Influence of exposure to light on some chemical and physical characteristics of virgin olive oil produced in Northern Libya

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Abstract:

Libyan Virgin olive oil is most often used as salad oil, cooking and for other foods such as home made products. Virgin olive oil contains phenols, and alpha-Tocopherol which act as antioxidants, reducing the oxidative stress throughout the bodies, and other health benefits. In order to investigate the influence of fluorescent light on the chemical and physical characteristics, three virgin olive oils from olive '*Roghiani*' cultivar were examined in three regions of Northern Libya in the crop year 2018. An experimental investigation was carried out to study the influence that exposure to light has on the Virgin olive oil during 35 days, storage period by comparing it with same sample (control) stored in the dark. The results showed that the oils stored in the light had significantly ($P \leq 0.05$) lower carotenoids and chlorophyll contents than that the same oils stored in the dark mainly contained primary oxidation products.

While the oils kept in the light contained secondary oxidation products as confirmed by Peroxide value, which exceeded the available limits, even after purification by means of an alumina as adsorption surface. Overall, the results obtained showed that the shelf life of the oils exposed to light is shorter than ($P \leq 0.05$) that of oils kept in the dark, and that only 6 days after oil samples were exposed to light, the oil they contained was no longer classified as virgin olive oil.

Keywords: pigment contents, virgin olive oils, oxidative degradation, shelf life.

Introduction

Olive is the common name for about 35 species of evergreen shrubs and trees of the genus *Olea* in the olive family, the *Oleaceae*, native to tropical and warm temperate regions. The name is especially used for *Olea Europaea*, the well-known olive which is grown for its edible fruits. Olive trees are native to Greece, Italy, Palestine, and Syria, but different species are native to different areas. It is believed that cultivation of olives started around the fourth millennium B.C. in the area which is today Syria and Palestine. The inhabitants of Crete during the Minoan civilization cultivated olives as early as 2500 B.C. Pottery items such as jars found in Knossos Palace were probably intended for storing olive oil. The botanical origin of the tree and the beginning of its cultivation has been a subject of dispute (Kapellakis, *et al*., 2008; Blazquez, 1996).

Moreover, it is widely known, that the quality of Virgin olive oil (VOO) is influenced by various agronomic factors such as olive cultivar, climatic conditions, degree of maturation and agronomic practices related to irrigation treatment. In fact, olive (*Olea europaea* L.) is one of the most important fruit crops throughout the Mediterranean Basin. The species include thousands of cultivars, most derived from empirical selections over many centuries, now well-adapted to different local conditions (Besnard *et al*., 2001; Dabbou *et al*., 2010).

Libya is one of the major olive oil producers in North Africa, where olive oil is the basic culture. Between 2008/2009 and 2013/2014,

The average oil production in the country amounted to 15,000 tons per year. Use olive oil in Libya is part of the culture and a large number of individual manufactures producing oil using the process of cold pressing (Elbeydi & Hamuda, 2016).

The colour of VOOs when just extracted, ranges between light yellow and a more or less deep green depending on the content of liposoluble pigments (chlorophylls and carotenoids) naturally occurring in the fruits. Chlorophylls give oils their yellow/green colour, whereas carotenoids determine shades between yellow and red. The level of these pigments is related to genetic factors, to the ripening degree of olives, and to the conditions adopted for oil extraction. Such pigments decrease in concentration as the fruit ripens and disappear at the moment of complete maturity (Aparicio, 2013).

Luminous radiation is another external factor to be considered in the lipid oxidation process during storage, since it initiates auto-oxidation and produces photo-oxidation. To observe these effects, the oil must contain photo-sensitizers, like chlorophylls, that are excited by light absorption. Prevention of light exposure during storage of VOO is absolutely necessary to extend its shelf life (Jadhav *et al.*, 1996); oils exposed to light are less stable than those kept in the dark (Caponio *et al.*, 2005). However, VOO is usually protected from exposure to light radiation from the time of its production until it is exposed as bottled oil on the supermarket shelves. From that time onwards the opacity to light of the packaging material is of fundamental importance for its preservation (Méndez & Falqué, 2007; Caponio *et al.*, 2005).

Olive oil is used primarily as salad oil, however, the culinary use of oil is often subjected to moderate temperatures, accompanied by physical and chemical changes in the quality that is reflected in the colour of the oil. Therefore, the aim of this study were to investigate the impact of florescent light on some physical and chemical characteristics such as colour stability by determine the natural pigments content and antioxidant capacity by using DPPH assay technique.

Material and methods

Chemicals

All standards, and reagents used in this study for determination of phytochemicals in VOO, and their bioactivities were purchased from (Sigma-Aldrich Co., St. Louis, USA; J.T. Baker, Deventer, Teugseweg 20 7400 AA Deventer Netherlands). Other chemicals and solvents were of the highest commercial grade were purchased from (Merck, Darmstadt, Germany).

VOO samples

The study was carried out on '*Roghiani*', the cultivar of olive fruits (*Olea europea* L.) from Libyan. Olive samples were handpicked at the beginning of January 2018, from three different geographic regions. The cultivar is growing in the North of Libya (Gharyan: 32° 10′ N, 13° 01′ E, Tarhuna: 32° 26′ N, 13° 10′ E, Msallata: 32° 35′ N, 14° 2′ E). The mean rain precipitation registered in the North was about 383 mm/year, with a mean temperature of approximately 27 °C.

Oil samples extraction

Olive fruit samples were collected and harvested from regions described above. Within 3 days after harvesting fruits were washed, milled and olive pastes were malaxed with a mixer for 40 min at 35- 40 °C. After separation at 3000 rpm by centrifugal separation process (Rapanelli, Foligno, Italy), extracted olive oil samples were decanted and filtered through filters. VOO samples were stored in the refrigerator at 8 °C in dark glass bottles until further analysis. Samples then were tempered at room temperature for 24 h before analysis.

Fluorescent light test

The photo stability of virgin olive oils was tested using the fluorescent light test, under conditions of constant temperature and brightness of 4x40 W. To test changes that can occur under the influence of light. The oil is filtered, stuffed in transparent and dark-brown glass containers, into bottles of 40 ml volume, with a plastic closure, without the presence of air.

Thereafter, it was constantly illuminated by a fluorescent light, with two neon tubes on both sides of the box, as shown in Figure 38, while simultaneously monitoring the temperature, ranging from 25 to 29 ˚C. The distance of the samples from the light source was about 10 cm. The sampling dynamics exposed to fluorescent light influences were as follows: initially (0day) and 6, 10, 18 and 35 days, after bottling and storage in the conditions already described, (Pavlović, 1981).

Peroxide value (PV)

The peroxide value (PV) of the oil is determined by the standard iodometric method (SRPS EN ISO 3960: 2011), according to the flowed formula:

$$\mathbf{PV\ (mmol/kg) = \frac{V \times C \times 5}{m}}$$

Where:

V– sodium thiosulfate solution volume($Na_2S_2O_3$) (ml) used for titration of sample.

C- exact concentration (mol /L) of used sodium thiosulfate solution ($Na_2S_2O_3$).

m - mass (g) of sample for examination.

Chlorophylls

Chlorophyll pigments content was determined by spectrophotometric method described by Pokorny *et al*., (1995). The content of total chlorophyll pigments was calculated using the formula:

$$\mathbf{Ch = 345.3 \times [A_{630} - (A_{670} + A_{710})/2] \times 10}$$

Where:

Ch – content of chlorophyll pigments in mg of pheophytin-a in 1 kg of oil,

A_{670} - absorbance value of the undiluted sample at a wavelength of 667 nm,

A_{630} – absorbance value of the undiluted sample at a wavelength of 630 nm,

A_{710} – absorbance value of the undiluted sample at a wavelength of 710 nm

Carotenoids

The total carotenoid content was determined according to the procedure described by Minguez Mosquera *et al*., (1990). The content of carotenoids is calculated according to the formula:

$$\textbf{Total carotenoids (mg / kg)} = \frac{A_{470nm} \times 10^3 \times 25}{2000 \times 7.5}$$

Where:

A_{470nm} - measured absorbance at 470 nm.

Transparency test

In order to obtain certain parameters for colour definition, oil transparencies in relation to CCl4 at a wavelength of 455 nm were measured in a 1 cm glass cuvette in relation to carbon tetrachloride (Dimić & Turkulov, 2000).

All pigments content and transparency measurements were conducted using UV/VIS spectrophotometer (Model T80+, PG Instruments Limited, London).

Antioxidant capacity by DPPH assay

The antioxidant capacity of VOO extracts in the current study was assessed by the evaluation of the free radical scavenging effect on 2. 2- diphenyl-1-picrylhydrazyl (DPPH) radical, according to the method proposed by Martínez & Maestri (2008). The absorbance was read at 515 nm using toluene as the blank.

The ability to scavenge DPPH radicals, i.e. AC_{DPPH} was calculated following the formula:

$$\textbf{AC}_{\textbf{DPPH}}\textbf{ (\%)} = [A_C - A_S / A_C] \times 100$$

Where:

A_C is the absorbance of the control.

A_S is the absorbance of the sample.

EC_{50}^{DPPH} values were calculated as inhibitory concentration of the oil necessary to decrease the initial DPPH• absorbance by 50 %. A lower EC_{50} value indicates a higher antiradical capacity. EC_{50}^{DPPH} (mg/ml) values were expressed into antioxidant capacity (AC) as $1/EC_{50}^{DPPH}$ (ml/mg).

Statistical analysis

All results are presented as a mean value ± standard deviation (n = 3). One way an analysis of variance (ANOVA) with a Tukey's test was used to determine significant differences among data. The statistically significant level was fixed at ($P \leq 0.05$). Effective Concentration by 50% (EC_{50}) values were calculated by using the best-fit regression model. All the data were analyzed using Microsoft Office Excel 2007 Software.

Results and discussion.

Peroxide value

Peroxide value (PV) indicates the level of primary oxidation of unsaturated fatty acids, that is, shows the amount of hydroperoxide as the primary products of autoxidation, it expresses the degree of rancidity. The peroxide value is closely related to the method of oil storage. Fat oxidation is one of the basic reactions affecting the health safety of triacylglycerol because the products of the oxidation reaction are detrimental to the health of consumers (Oštrić-Matijašević &Turkulov, 1980).

Peroxide values are shown in Table (1). The PV of the initial Tarhuna VOO was significantly higher ($P \leq 0.05$) 6.74 ± 0.05 compared with Msallata and Gharyan VOOs which were 6.30 ± 0.32 and 5.10 ± 0.01 mmol/kg, respectively. The largest difference was observed in the Msallata VOO, where the PV in transparent packaging was 12.37 ± 0.42 mmol/kg, after 18 days of light exposure, while the PV in the dark packaging was 7.63 ± 0.49 mmol / kg at the same time. At the end of time storage (35 days), PV of Msallata VOO was reached up to 15.20 ± 0.00, which was the highest value ($P \leq 0.05$) compared to both Tarhuna and Gharyan VOOs.

The result in table (1) also shows that the smallest PV was in the Gharyan VOO in both transparent and dark packaging. The change in the peroxide value during exposing the oils to light and different storage conditions may be due to the presence of a variation in the initial amount of antioxidants in VOOs, which has a significant effect on the change in the PV.

Through the results recorded in Table (1), it can be conclude that the exposure of oil samples to fluorescent light led to the occurrence of oxidation, and during continued to storage. The rate of oxidation has increased, and this can be observed from the upward increase in the PV, and this increase varies from one cultivar to another depend on the initial content of antioxidants. Oxidation rate also related to different regions and the climate changes.

The PV of oils in this study are approximate similar to the values of virgin olive oils in both varieties of *Leccino* and *Frantoio*, according to the results published by Najafi*et al*., (2015) the results of the test show the fluctuation of the peroxide value over the duration of the test under the influence of the fluorescent light. This may be the result of a partial decomposition of hydroperoxide into secondary oxidation products. The results also show differences in PV values between samples in transparent and those in dark packaging.

Table (1). Effect of exposure to fluorescent light on peroxide value (mmol/kg) from different regions of VOOs stored for 35 days.

Samples location	Time of F.L test (days)				
	0	6	10	18	35
Light bottles	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
Gharyan	5.10 ± 0.01^{a}	6.80 ± 0.42^{b}	7.50 ± 0.71^{b}	8.80 ± 0.21^{c}	10.70 ± 0.00^{d}
Tarhuna	6.74 ± 0.05^{a}	7.70 ± 0.14^{b}	8.40 ± 0.28^{b}	10.10 ± 0.92^{c}	12.50 ± 0.00^{d}
Msallata	6.30 ± 0.32^{a}	8.80 ± 0.07^{b}	12.0 ± 0.57^{c}	12.37 ± 0.42^{c}	15.20 ± 0.00^{d}

Dark bottles					
Gharyan	5.10 ± 0.01[a]	5.60 ± 0.00[a]	5.90 ± 0.28[a]	7.30 ± 0.92[b]	8.76 ± 0.00[c]
Tarhuna	6.74 ± 0.05[a]	6.97 ± 0.14[a]	7.10 ± 0.14[a]	8.10 ± 0.85[b]	9.00 ± 0.00[c]
Msallata	6.30 ± 0.32[a]	6.61 ± 0.28[ab]	7..31 ± 0.16[b]	7.63 ± 0.49[bc]	8.93 ± 0.00[c]

Comparison between each row of cultivar during period of storage M: Mean of three replication, SD: Standard deviation. Different letters has significance difference at ($P \leq 0.05$)

The data obtained by fluorescent light test were in accordance with Manzocco *et al*. (2010) who examined the sustainability of photosensitive oils by exposing samples to the effect of light of varying intensity, compared to a light-controlled control pattern. The results showed that oils kept in the dark have a slow increase in PV compared with the samples that were exposed to the light; PV was larger than the intensity of the light was stronger. Bilancia, *et al*., (2007) also studied quality parameters in extra virgin olive oil during storage, those authors found that the PV was 7.50 mg/kg, while after storage in the dark glass was 9.00 mg/kg, and in the clear glass was 15.30 mg/kg. These results are in similar with the results in this study. These results are in agreement with those of other researchers (Caponio, *et al.*, 2005) decreased during storage specially in the light and in the dark, and their significant differences were observed between the two storage conditions.(Kanavouras, *et al.*, 2006; Pristouri, *et al.*, 2010) these researchers ran a shelf-life study carried out on olive oils produced from the '*Picual*', '*Hojiblanca*', and '*Arbequina'* cultivars packaged in dark and transparent glass bottles. The oils showed a decrease in some quality parameters during storage (the variation of peroxide value PV was significant in EVOOs stored in transparent glass).

Total chlorophyll content

Chlorophyll pigment play an important role in stability, related to their antioxidant nature in the dark and pro-oxidant activity in the light,

Which depends on their concentration in the fruit. Dependence on the total chlorophyll (TChl) content of oil samples and the time spent under fluorescent light conditions. Figure (1) showed that the highest value of (TChl) content was registered in Gharyan VOO ($P \leq 0.05$), following by Tarhuna and Msallata respectively. The results of (TChl) content in VOOs samples (Fig. 1) showed significant differences between all those VOOs samples. The lowest ($P \leq 0.05$) TChl content is present in Msallata VOO. Gharyan VOO possesses about 2-fold higher values for TChl comparable with Tarhuna VOO, and 4-fold higher values TChl comparable with Msallata VOO which had the lowest content, where as Tarhuna VOO possesses about twice TChl content comparable with Msallata VOO, and this fact suggests that the oil in Tarhuna and Msallata were produced from mature olives, while Gharyan VOO are produced form Immature fruits according to the amount of initial total chlorophyll content.

In Gharyan samples, the initial content of the TChl pigment was 37.77 ± 0.17 mg/kg, while the values of the TChl content from 6 days of storage was destroyed completely and became in very small amounts till the end of storage (35 days) as showed in (Fig,1 a). In Tarhuna samples the initial content of the TChl pigment was 18.27 ± 0.23 also started in 6 days of storage it was destroyed and became in very small amount during all the periods of storage (Fig,1 b), while Msallata VOO had initial content of the TChl pigment which it was 8.3 ± 0.00 mg/kg, as with previous oil samples, the TChl pigment was in very small amount from six days during exposure to light (Fig,1 c). As results show, after exposure to a fluorescent light effect of 35 days, chlorophyll remains in this oil, but in very small amounts. In the remaining three samples originating in Libya using the above-mentioned chlorophyll method, after 6 days of illumination, they were not detected in oil.

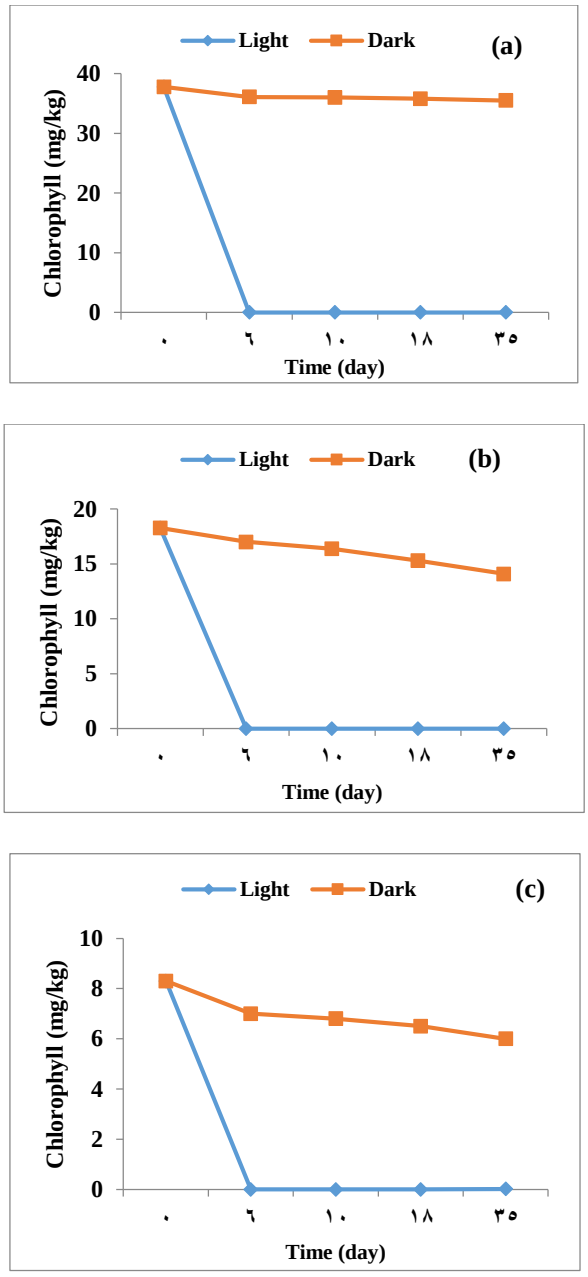


Figure (1). Effect of exposure to fluorescent light on total chlorophyll content (mg/kg) of different samples of VOOs stored for 35 days from different location. (a); Gharyan, (b); Tarhuna, (c); Msallata.

According to the results shown in (Fig. 1) in both a , b and c , when samples are kept in the dark, and in various oil samples from different regions, there is a slight decrease not significant ($P \leq 0.05$) in TChl pigment across all stages of storage until the

end of the period (35 days). Based on the results, it can be concluded that there has been a complete degradation of chlorophyll pigment content under the influence of light. Chlorophylls are important bioactive components of olive oil that significantly contribute to the quality, and are also the most susceptible to degradation under the influence of fluorescent light, as extremely photosensitive compounds.

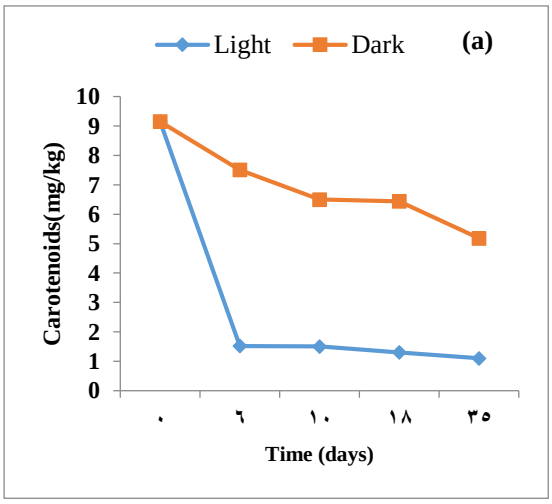
Food is often packaged in transparent packaging and high on shelves in markets to attract consumers attention. This is a practice even with photosensitive products, such as olive oil. In general, sustainability tests are carried out to obtain information on product sustainability during storage under controlled conditions. However, if the oils in markets are exposed to inappropriate conditions, their sustainability is wrong (Manzocco, *et al*., 2011). Bilancia, *et al*., (2007) also studied quality parameters in extra virgin olive oil during storage, those authors found that the total chlorophyll content was 25.70 mg/kg, while after storage in the dark glass was 24.41 mg/kg, and in the clear glass was 0.16 mg/kg. These results are in an agreement with the results in this study. Vacca *et al*. (2006) studied changes in the quality parameters, antioxidant compounds, oxidative stability, and antioxidant activity of extra Virgin olive oil from the '*Bosana*' cultivar, exposed to light and dark during storage for a period of 18 months. Analysis of data showed that all the parameters underwent significant changes during storage chlorophylls and carotenoids underwent a decrease until eight months of storage (49 %). Regarding exposure conditions, storage in the dark was more effective in retaining the quality of the oil.

Total carotenoids content

Carotenoids included carotenes and xanthophylls are yellow, orange and red lipid-soluble pigments that occur widely in plants, fruits and vegetables. Several of them are antioxidant nutrients that act mainly as secondary antioxidants in foods by quenching singlet oxygen. They may also prevent oxidation by trapping free radicals in the absence of singlet oxygen. Carotenoids play an important role in stability, related to their antioxidant nature in the dark and pro-oxidant activity in the light,

Which depends on their concentration in the fruit. Figure 2 shows the dependence of total carotenoids content on oil samples and the time spent under fluorescent light conditions.

The highest content of total carotenoids in the starting samples was found in a sample of Gharyan VOO which was 9.15 ± 0.01 mg/kg it was 3-fold higher compared with the content of carotenoids in Tarhuna and about 5-fold higher compared with Msallata VOOs, which was very clear in the values of the total carotenoids content in the Tarhuna and Msallata samples which were 3.35 ± 0.23 and 1.78 ± 0.00 mg/kg, respectively. As results showed, after exposure to a fluorescent light effect up to 35 days, carotenoids remains in those oils, but in very small amounts the degradation was very sharp after 6 days and then was very slowly till the end of exposure to fluorescent light (35 days) in all parts of Fig. 1(a, b and c) . In the dark conditions of storing oils. There was no significant difference ($P \leq 0.05$) in the carotenoids content throughout the storage period it was observed that the degradation of crotenoids content was higher in light condition, while in dark condition was very slightly.



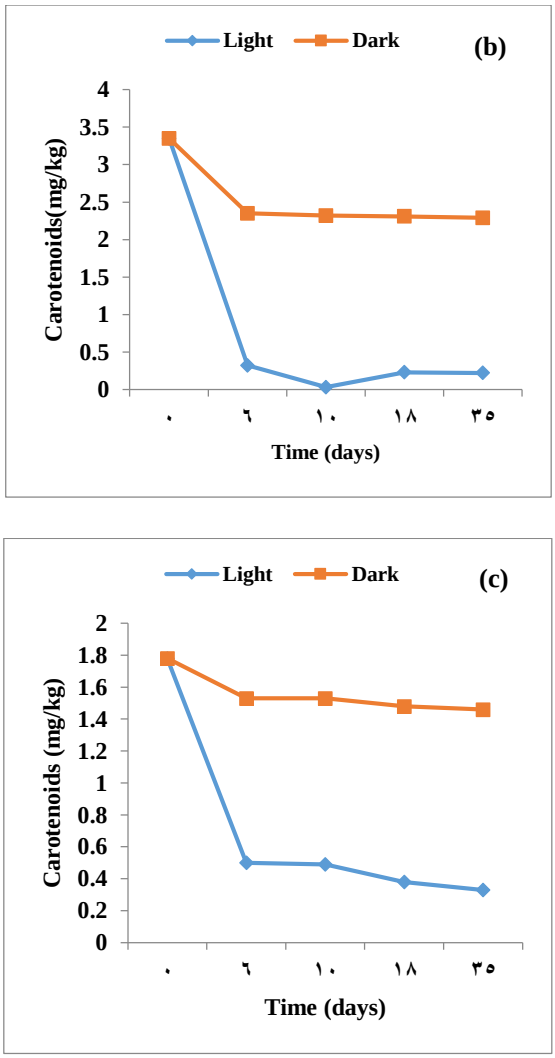


Figure (2). Effect of exposure to fluorescent light on total carotenoids content (mg/kg) of different types of VOOs stored for 35 days. (a); Gharyan, (b); Tarhuna, (c); Msallata.

Based on the results, it can be concluded that there has been a degradation for carotenoids under the influence of light. Carotenoids are important bioactive components of olive oil that significantly contribute to the quality, and are also the most susceptible to degradation under the influence of fluorescent light, as extremely photosensitive compounds. Bilancia, *et al.*, (2007) also studied the quality parameters in extra virgin olive oil during storage, those authors found that the total carotenoids content was 9.80 mg/kg,

While after storage in the dark glass was 8.57 mg/kg, and in the clear glass was 7.22 mg/kg. These results are similar with the results in this study. Dabbou, *et al*., (2011) also focused on the impact of packaging material and storage time on olive oil quality, those authors found that of carotenoids decreased from 18 mg/kg to 13 and then to 4 mg/kg during the first three months of storage. These results are in agreement with the results in this study. Vacca*et al*. (2006) studied changes in quality parameters, antioxidant compounds, oxidative stability, and antioxidant activity of extra VOO from the '*Bosana*' cultivar, exposed to light and dark during storage for a period of 18 months. Analysis of data showed that all the parameters underwent significant changes during storage; carotenoids underwent a decrease until eight months of storage (30%). Regarding exposure conditions, storage in the dark was more effective in retaining the quality of the oil.

Transparency test

In most edible vegetable oils, colour carriers are carotenoids. They show the maximum absorption at a wavelength of 440 to 460 nm, so transparency is determined in the range of these wavelengths. The values in (Table 2) shown that the Gharyan VOO had the lowest transparency among the initial samples. The reason for that is the highest content of pigments in Gharyan VOO compared to other oil tested, while Tarhuna VOO have a higher ($P \leq 0.05$) transparency value followed by Msallata VOO. The change in transparency in the samples could also be seen visually even after six days of exposure to the fluorescent test. As shown in Table 2 all the samples in transparent packaging increase during storage, it was seen that the transparency of oil samples increases with the increase in illumination time, with a notable difference in the increase between oil samples in transparent packaging and those in dark packaging. According to the starting samples, the transparency of the Gharyan VOO in transparent packaging increased by 76.17 % during exposure to fluorescent light, but it was 68.64% when it during dark packaging after 35 days. The increase in the transparency value is conditioned by the loss of pigments since the transparency is in reverse proportion to the content of pigments in the oil,

Whereas Tarhuna VOO in transparent packaging increased by 38.00 % during exposure to fluorescent light, but it was 19.00% when it was during dark packaging after 35 days. The transparency percentage % of Msallata VOO during exposure to fluorescent light was 43.81% while in dark was 28.95 % after 35 days.

Table 2.Change of transparency (T %) for VOO during F.L test.

sample	Time of F.L test (days)				
	0	6 (20)	10 (30)	18 (55)	35 (106)
Light bottles	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
Gharyan	4.17 ± 0.05^{a}	6.30 ± 0.21^{a}	6.80 ± 0.21^{a}	8.60 ± 0.14^{a}	17.50 ± 0.00^{a}
Tarhuna	46.10 ± 0.11^{b}	52.50 ± 1.34^{b}	54.50 ± 18.65^{b}	58.10 ± 0.71^{b}	74.40 ± 0.05^{b}
Msallata	31.97 ± 0.56^{c}	39.40 ± 1.20^{c}	42.70 ± 15.77^{c}	45.80 ± 0.57^{c}	56.90 ± 0.00^{c}
Dark bottles					
Gharyan	4.17 ± 0.05^{a}	6.10 ± 0.21^{a}	6.40 ± 0.07^{a}	7.10 ± 0.00^{a}	13.30 ± 6.36^{a}
Tarhuna	46.10 ± 0.11^{b}	49.10 ± 0.64^{b}	51.15 ± 17.75^{b}	54.70 ± 1.65^{b}	57.10 ± 0.07^{b}
Msallata	31.97 ± 0.56^{c}	36.30 ± 1.06^{c}	37.70 ± 0.49^{c}	42.40 ± 13.43^{c}	45.00 ± 0.00^{c}

Comparison between each row of cultivar during period of storage M: Mean of three replication, SD: Standard deviation. Different letters has significance difference at ($P \leq 0.05$)

The best protection against the impact of light gives the product a brown glass, then green, while it is translucent very transparent, as can be seen from the results of the fluorescence test. According to Lazić *et al*. (2003), the lightness of the wavelength of 350 nm of transparent bottles passes up to 60%, green to 45% and brown to 15%. The brightness of the wavelengths of 400 to 800 nm of the transparent bottles passes 80-90%, the green of 45 - 70%, while the brown glass, except for the light of the wavelength of 600 nm, passes 15 - 60%. Since most of the energy of the ultraviolet part of the spectrum, which causes the greatest change within the contents of the package,

emits within the limits of 200 - 400 nm, it can be concluded that the transparent packaging for virgin olive oils is less suitable. The thickness of the packing glass does not have a significant influence on the permeability of light (Lazić, *et al.*, 2003). The colour of the packaging plays a major role in the preservation of nutritionally valuable products, which was confirmed by the results obtained by these studies. The increase of the transparency is one of the changes that occur with the exposure of olive oil to the fluorescent light. By examining the values obtained for changing transparency by this test, it can be seen that the losses in the content of pigments significantly reflect the results. Chlorophyll and β-carotene are photosensitive components whose losses lead to deterioration in the quality of olive oil. Examination showed greater losses in the content of these pigments in all samples in transparent packaging. In order to preserve the ingredients that make olive oil one of the healthiest products of today, you should take special care in the choice of packaging.

DPPH assay for antioxidant capacity.

In order to test the bioactivity potential of VOOs in this study antioxidant capacity using DPPH assay was investigated. In vitro assay based on DPPH$^{\bullet}$ was used to determine the antioxidant capacity (AC) of VOOs. This assay has been widely used to determine the free radical-scavenging capacity of various plants. The capacity to capture the free radicals of the tested samples was determined by measuring their ability to neutralize the DPPH radicals. Effective Concentration by 50% (EC50), defined as the necessary concentration at which the radicals generated by the reaction systems were scavenged by 50%, could be served as an indicator of radical-scavenging activity. The EC50 value expresses the amount of VOO's extract necessary to decrease the absorbance of DPPH by 50% (Antolovich et al., 2002). The value can be determined graphically by plotting the % DPPH inhibition against the concentration of VOOs extract than using the regression equation to calculate EC50.The higher EC50 value corresponds to a lower scavenging activity on DPPH radicals.

(Fig. 3) showed the dependence of antiradical power ARP ($1/EC50^{DPPH}$) values of virgin olive oils in the function of exposure time to the fluorescent light.

It can be seen in the (Fig. 3) that during the first six days there were significant changes which can be observed in the ARP values and in the samples in transparent and in the dark packaging. Perhaps the main reason for that was the loss occurred in the pigments and polyphenols it work as antioxidants as noted in (Fig. 3) generally the ability to oxidize was less in oils exposed to light, while it was more in oils stored in the dark and that is evident through the values of ARP ($1/EC50^{DPPH}$). Olive oil also contains another, functionally compounds that also affect the change of the ARP ($1/EC50^{DPPH}$). Accordingly, it can be expected that, with the inevitable changes that would have occurred during prolonged exposure to the fluorescent light, there would be major changes in the ARP values due to changes in the content of certain components of olive oil. The best oil in order to preserve the anti-oxidant ability is the Gharyan VOO. While for the Msallata VOO, and after 6 days of storage, it is noted that the oil stored in the darkness decreased in its ability as an antioxidant compared to the same samples that was exposed to the fluorescent light. Msallata VOO until the end of storage, there was no significant difference between the oil exposed to light or stored in the dark regarding. The samples of Msallata VOO Shown strange behaviour during storage related to antiradical power.

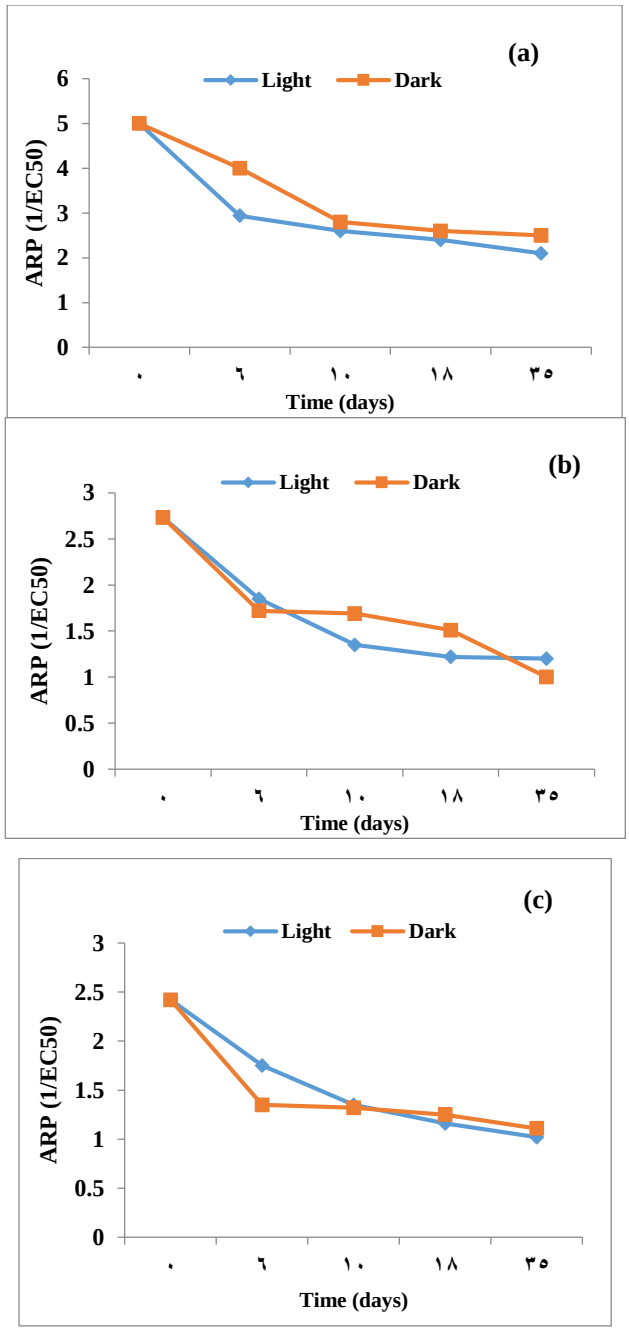


Figure (3). Effect of exposure to fluorescent light on ARP ($1/EC_{50}^{DPPH}$ (ml/mg) of different types of VOO stored for 35 days. (a); Gharyan, (b); Tarhuna, (c); Msallata.

The process of auto oxidation of vegetable oils is inevitable and will occur slower or faster depending on the composition of the oil, the conditions of storage and the presence of ingredients that accelerate or slow down this reaction (Vujasinović, 2011).

Many literature data talk about the barrier properties of the packaging, that is, the lightness of glass bottles most commonly used for the packaging of olive oil.

Conclusion.

This study evaluated the change occurring in the hydrolytic and oxidative degradation of VOO during storage in the dark or in the light and led to the following conclusions:

The VOO stored in the light displayed significantly lower carotenoids and chlorophyll contents than the VOO kept in the dark. The VOO kept in the dark mainly contained products of primary oxidation, while the VOO kept in the light contained products of secondary oxidation, as also shown by peroxide values. Exceeding the legal limits. Even after purification by means of an alumina. The VOO in the light showed significantly ($P \leq 0.05$) higher values of DPPH. The results obtained suggest that the shelf life of oils exposed to light is shorter than that of VOO kept in the dark and that after only 6 days in the light the oils examined could no longer be consideration VOO. Finally, in consideration of the results reported herein, packaging techniques ensuring more effective protection from light showed be recommended to together with bottling procedures that do not jeopardize the quality of the VOO, such as bottling under an inert atmosphere, and packaging in deep dark bottles.

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