99±0.25^{b}
T_3	35.84±0.76^{b}	30.25±0.70^{b}	16.54±0.32^{bc}	13.45±0.25^{b}
T_4	40.91±0.95^{a}	35.2±0.72^{a}	18.23±0.41^{a}	15.11±0.23^{a}
T_5	30.62±0.85^{c}	26.26±0.72^{c}	15.47±0.28^{c}	12.35±0.31^{c}
T_6	33.19±0.74^{c}	28.24±0.70^{bc}	16.25±0.36^{bc}	12.2±0.214^{c}
LSD value at p > 0.05	2.55	2.21	1.05	0.80

*T_1: Control without treatment, T_2: Gibberellic acid (GA3) at 50 ppm. T_3: Gibberellic acid (GA3) at 75 ppm. T_4: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 50 ppm. T_5: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 75 ppm. T_6: Naphthaleneacetic acid (NAA) at 75 ppm. LSD: Least Significant Difference at 0.05. *M±SE: Mean of triplicate determinations results ± standard error; the means, within the same column, having different superscripts are significantly.*

Effect of natural fermentation process on Polyphenol compounds content of Manzanillo table olive (fresh and after fermentation) treated with GA3 and NAA (mg/kg) .

Olive flesh contains 1–14% (d.w.) phenolic compounds, depending on the variety (Amiot *et al.,* 1986). The phenolic compounds are important in many aspects, such as protection of plants from bacteria, fungi and viruses (Hanbury, 1954) and fruit browning as maturation proceeds. Furthermore, phenolic compounds play significant roles in human nutrition (Bravo, 1999) and health (Christakis *et al.,* 1982 and Manna *et al.,* 1999). The bitter taste of raw olives is due to phenols and, especially, oleuropein, which is water soluble (Gutiérrez *et al.,* 1992). Other phenols include ligstroside, verbascoside, 4-hydroxytyrosol, tyrosol and glucosides or aglycones, 3,4-dihydroxyphenyl glycol and flavonoids (Manna *et al.,* 1999). The concentration of phenols is a function of fruit maturity and can be as high as 14% (d.w.) in young fruits, though it may be close to zero in black-type fruits.

Fifteen of polyphenol compounds qualitative and quantitative were identified by High performance liquid chromatography (HPLC) in fresh and fermented Manzanillo table olive including gallic, protocatechuic acid, catechin, tyrosol, hydroxytyrosol, caffeic acid ,vanillic acid, caffien, ferrulic acid, *p*-oH-benzoic acid, reseratol, salicylic, ellagic, coumarin and cinnamic acid and the results are given in Table (5).

As illustrated in Table (5), p-oH-benzoic acid was the major of phenolic compounds detected in fresh Manzanillo table olive samples T1(191.9 mg/kg), T2(204.86 mg/kg), T5(92.26 mg/kg) and T6(61.52 mg/kg) and fermented samples T1(69.03mg/kg), T2 (62.78mg/kg), T5 (88.29mg/kg) and T6(51.03mg/kg) as well as the major of phenolic compounds detected in fresh Manzanillo table olive samples T3 was ellagic (145.89mg/kg) and T4 was cinnamic acid(71.13 mg/kg ,while the major of phenolic compounds was

Protocatechuic acid (75.3 mg/kg) in fermented sample T3, and Vanillic acid (64.81 mg/kg) in fermented sample T4.

These results are in accordance with the trend of polyphenol compound contents in Manzanillo table olive obtained by many researchers. (Manna *et al.,* 1999; Bianchi, 2003; Romero *el al.,* 2004a and b; Poiana and Romero, 2006; Kailis and Harris, 2007and Ben Othman *et a!.,* 2009),

Concerning the effect of the natural fermentation process on polyphenol compounds content of Manzanillo table olive (fresh and after fermentation) treated with GA3 and NAA, it could be noticed from Table (5) that polyphenol compounds content of Manzanillo table olive decreased as affected by treating with GA3 and NAA when compared to the control samples by other treated Manzanillo table olive. Also, as can be seen from Table (5) natural fermentation process led to the decreasing effects in most polyphenol compounds and increased in little of other polyphenol compounds in control and treated Manzanillo table olive as affected by natural fermentation.

In this concerning in table olives, phenolic compounds have a significant influence on color, and they play a crucial role in the sensory and nutritional characteristics (Marsilio *et al.,* 2001; Ben Othman *et al.,* 2009; Rodriguez *el al.,* 2009; Romeo *et al.,* 2009; and Fadda *et al.,* 2014) ,reported that differences between the phenol composition of fresh and processed fruits are the result of reactions occurring during the fermentation period (Romero *el al.,* 2004_a); polyphenols undergo chemical transformations and, in general, their concentration in olives diminishes (Romero *el al.,* 2004_b). During the fermentation period an increase in hydroxytyrosol to the detrirnent of oleuropein was observed Esti *et al.* (1998) and Romero *el al.* (2004.), Hydroxytyrosol is the most important phenolic compound detected in the final products (Romero *el al.,* 2004a). Browning reactions that occur during processing are often caused by polymerization of phenolic compounds (Romeo *et al.,* 2009).

Table 5: Phenolic compounds (mg/kg) of Manzanillo table olive treated with gibberellic acid (GA3) and naphthaleneacetic acid (NAA) (fresh and after fermentation) on fresh weight basis.

Phenolic compounds (mg/kg)	T_1		T_2		T_3		T_4		T_5		T_6	
	Fresh	Ferm.	Fresh	Ferm.	Fresh	Ferm.	Fresh	Ferm.	Fresh	Ferm.	Fresh	Ferm.
Gallic	4.32	20.76±	4.69	57.28	11.13	69.28	4.13	6.97	10.5	ND	32.15	1.35
Protocatechuic acid	13.64	59.62	47.78	61.35	10.06	75.3	12.2	16.78	16.69	2.06	4.13	5.89
Catechin	ND	ND	ND	ND	33.52	ND	1.53	ND	ND	ND	ND	ND
Tyroso	28.8	23.1	33.72	22.21	95.26	6.25	2.54	15.33	19.12	7.67	3.04	27.54
Hydroxytyroso	139.32	7.77	21.54	16.19	ND	4.95	65.71	25.94	74.97	9.8	6.91	22.34
Caffeic acid	15.43	43.11	6.3	17.65	8.08	ND	2.36	1.69	8.75	ND	54.36	ND
Vanillic acid	23.15	33.72	14.85	6.07	16.52	1.07	ND	64.81	39.09	2.32	22.04	5.93
Caffien	26.97	1.72	30.52	12.63	18.29	2.93	19.86	1.29	11.07	1.55	11.01	2.38
Ferrulic acid	38.96	67.66	ND	14.28	13.82	4.72	ND	9.97	17.86	1.59	ND	11.13
p-oH-benzoic cid	191.9	69.03	204.86	62.78	ND	ND	49.73	14.39	92.65	88.29	61.52	51.03
Reseratol	ND	ND	ND	ND	ND	ND	1.99	ND	ND	ND	ND	ND
Salicylic	ND	22.02	61.35	18.76	ND	25.6	55.49	3.97	23.87	26.52	ND	ND
Ellagic	51.87	36.56	60.13	30.78	145.89	38.14	22.54	8.1	ND	39.74	13.88	45.23
Coumarin	27.6	2.84	9.06	6.26	ND	12.91	35.23	ND	12.35	5.66	41.1	7.16
Cinnamic acid	2.94	11.73	ND	15.71	6.97	0.78	71.13	56.59	2.84	29.51	30.28	0.87

T_1: Control without treatment, T_2: Gibberellic acid (GA3) at 50 ppm. T_3: Gibberellic acid (GA3) at 75 ppm. T_4: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 50 ppm. T_5: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 75 ppm. T_6: Naphthaleneacetic acid (NAA) at 75 ppm. ND: not detect Ferm.: fermented.

Change in pH, total acidity and sodium chloride content of brine during fermentation process of Manzanillo table olive treated with GA3 and NAA.

When olives are placed in brine, they were subjected to physical and chemical changes that modified the fruits and their brine solution. The water soluble components are withdrawn from olive flesh due to diffusion of these components into brine. This process is influenced by the salt concentration. The sugars diffused in brine are being used by microorganisms and converted to organic acids. Changes in chemical components are related to concomitant microbiological populations developed during spontaneous fermentation. (Poiana and Romeo, 2006).

Brine salt concentrations, pH and acidity levels are important parameters used to monitoring the olives during and after processing. Lactic fermentation produces more free acid and a lower brine pH than mixed fermentations producing lactic and acetic acid. The balance of salt, pH and acid are also important in the preservation and safety of the olives as recommended by Codex Alimentarius (1981) and IOOC. (1990).

Brine salt concentrations, pH and titratable acidity levels during natural spontaneous fermentation of Manzanillo table olive treated by GA3 and NAA for 12 months were carried out periodically for determination. The results were showed in table (6).

From data in table (6) it could be appear that the pH values at zero time were approximately convergent values from control sample of Manzanillo table olive. As can be seen in table (6) the pH in brine was progressively decreased during the storage period for 12 months in all tested Manzanillo table olive. On contrast titratable acidity was progressively slight increased during the storage period for 12 months in all tested Manzanillo table olive. The producing of acidity in brine solution during the fermentation process led to lowering in pH.

Statistically no significantly differences were noticed between control samples and other tested treatments in pH value at the zero time, as well as the same effect were shown during and at the end of spontaneous fermentation process. On the contrary, total acidity contents of brine sample T6 was different from other tested brine samples (significantly differences) which was increased from 0.10 after one month to 1.15 after 12 months.

Also, data in Table (6) revealed that the effect of spontaneous fermentation process as well as the fermentation time for 12 months on the salt contents of the brine. The Nacl content of brine was about 10 % at the start of fermentation process and after a few days, more food grade salt is added to increase the strength of the brine to 10% w/v salt . The sodium chloride content was showed slight decreased during fermentation time in all tested sample and found to be no significantly difference between all samples which was reached to 8.42 – 8.65 % after 12 month.

Fermentation of olives involves the action of lactic acid producing bacteria e.g. *Lactobacillus* species and/or yeasts on fermentable substrates, such as sugars, released from the olives during soaking. During the fermentation, the acids such as lactic and acetic acid are produced which increase the acidity level of the brine and lower in pH. The combination of high salt and low pH greatly reduces the risk of microbial spoilage of the olives. Here controls are essential to reduce the risk of overgrowth of undesirable or harmful microbes that can lead to product deterioration or food poisoning. Process control involves maintaining the salt and acid levels by targeted additions of sodium chloride and food acids (Kailis and Harris, 2004) and

Table 6: Changes in pH, total acidity and sodium chloride of brine during fermentation process of Manzanillo table olive treated with growth regulators as affected by storage time (*Mean ± SE).

Treatments	Storage time (month)								
pH	0	1	2	3	4	6	8	10	12
T1	7.61±0.064^{a}	6.98±0.069^{a}	6.62±0.081^{a}	5.92±0.104^{b}	5.62±0.064^{b}	5.11±0.058^{b}	4.84±0.064^{b}	4.61±0.064^{a}	4.38±0.058^{a}
T2	7.62±0.081^{a}	7.02±0.052^{a}	6.79±0.058^{a}	6.41±0.069^{a}	6.11±0.075^{a}	5.58±0.092^{a}	5.21±0.075^{a}	4.64±0.075^{a}	4.34±0.092^{a}
T3	7.64±0.058^{a}	6.97±0.081^{a}	6.72±0.081^{a}	6.48±0.121^{a}	6.24±0.081^{a}	5.74±0.075^{a}	5.32±0.081^{a}	4.73±0.081^{a}	4.42±0.075^{a}
T4	7.55±0.081^{a}	6.91±0.075^{a}	6.69±0.058^{a}	6.32±0.092^{a}	6.03±0.058^{a}	5.51±0.058^{a}	5.11±0.^{081ab}	4.72±0.058^{a}	4.29±0.058^{a}
T5	7.69±0.058^{a}	7.01±0.069^{a}	6.71±0.092^{a}	6.37±0.081^{a}	6.22±0.092^{a}	5.81±0.092^{a}	5.42±0.058^{a}	4.82±0.092^{a}	4.4±0.092^{a}
T6	7.65±0.092^{a}	7.11±0.052^{a}	6.58±0.127^{a}	6.44±0.127^{a}	6.11±0.075^{a}	5.85±0.075 ^{a}	5.1±0.092^{ab}	4.76±0.075^{a}	4.3±0.075^{a}
LSD at 0.05	0.22	0.207	0.26	0.311	0.230	0.235	0.233	0.230	0.235
acidity									
T1	-	0.09±0.012^{b}	0.17±0.006^{ab}	0.23±0.012^{c}	0.41±0.023^{abc}	0.54±0.023^{ab}	0.61±0.023^{c}	0.79±0.029^{b}	0.91±0.035^{c}
T2	-	0.08±0.012^{b}	0.18±0.012^{ab}	0.34±0.006^{a}	0.44±0.017^{ab}	0.62±0.012^{a}	0.78±0.012^{ab}	0.85±0.017^{b}	0.94±0.029^{c}
T3	-	0.10±0.017^{b}	0.17±0.006^{ab}	0.26±0.017^{bc}	0.34±0.029^{c}	0.51±0.029^{b}	0.68±0.035^{bc}	0.82±0.012^{b}	0.97±0.017^{bc}
T4	-	0.11±0.006^{b}	0.21±0.017^{a}	0.33±0.023^{a}	0.48±0.023^{a}	0.61±0.017^{a}	0.75±0.029^{ab}	0.89±0.035^{b}	1.05±0.012^{ab}
T5	-	0.90±0.017^{a}	0.14±0.006^{b}	0.29±0.012^{ab}	0.37±0.017^{bc}	0.52±0.012^{b}	0.71±0.017^{ab}	0.84±0.029^{b}	1.09±0.035 ^{a}
T6	-	0.10±0.006^{b}	0.19±0.012^{a}	0.32±0.006^{a}	0.48±0.029^{a}	0.62±0.023^{a}	0.79±0.023^{a}	0.97±0.017^{a}	1.15±0.029 ^{a}
LSD at 0.05		0.038	0.032	0.042	0.072	0.062	0.074	0.075	0.084
Nacl									
T1	9.98±0.231^{a}	9.11±0.179^{a}	9.25±0.139^{a}	9.51±0.162^{a}	9.21±0.156^{a}	9.41±0.260^{a}	8.9±0.139^{a}	8.52±0.214^{a}	8.32±0.237^{a}
T2	9.98±0.294^{a}	9.42±0.185^{a}	9.11±0.167^{a}	9.26±0.185^{a}	9.61±0.202^{a}	9.12±0.352^{a}	8.82±0.237^{a}	8.75±0.167^{a}	8.65±0.237^{a}
T3	9.98±0.260^{a}	9.15±0.139^{a}	9.54±0.185^{a}	9.25±0.237^{a}	9.21±0.214^{a}	9.34±0.179^{a}	8.92±0.127^{a}	8.2±0.156^{a}	8.52±0.127^{a}
T4	9.98±0.352^{a}	8.99±0.162^{a}	9.24±0.139^{a}	9.32±0.127^{a}	9.11±0.144^{a}	8.85±0.167^{a}	8.74±0.167^{a}	8.55±0.202^{a}	8.42±0.162^{a}
T5	9.98±0.225^{a}	9.02±0.196^{a}	9.41±0.237^{a}	9.21±0.214^{a}	9.05±0.196^{a}	8.98±0.237^{a}	8.85±0.156^{a}	8.64±0.214^{a}	8.48±0.214^{a}
T6	9.98±0.358^{a}	9.21±0.191^{a}	9.52±0.127^{a}	9.32±0.237^{a}	9.14±0.237^{a}	8.89±0.139^{a}	8.71±0.294^{a}	8.54±0.144^{a}	8.45±0.167^{a}
LSD at 0.05	0.898	0.543	0.522	0.608	0.598	0.719	0.603	0.569	0.600

*T_1: Control without treatment, T_2: Gibberellic acid (GA3) at 50 ppm. T_3: Gibberellic acid (GA3) at 75 ppm. T_4: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 50 ppm. T_5: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 75 ppm. T_6: Naphthaleneacetic acid (NAA) at 75 ppm. LSD: Least Significant Difference at 0.05. *M±SE: Mean of triplicate determinations results ± standard error; the means, within the same column, having different superscripts are significantly.*

(Kailis and Harris, 2007). It is generally accepted that yeasts are able to produce compounds with important organoleptic attributes (Querol and Fleet, 2006). Homofermentative LAB (*Lactobacillus* species) Metabolize the sugars available during fermentation, producing lactic acid which originates the rapid and safe acidification of brines (Fernandez-Diez *et al.,* 1985; Garrido- Fernández *et al.,* 1997).

Sensorial Evaluation

The field of sensory evaluation grew rapidly in the second half of the twentieth century, along with the expansion of the processed food and consumer products industries. Sensory evaluation comprises a set of techniques for accurate measurement of human responses to foods and minimizes the potentially biasing effects of brand identity and other information influences on consumer perception. As such, it attempts to isolate the sensory properties of foods themselves and provides important and useful information to product developers, food scientists, and managers about the sensory characteristics of their products. Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing. Stone and Sidel, 2004 ; Moskowitz *et al.,* 2006 and Meilgaard *et al.,* 2006).

Therefore the sensory evaluation is important in the evaluation of some new types of food and the impact of some the new treatments on the quality of these foods, such as treatment of some types of olives fruit by GA3 and NAA during growth on the quality and behavior of these fruits during processing and quality of these fruits and the impact of fermentation process on the sensory properties as well as consumer acceptance of these foods.

Average values of the sensory parameters evaluated (appearance, color, taste, odor, flavor, aroma, texture and global appreciation) of Manzanillo table olive treated by GA3 and NAA are reported in Table 7.

From data in table 7 it could be revealed that global appreciation of tested samples T1, T4 and T5 fermented table olives were preferred by the sensory panel, with an average score of 9.22, 9.1 and 9.29, respectively, in a scale from 1 to 10. The presents table olives samples highly appreciated by the consumers, due to being good appearance, color, taste, odor, flavor, aroma and texture, what probably influenced the sensory panel's.

Concerning tested Manzanillo olives aroma, sensory panels showed no significant differences were found mainly between the control samples and other Manzanillo treatments in all sensory parameters (appearance, color, odor, flavor and aroma) except taste in samples T2 and T3 ,texture in samples T6 and global appreciation in samples T6 showed significant differences.

Based on the results obtained, sample T5 is highly appreciated by the local consumers. This fact indicates that this treatment could be used for table olive production which was recorded high score in most sensory parameters furthermore global appreciation. On the other hand, tested samples T6 was negatively evaluated in most the sensorial parameters and significant statistical differences were found between this table olive treatment and the others.

Table 7: Sensory evaluation of fermented Manzanillo table olive treated with growth regulators. (mean ± SE)

Treatments	Attributes							
	Appearance	Color	Taste	Odor	Flavor	Aroma	Texture	Global appreciation
T1	9.34±0.29^{a}	9.12±0.58^{a}	9.55±0.29^{a}	9.24±0.29^{a}	9.34±0.29^{a}	9.44±0.58^{a}	9.02±0.58a	9.22±0.29^{a}
T2	8.77±0.58^{a}	8.41±0.29^{a}	8.00±0.29^{b}	8.63±0.58^{a}	8.66 ±0.29^{a}	8.89±0.58^{a}	7.78 ±0.58^{a}	8.92 ±0.58^{a}
T3	8.23±0.29^{a}	8.35±0.29^{a}	8.00±0.58^{b}	8.74±0.29^{a}	8.84±0.58^{a}	8.78±0.58^{a}	7.99 ±0.58^{a}	8.84±0.29^{a}
T4	9.65± 0.58^{a}	9.21±0.58^{a}	9.54±0.29^{a}	9.35±0.58^{a}	9.36 ±0.58^{a}	9.29±0.58^{a}	8.664±0.29^{a}	9.1 ±0.58^{a}
T5	9.52±0.58^{a}	9.11±0.29^{a}	9.41±0.58^{a}	9.43±0.58^{a}	9.25 ±0.58^{a}	9.38±0.29^{a}	9.11±0.58^{a}	9.29±0.58^{a}
T6	7.78±0.29^{a}	8.46±0.58^{a}	7.11±0.29^{b}	8.81±0.58^{a}	8.76±0.29^{a}	8.84±0.29^{a}	6.36 ±0.58^{b}	7.42 ±0.29^{b}
LSD at 0.05	1.4	1.40	1.25	1.54	1.40	1.54	1.66	1.40

*T_1: Control without treatment, T_2: Gibberellic acid (GA3) at 50 ppm. T_3: Gibberellic acid (GA3) at 75 ppm. T_4: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 50 ppm. T_5: Gibberellic acid (GA3) at 75 ppm + Naphthaleneacetic acid (NAA) at 75 ppm. T_6: Naphthaleneacetic acid (NAA) at 75 ppm. LSD: Least Significant Difference at 0.05. *M±SE: Mean of triplicate determinations results ± standard error; the means, within the same column, having different superscripts are significantly.*

Generally**,** the treating of Manzanillo olive fruit with GA3 either individually or in combination with NAA as described previously was resulted to some chemical, physical and sensory differences in olives fruit. in the first place positively influenced fruit physical quality, i. e., fruit weight, flesh weight, stone weight, flesh /stone ratio and the percent of flesh.

Manzanillo olive fruits treated by GA3 and /or NAA decreasing in moisture contents, protein content, total sugars, crude fiber and total carbohydrates on contrast dry matter, oil content and ash content were found to be increased in all tested samples.

On the other hand Manzanillo olive fruits negatively affect with respect to decreasing in total phenols, total carotenoids and chlorophyll A and B in fresh treated samples when compared with fresh control samples, as well as treated samples was showed more decreasing than control samples in total phenols, total carotenoids and chlorophyll A and B as affected by fermentation process.

As regards sensory evaluation**,** sample T5 is highly appreciated by the local consumers. This fact indicates that this treatment could be used for table olive production which was recorded high score in most sensory parameters furthermore global appreciation**.** On the other hand, tested samples T6 was negatively evaluated in most the sensorial parameters and significant statistical differences were found between this table olive treatment and the others.

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