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Evaluation of Selected Quality Degradation Indices for Palestinian Extra Virgin Olive Oil Bottled in Different Packaging Materials upon Storage under Different Lighting Conditions

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Abstract: The effect of packaging materials and lighting conditions on quality of extra virgin olive oil (EVOO) was investigated during six months. The results highlighted an influence of light and type of packaging material on EVOO-quality with storage time. At shelf, all packages maintained EVOO at the end of storage in terms of acidity, peroxide value, K_{232}, while K_{270} exceeded limit of EVOO in glass and PET-stored oil. Loss of phenols was the highest in glass-stored oil and the lowest in high-density polyethylene (HDPE)-stored oil. In terms of sensory evaluation, glass-stored oil lost EVOO grade after three months and its edible compliance after six months, while HDPE-stored oil maintained EVOO grade 90 days and was virgin after six months. In extended lighting, acidity, peroxide value and K_{232} did not exceed EVOO grade, while K_{270} exceeded EVOO grade after 30 days in glass and polyethylene terephthalate (PET)-stored oil and after 90 days in HDPE. The loss of phenols was the largest in glass and smallest in HDPE-stored oil. Glass stored-oil lost organoleptic edible compliance before 90 days, while that in PET was virgin at 90 days and that in HDPE maintained EVOO quality 90 days. At the end of experiment, oils in all packages were not edible. In dark, all packages maintained oil in EVOO quality in terms of all indices. The loss of phenols was marginal but was the least in glass and the highest in HDPE. It was concluded that HDPE bottles conserve stored olive oil at shelf or illumination better than PET or glass, while in dark, glass was superior over plastic.

Key words: Acidity, oil oxidation, olive oil, stability indicators, storage conditions.

1. Introduction

Olive tree is one of the most important trees internationally, from which high quality olive oil is produced [1]. From more than 750 million olive trees cultivated worldwide, 95% of which, are planted in the Mediterranean region [2]. The global production of olive fruits in 2011 was around 19.9 million tons, and 115,551 tons are produced in Palestine [3], from which around 30% olive oil is normally extracted. Olive oil plays a special role among vegetable oils because of its balanced fatty acid composition [4-7], which rank this product as the best among dietary fats [8]. Olive oil is categorized according to its organoleptic properties (sensory attributes) and chemical tests into extra virgin, virgin and lampant oil in terms of decreasing its edible quality, hence its healthy and marketable values. The highest grade extra virgin olive oil (EVOO) must

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contain zero defects and greater than zero positive attributes as evaluated by a certified taste panel, and must have a free acidity percentage of less than 0.8, and peroxide value does not exceed 20 milliequivalent peroxide O_2 per kg oil and conform to all the standards listed in its category. EVOO should have clear flavor characteristics that reflect the fruit from which it is made. In relation to the complex matrix of variety, fruit maturity, growing region and extraction technique, extra virgin olive oils can be very different from one another [9].

Specific sensory characteristics including color, aroma, and taste distinguish the extra virgin olive oil from other edible vegetable oils and other grades of olive oil [10] and accounts for its nutritive and health-giving properties [11, 12]. Therefore, its excellent organoleptic and nutrient properties, together with the current tendency of consumers to choose the least-processed foods, have enhanced its presence in consumers' diets and its marketable value [13, 14]. The antioxidant effects of extra-virgin olive oil seem to be a result of the phenolic compounds [15, 16], of which content depends on the cultivar, climate and degree of ripeness of the fruit [8]. Other factors which influence the quality of the oil include the cultural and harvesting practices, the health of the drupe, and the interval between harvest and processing [17], and accordingly, only 50% of the world olive oil production is classified as grade EVOO [18]. As in other foods, the quality of olive oil decreases during storage, and is attributable to lipid oxidation mechanisms which lead to rancidity [8], and hydrolytic degradations causing the partial loss of minor constituents having health-promoting effects [19, 20]. Therefore, it would be a good practice to consume the extra virgin olive oil produced during one crop season before the following crop season [14]. It is a matter of great concern for the olive oil industry to preserve the positive attributes of oil during the time elapsing from production to bottling, and up to purchasing [13, 14]. Accordingly, variation of storage conditions during olive oil storage and transportation, affecting its quality, is common and may be attributed to natural climatic changes as well as bad storage techniques [21, 22].

During the shelf-life of bottled extra virgin olive oil, the packaging must adequately protective against autoxidation processes that cause rancidity [10]. Therefore, several types of plastic films or metal containers can be used, but glass bottles of different shape and color are the most common [14, 23]. For example, in Spain, 90% of virgin olive oil is packaged in bio-use PVC, PET and clear glass, with the latter being increasingly used for the packaging and marketing of "extra quality" olive oils [10]. Although, extra-virgin olive oil is usually packaged in glass, or plastic bottles, these packages have some disadvantages because their bottled contents may be subjected to photo-oxidation [23]. The effect of different packaging materials on the quality of olive oil is widely reported [10, 14, 23-27]. Furthermore, the non-optimal storage conditions, such as those occurring on a store shelf, may alter the qualitative characteristics of the product to the extent that they may eventually differ from those indicated on the label, which, as legally, should maintain the analytical characteristics of the oil at the time of bottling. Thus, an investigation into the type and magnitude of the alterations in oil undergoes during its shelf life by comparing the changes occurring during storage in the light and in the dark may provide useful information [14]. In real time storage of oil in super- and hyper-markets, bottled oils are may exposed to light and high temperatures (typically 28-30 °C), which are not optimum conditions of preservation for the virgin olive oil [10]. It is known that oxidative reactions are catalyzed by light and heat and are partly slowed down by compounds belonging to the unsaponifiable fraction (phenolic compounds, carotenoids and tocopherols) naturally found in olives [28-32].

Accordingly, oil producers need to pay a great deal of attention to the type of containers they place the oils after production and to the storage conditions they are

kept in before sale [14]. The influence of glass and high density polyethylene on oil quality during storage was frequently studied [23], while little information is known about the effect of high density polyethylene (HDPE). Some investigators studied the changes occurring in few quality parameters over either short periods of time [26, 27], or long time as 12 months [14, 27] as the maximum storage period considered from bottling to consumption as real time stability studies. Therefore, the aim of this study is to determine which of the standard quality indices of oil may be used as markers to predict the time when a stored bottled virgin olive oil loses its "extra" quality (acidity 0.8%, peroxide value 20 mequiv kg^{-1}, K_{232} 2.50, K_{270} 0.25, sensory score 6.5) in Glass bottles, PET plastic bottles and HDPE plastic bottles in an accelerated stability study in terms of different lighting conditions (dark, diffused day light, and extra-lighting conditions). Furthermore, we studied the effect of these selected packaging materials and lighting conditions on the loss of phenol compounds of the stored oil during six months of storage.

2. Materials and Methods

2.1 Experimental Design

A homogeneous sample of olives (*Olea europea* L.) of the cultivar "Nabali Baladi" were handpicked with no defects and at an optimal stage of ripening (5.5 N detachment force, 3.8 pigmentation index, 57.5% water content) in late October from trees located in Salfeet district of a Mediterranean climatic region of Palestine. Olives were processed (stone mill and hydraulic press), after defoliation and washing the drupes. The initial whole oil sample was filled in two 20 L HDPE containers and directly transported to the laboratories of Al-Quds University. EVOO quality at the beginning of the experiment (November, 2008) was tested initially for its quality indexes and confirmed as extra quality virgin olive oil (peroxide value < 20, acidity < 0.8%, K_{232} < 2.5 and K_{270} < 0.25, iodine value 75-94, refractive index 1.4677-1.4700 and oil density). The 40 L extra virgin olive oil sample was divided into small subsamples (200 mL each) that were bottled in different packaging materials maintaining 2% head space in each bottle: non colored glass bottles, plastic polyethylene terephthalate (PET) and HDPE. Bottled EVOO small samples were stored under different illumination conditions at room temperature (25 °C ± 3 °C); firstly diffused day light, secondly continuous extended illumination (400 Lux white lamp) in white painted room (12 h daily), where the samples and were rearranged weakly to insure uniform exposure to light to avoid unequal spacial distribution of the bottles, and finally in dark (in a completely closed woody box having 1.5 cm wall thickness, painted with gray color from inside). The bottles (in three replicates for each treatment) of different packaging materials were randomized in a complete randomized design (CRD) in each storage condition. The effect of each of these factors (packaging materials and illumination conditions) on the stability of Palestinian extra virgin olive oil was studied in a non orthogonal design by monitoring oil quality indicators that include: acidity percent (as oleic acid), peroxide value, extinction coefficients (K_{232} and K_{270}), total phenolic contents (expressed as mg of gallic acid kg^{-1} oil), and sensory attributes (Panel test) in consequent days during six months of the experimental period (0, 30, 60, 90 and 180 days of storage).

2.2 Statistical Analysis

Three bottles of each treatment were independently analyzed in each sampling, and all of the determinations were carried out in triplicate. The results are expressed as mean ± standard deviation. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of different storage conditions and different packaging materials were carried out using the GLM procedure considering a fully randomized design, treating main factors (packaging materials and storage conditions)

separately using one-way analysis of variance. The Bonferroni procedure was employed with multiple *t*-tests in order to maintain an experiment wise of 5%.

2.3 Oil Quality Indicators

Acidity and peroxide values were performed according to the methods described in AOAC [33]. Data obtained were expressed as g oleic acid (100 g)$^{-1}$ oil for the former and as milliequivalent O_2 kg^{-1} oil for the later. Ultraviolet light absorption K_{232} and K_{270} indexes (K_{232} and K_{270} extinction coefficients) were determined using the methods described in IOOC [34]. Total phenol compounds were extracted according to Georgios et al. [33]. The total polar phenol content was determined spectrophotometrically at 765 nm and its concentration was expressed as mg gallic acid kg^{-1} oil. Sensory evaluation test was run by taster team for sensory analysis in the Palestinian standard institution laboratory, Ramallah, Palestine. The test was performed by the analytical panel done by 13 trained technicians, working according to the method defined by the Standard IOOC/T.15/NC No 3/rev. 2. The results obtained based on the ranking based on the median of notes from the tasters. Each bottle in each treatment was analyzed monthly for each mentioned quality indicators up to six months, except the sensory evaluation which were inspected in three periods (0, 3 and 6 months).

3. Results

3.1 Storage at Diffused Normal Day Light (Shelf)

3.1.1 Effect of Different Packages on Acidity

Free acidity as an important parameter for assessment of hydrolysis of triacylglycerols in virgin olive oil (VOO) as shown in Table 1 increased significantly with increasing time of storage in all types of packaging materials under study. The increase in acidity values in glass-bottled samples was significantly higher than that stored in PET and HDPE bottles at all respective sampling dates. Comparing the effect of PET and HDPE packaging on acidity of stored oil reveals that both storage materials affected acidity in similar way until 45 days after storage, but acidity of oil stored in HDPE bottles out-yielded that of oil stored

Table 1 Evolution of stability indexes: acidity, peroxide value (PV), extinction coefficient and polar phenols for different packaging materials during the storage time at shelf (room temperature). SD: standard deviation.

Source of variation	Storage time (days)	Acidity % ± SD*	PV ± SD	K_{232} ± SD	K_{270} ± SD	Polar phenols ± SD
Glass	0	0.38 ± 0.008 e	10.49 ± 0.84 b	2.02 ± 0.01 c	0.16 ± 0.002 f	214 ± 1.46 a
	30	0.44 ± 0.005 de	9.36 ± 0.20 bc	2.12 ± 0.01 a	0.20 ± 0.005 e	197 ± 0.44 b
	45	0.48 ± 0.020 dc	12.63 ± 0.85 a	1.91 ± 0.00 d	0.21 ± 0.000 d	198 ± 10.37 b
	90	0.51 ± 0.020 c	8.42 ± 0.20 c	1.82 ± 0.00 e	0.22 ± 0.000 c	171 ± 1.87 c
	135	0.58 ± 0.020 b	8.11 ± 0.05 c	2.01 ± 0.01 c	0.23 ± 0.001 b	164 ± 0.72 c
	180	0.66 ± 0.020 a	8.23 ± 0.26 c	2.07 ± 0.01 b	0.27 ± 0.002 a	155 ± 6.25 d
PET	0	0.38 ± 0.008 c	10.49 ± 0.84 b	2.02 ± 0.01 a	0.16 ± 0.002 c	214 ± 1.46 a
	30	0.42 ± 0.009 bc	14.34 ± 0.51 a	2.01 ± 0.06 a	0.22 ± 0.010 b	200 ± 9.05 a
	45	0.41 ± 0.010 cb	14.30 ± 0.22 a	2.10 ± 0.00 a	0.23 ± 0.000 a	202 ± 0.66 ab
	90	0.43 ± 0.020 b	7.99 ± 0.51 c	2.03 ± 0.00 b	0.24 ± 0.002 a	198 ± 2.23 b
	135	0.51 ± 0.010 a	7.24 ± 0.22 c	1.85 ± 0.03 b	0.23 ± 0.003 a	184 ± 3.82 c
	180	0.52 ± 0.030 a	8.55 ± 0.26 c	1.85 ± 0.03 b	0.23 ± 0.003 a	166 ± 2.35 d
HDPE	0	0.38 ± 0.008 b	10.49 ± 0.84 b	2.02 ± 0.01 a	0.16 ± 0.002 c	214 ± 1.46 a
	30	0.43 ± 0.003 cb	9.87 ± 0.02 b	1.56 ± 0.01 c	0.15 ± 0.010 c	209 ± 1.35 b
	45	0.42 ± 0.020 cb	13.04 ± 0.50 a	1.87 ± 0.27 bc	0.19 ± 0.005 b	202 ± 0.92 c
	90	0.49 ± 0.050 b	9.42 ± 0.21 b	1.73 ± 0.08 c	0.18 ± 0.009 b	192 ± 0.33 d
	135	0.58 ± 0.020 a	9.91 ± 1.03 b	1.93 ± 0.00 b	0.22 ± 0.001 a	190 ± 0.87 d
	180	0.56 ± 0.003 a	10.84 ± 0.08 b	1.80 ± 0.01 bc	0.21 ± 0.010 a	183 ± 0.16 e

in PET bottles after 45, 135 and 180 days of storage. At the end of storage period, acidity values were higher in glass bottles, followed by HDPE bottles followed by PET, but all types of packaging materials maintained the acidity of stored olive oil in its extra virgin grade (< 0.8% as oleic acid).

3.1.2 Effect of Different Packages on Peroxide Values

Evolution of peroxide value which indicates the state of primary oxidation products in EVOO stored in glass increased significantly after 45 days of storage, decreased significantly compared to the initial value after 90 days of storage and stayed stable until the end of the storage time. In PET bottles, peroxide values increased significantly after 30 days of storage, stayed at the highest level at 45 days of storage then was reduced significantly compared to the initial value after 90 days of storage and this reduced value was maintained until the end of the experiment. Peroxide values in olive oil stored in HDPE increased significantly after 90 days, then was reduced to values not significantly different from the initial value at the rest period of storage. Comparing different packages, the peroxide value increment was reported in PET bottles and was significantly higher than that in glass and HDPE. At the end of the experiment, peroxide values in oil stored in glass and PET were similar but were significantly lower than that in HDPE, and none of samples exceeded the official limit of extra virgin olive oil (20 meq O_2 kg^{-1} oil).

3.1.3 Effect of Different Packages on Extinction Coefficients (K_{232} and K_{270})

Spectroscopic values of K_{232} and K_{270} extinction coefficients in ultraviolet indicate the level of oxidation to produce primary and secondary products incurred during production and/or storage. Inspection of the results reveals differences within different packaging materials during storage at shelf (Table 1). It was clearly observed that K_{232} values in EVOO stored in glass fluctuated with increasing time of storage without a clear trend, while the values of this quality indicator in olive oil stored in plastic bottles (PET and HDPE) decreased marginally but significantly with increasing time of storage. After six months, none of the packaging materials under investigation exceeded the official limit in terms of extinction coefficient K_{232} < 2.5, these results highlighted that K_{232} was correlated with PV not only at zero time but also during storage for different types of bottles. Extinction coefficient K_{270} increased significantly during storage in all types of bottles used for storage and exceeded the official limits of the EVOO grade (< 0.22) in glass and PET, while HDPE marginally reached the critical limit after 135 day of storage then decreased to below the critical limit at the end of storage period. K_{270} of oil samples stored in glass showed higher values at the end of storage period compared to plastic bottles (PET and HDPE) and exceeded the limits for even virgin olive oil quality (0.25). The least values of K_{270} were found in oil stored in HDPE compared to glass and PET at all respective testing dates during storage period. This indicates that HDPE protects EVOO better than glass and PET when K_{270} was used as quality indicator. Furthermore, the PET bottles provide more protection for EVOO in the presence of light than glass in terms of mentioned coefficients. Glass was found to be the worst storage packaging material at shelf in terms of K_{232} and K_{270} since glass is permeable to light more than the other materials under study.

3.1.4 Effect of Different Packages on Phenol Compounds

Total polar phenolic compounds which are considered as natural antioxidants in EVOO decreased during storage time at shelf in all types of packaging materials under study (Table 1). In particular for EVOO stored in glass bottles which showed dramatic decrease during storage period and their values were the least compared to EVOO stored in PET and HDPE at all respective testing dates. The loss of polar phenols was the largest and more rapid in oil stored in glass (from 214 mg to 166 mg gallic acid kg^{-1} of olive oil), while plastic bottles maintained these antioxidants

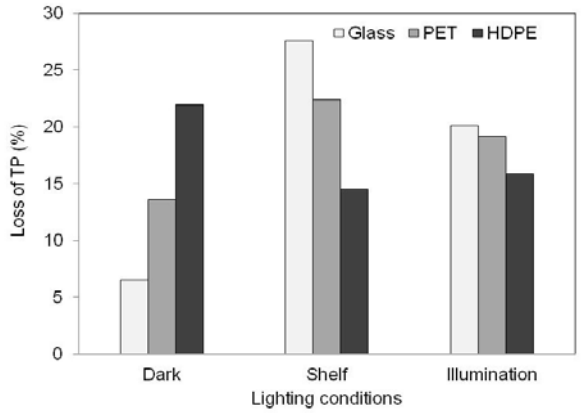

Fig. 1 Loss of total phenols as affected by different means of packaging conditions and lighting storage conditions (percentage lost after 6 months of storage compared to the initial values at the beginning).

better than glass. The reduction of this quality indicator was more sharp (from 214 mg to 166 mg Gallic acid kg^{-1} of olive oil) in PET compared to HDPE (from 214 to 183 mg Gallic acid/kg of olive oil). At the end of the storage period, the loss of phenolic compounds concentration in stored EVOO was higher in glass, followed by PET followed by HDPE (Fig. 1).

3.1.5 Effect of Different Packages on Sensory Evaluation

Olive oil legislations refer to four groups of off-flavors: fusty, mustiness-humidity, winey-vinegary, and rancid. The three first groups are related to olive quality whereas the last one, rancid, develops in storage. Sensory evaluation of olive oil under investigation (Table 2 and Fig. 2) showed that samples stored in glass bottles maintained their extra virgin category in the first three months of storage, while become virgin after this time of storage and quit from the virgin grade at the end of storage period. For samples, stored in PET bottles, the oil quit the EVOO grade after 30 days and stayed in the VOO category till the end of storage period, while HDPE maintained the extra virgin quality of stored oil for 90 days, and the oil stayed as virgin till the end of the storage period. These results indicated that sensory evaluation test correlates with the results of K_{270} which was also failed out of extra virgin category for EVOO stored in glass bottles.

3.2 Storage under Extended Fluorescent Light

3.2.1 Effect of Different Packages on Acidity

Acidity of EVOO stored under extended illumination increased significantly during storage in all types of packaging materials under study (Table 3). At the end of storage period of 180 days, glass bottles showed significantly higher acidity in stored oil compared to PET and HDPE. Furthermore, acidity of stored oil was significantly higher in PET compared to HDPE at the end of storage period. All packaging materials under study maintained stored oil in its EVOO grade (< 0.8%) at all testing intervals during time of storage.

3.2.2 Effect of Different Packages on Peroxide Values

Peroxide values of EVOO stored in glass and PET decreased with increasing storage time at extended illumination conditions, while that of oil stored in HDPE was marginally and insignificantly reduced (Table 3). At the end of storage period, peroxide value

Table 2 Sensory evaluation and other stability indexes for olive oil samples stored in different packaging materials on shelf.

Source of variation	Storage time (Days)	Sensory evaluation (Defects)	Sensory evaluation (Fruity)	Olive oil grade
Glass	0	0.0	4.9	EVOO
	90	0.0	2.0	VOO
	180	0.8	1.0	Not VOO
PET	0	0.0	4.9	EVOO
	90	0.0	3.0	VOO
	180	0.0	2.55	VOO
HDPE	0	0.0	4.90	EVOO
	90	0.0	1.19	VOO
	180	1.85	1.30	VOO

EVOO: extra virgin olive oil; VOO: virgin olive oil.

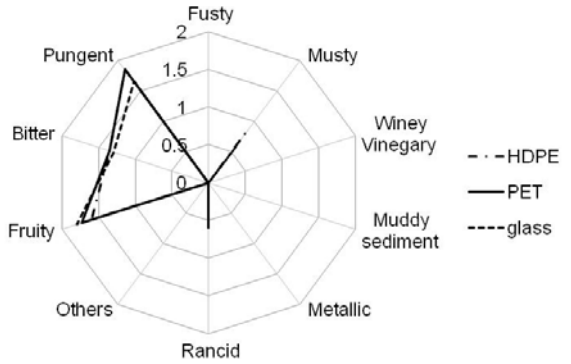

Fig. 2 Evaluation of sensory attributes for EVOO stored in different packaging materials at shelf (normal diffused day light) after six months of storage.

of oil stored in glass bottles was not significantly different from that of oil stored in PET bottles, while that of oil stored in HDPE bottles was maintained in a significant higher value compared with the other two types of packaging materials under investigation.

3.2.3 Effect of Different Packages on Extinction Coefficients (K_{232} and K_{270})

The extinction coefficients K_{232} of olive oil samples stored in glass bottle under florescent light increased significantly at the end of the storage period compared to that at the beginning of the experiment, but values fluctuated within the time borders of the experiment (Table 3). Although K_{232} of oil stored in glass increased slightly at the end of storage period, the unclear trend between the beginning and the end of the storage period was also the case in terms of K_{232} extinction coefficient values for oil stored in both types of plastic packages (PET and HDPE). All types of packaging materials maintained the oil in its extra virgin quality in terms of $K_{232} < 2.5$. The extinction coefficient K_{270} increased significantly with increasing time of storage in all types of packaging materials under study. Oil stored in glass and PET quit the EVOO grade (< 0.2) after a period of less than 30 days, while HDPE maintained the oil in its extra virgin quality for more than 90 days under accelerated stability study in terms of extra light condition. At the end of the experiment, oil stored in glass showed the highest K_{270} value.

3.2.4 Effect of Different Packages on Phenol Compounds

Total polar phenols decreased significantly with increasing time of storage under florescent illumination (Table 3). The loss of polar phenols was faster in oil stored in glass compared to that stored in PET and HDPE

Table 3 evolution of stability indexes: acidity, peroxide value (PV), extinction coefficient and polar phenols for different packaging materials during the storage time under florescent light illumination (400 Lux). SD: standard deviation.

Source of variation	Storage time (days)	Acidity % ± S.D*	PV ± S.D	K_{232} ± S.D	K_{270} ± S.D	Polar phenols ± SD
Glass	0	0.38 ± 0.008 b	10.49±0.84 a	2.02±0.01 c	0.16±0.002 c	214±1.46 a
	30	0.38 ± 0.030 b	8.56±0.18 ab	2.10±0.01 b	0.23±0.004 b	184±2.71 b
	45	0.50 ± 0.030 b	9.34±1.18 ab	1.97±0.04 d	0.23±0.010 b	182±0.91 b
	90	0.50 ± 0.000 a	9.30±0.05 ab	1.92±0.01 e	0.23±0.000 b	176±0.49 c
	135	0.57 ± 0.006 a	8.96±1.00 ab	2.01±0.01 c	0.26±0.002 a	172±0.49 d
	180	0.58 ± 0.040 a	8.18±0.32 b	2.17±0.00 a	0.28±0.010 a	171±0.16 d
PET	0	0.38 ± 0.008 c	10.49±0.84 a	2.02±0.01 bc	0.16±0.002 d	214±1.46 a
	30	0.40 ± 0.008 c	9.64±0.49 a	1.99±0.02 ba	0.24±0.010 c	204±0.49 b
	45	0.46 ± 0.020 b	7.71±0.26 b	2.08±0.02 a	0.23±0.010 c	189±0.49 c
	90	0.50 ± 0.001ab	7.52±0.53 b	1.95±0.01 c	0.23±0.035 c	176±0.00 d
	135	0.51 ± 0.020 a	8.06±0.22 b	1.99±0.00 bc	0.25±0.002 b	175±0.33 d
	180	0.53 ± 0.000 a	7.67±0.69 b	2.02±0.02 ab	0.26±0.001 a	173±0.30 e
HDPE	0	0.38 ± 0.008 d	10.49±0.84 a	2.02±0.01 bc	0.16±0.002 e	214±1.46 a
	30	0.40 ± 0.020 cd	8.94±0.33 b	1.99±0.02 ab	0.18±0.010 d	193±1.35 b
	45	0.45 ± 0.008 bc	8.49±0.12 ab	2.08±0.02 a	0.22±0.010 c	187±0.44 c
	90	0.47 ± 0.000 ba	8.79±0.09 ab	1.95±0.01 c	0.21±0.020 c	184±1.43 c
	135	0.53 ± 0.003 a	8.84±0.32 ab	1.99±0.00 bc	0.23±0.002 b	183±0.72 c
	180	0.51 ± 0.020 a	9.49±1.10 ab	2.02±0.02 ab	0.27±.002 a	180±0.82 d

Table 4 Sensory evaluation and other stability indexes for olive oil samples stored in different packaging materials under florescent light.

Source of variation	Storage time (Days)	Sensory evaluation (Defects)	Sensory evaluation (Fruity)	Olive oil grade
Glass	0	0	4.9	EVOO
	90	2.56	2	Not VOO
	180	2.55	1.65	Not VOO
PET	0	0	4.9	EVOO
	90	0	2.3	VOO
	180	2.3	0.65	Not VOO
HDPE	0	0	4.9	EVOO
	90	0	2.6	EVOO
	180	1.9	1.9	Not VOO

EVOO: Extra virgin olive oil; VOO: virgin olive oil.

bottles in the first 45 days of storage. After 45 days of storage, total polar phenols of oil stored in glass were reduced in the same scale as that stored in PET bottles, while HDPE bottles maintained higher total polar phenols at all testing times throughout the storage period. At the end of the experiment, total polar phenols were maintained in larger contents in oil preserved in HDPE followed by that stored in PET bottles, and the least was found in oil stored in glass (Fig. 1).

3.2.5 Effect of Different Packages on Sensory Evaluation

Sensory evaluation of olive oil stored in different packaging materials under study shows a great effect of light in the deterioration of sensory attributes of olive oil (Table 4, Fig. 3). Extended artificial illumination largely affected the organoleptic properties of oil stored in glass bottles more than that stored in PET and HDPE bottles. Oil stored in glass under this extreme condition lost its compliance as edible oil before 90 days and become not virgin olive oil, while oil stored in PET was found virgin after 90 day of storage and that stored in HDPE maintained its extra virgin quality. At the end of the storage period, oil stored in all packaging materials under study lost its virginity and hence its compliance as edible oil.

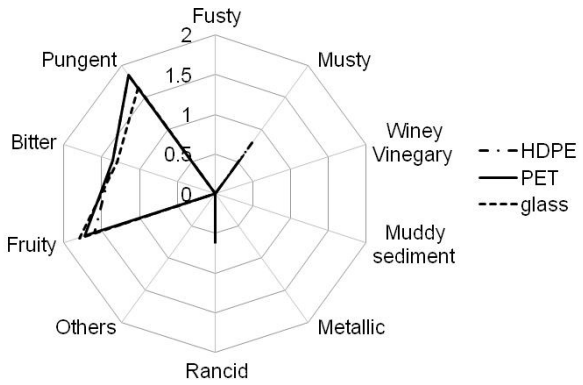

Fig. 3 Evaluation of sensory attributes for EVOO stored in different packaging materials at extended illumination after six months of storage.

3.3 Storage in Dark Conditions

3.3.1 Effect of Different Packages on Acidity

The acidity of oil stored in all packaging materials under study at dark conditions increased significantly with increasing time of storage (Table 5). The significant increase in acidity began after 90 days of storage in oil stored in glass while significant increase of this indicator began after 30 days of storage in oil stored in plastic bottles (PET and HDPE). At the end of the experiment, oil stored in PET bottles showed the lowest acidity value, followed by oil bottled in glass, and the highest acidity was found in oil stored in HDPE bottles. Oil stored in all packaging material under investigation did not exceed the limits for the extra virgin quality (< 0.8%).

3.3.2 Effect of Different Packages on Peroxide Values

Peroxide values responded in different ways among different packaging materials under study (Table 5). Peroxide value of oil stored in both glass and PET bottles began to decrease significantly after 45 days of

Table 5 Evolution of stability indexes: acidity, peroxide value, extinction coefficient and polar phenols for different packaging materials during the storage time indark. SD: standard deviation.

Source of variation	Storage time (days)	Acidity % ± SD*	Peroxide Value ± SD	K_{232} ± SD	K_{270} ± SD	Polar phenol ± SD
Glass	0	0.38 ± 0.008 c	10.49±0.84 a	2.02±0.010 a	0.16±0.002 b	214±1.46 a
	30	0.42 ± 0.020 bc	9.88±0.52 a	1.77±0.004 d	0.16±0.002 b	203±2.80 b
	45	0.42 ± 0.010 bc	7.36±0.06 b	1.74±0.010 d	0.18±0.010 a	203±2.80 b
	90	0.43 ± 0.020 b	8.23±0.32 b	1.85±0.030 c	0.16±0.000 b	196±0.16 d
	135	0.52 ± 0.020 a	8.38±0.10 b	1.90±0.005 bc	0.16±0.010 b	201±1.15 bc
	180	0.53 ± 0.003 a	8.42±0.36 b	1.96±0.050 ba	0.16±0.001 b	200±0.82 c
PET	0	0.38 ± 0.008 c	10.49±0.84 a	2.02±0.010 a	0.16±0.002 c	214±1.46 a
	30	0.42 ± 0.020 b	10.40±0.40 a	2.03±0.020 a	0.19±0.004 a	206±3.97 b
	45	0.42 ± 0.010 b	8.25±0.01 b	2.01±0.000 ab	0.19±0.000 a	202±0.81 bc
	90	0.49 ± 0.002 a	8.46±0.14 b	2.02±0.013 a	0.19±0.010 a	199±3.21 bc
	135	0.50 ± 0.002 a	8.62±0.01 b	1.98±0.014 bc	0.18±0.006 b	195±3.61 c
	180	0.51 ± 0.020 a	8.67±0.36 b	1.96±0.010 c	0.18±0.010 b	185±2.83 d
HDPE	0	0.38 ± 0.008 e	10.49±0.84 a	2.02±0.010 ba	0.16±0.002 c	214±1.46 a
	30	0.43 ± 0.010 d	8.68±1.14 a	2.02±0.010 ba	0.18±0.010 ba	208±1.45 b
	45	0.50 ± 0.020 c	8.64±0.08 a	2.03±0.014 a	0.20±0.010 a	202±0.42 c
	90	0.53 ± 0.004 b	9.37±0.93 a	2.08±0.005 c	0.15±0.010 cd	183±1.32 d
	135	0.53 ± 0.004 b	9.22±0.10 a	1.75±0.140 ba	0.15±0.004 cd	192±0.57 e
	180	0.57 ± 0.004 a	10.04±0.13 a	1.95±0.005 bc	0.14±0.002 d	167±0.28 f

storage, while that of oil stored in HDPE did not change significantly within storage time. At the end of the storage period, peroxide value in oil stored in glass and PET bottles share similar values, while that of oil stored in HDPE was higher significantly. Peroxide values of oil samples bottled in all types of packaging materials under study did not exceed the limit of the extra virgin grade of olive oil during the storage period (20 meq O_2 kg^{-1}).

3.3.3 Effect of Different Packages on Extinction Coefficients (K_{232} and K_{270})

There was no clear trend in the response of K_{232} in oil stored different types of packaging materials under investigation, as the values of this extinction coefficient fluctuated with storage time (Table 5) within a very narrow range and no oil sample exceeded the limit of extra virgin quality (2.5). The extinction coefficient K_{270} of oil stored in glass bottles increased significantly after 45 days of storage then returned to its initial value till the end of storage period, the same response was observed in oil stored in both PET and HDPE bottles. The values of this indicator were sustained below the limit for the extra virgin grade of olive oil and all oil samples stored in all packaging materials were sustained under the critical limit of extra virgin olive oil (0.22).

3.3.4 Effect of Different Packages on Phenol Compounds

Total polar phenols decreased significantly during storage at dark conditions in oil stored in all packaging materials under study (Table 5). The loss of polar phenols at the end of storage period (Fig. 1) was more pronounced in oil stored in HDPE (22% reduction) followed by PET (13.6% reduction) followed by glass (6.5% reduction).

3.3.5 Effect of Different Packages on Sensory Evaluation

Olive oil stored in all types of packaging materials was maintained their extra virgin category without any sensory defects (Table 6, Fig. 4).

4. Discussion

One of the most fundamental reactions in lipid chemistry is oxidation, in which a series of compounds are formed, causing off-flavors and rancidity, loss of nutritional value and finally consumer rejection of the

Table 6 Sensory evaluation and other stability indexes for olive oil samples stored in different packaging materials in dark.

Source of variation	Storage time (Days)	Sensory evaluation (Defects)	Sensory evaluation (Fruity)	Olive oil grade
Glass	0	0	4.9	EVOO
	90	0	2.5	EVOO
	180	0	1.65	EVOO
PET	0	0	4.9	EVOO
	90	0	2.3	EVOO
	180	0	0.65	EVOO
HDPE	0	0	4.9	EVOO
	90	0	2.6	EVOO
	180	0	1.6	EVOO

EVOO: extra virgin olive oil.

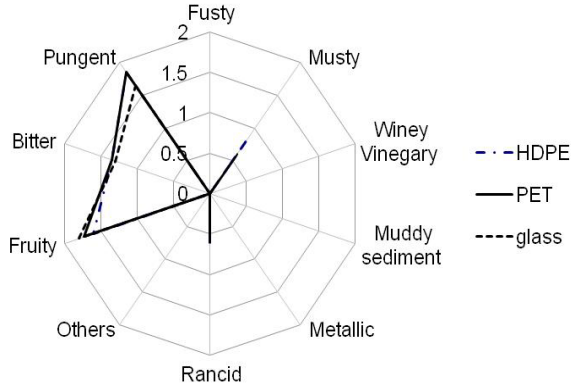

Fig. 4 Evaluation of sensory attributes for EVOO stored in different packaging materials in dark after six months of storage.

food product. Auto-oxidation-occurs in the absence of air by reactive oxygen species or "free radicals" is temporarily prevented by the natural antioxidants in the oil that absorb these free radicals. When the antioxidants are used up, the oil ages quickly. Studies of the autoxidation of oleic acid process date back to 1943 [34]. Autoxidation is therefore the main cause of olive oil quality deterioration and its reaction rate determines the shelf-life of this product [35]. In the case of virgin olive oil, upper limit values for different oxidation indexes were established (peroxide value: 20 meq kg^{-1}, K_{232}: 2.50 and K_{270}: 0.20) which could be employed as end points for its shelf-life [36].

4.1 Acidity

Comparing the influence of previously mentioned packaging materials in terms of their effect on acidity of olive oil stored in dark, glass showed the least (best results) acidity values, where in contrast to plastic material, glass is not permeable to oxygen and humidity which could increase the acidity of the oil through increasing the rate of hydrolysis of triglyceride to liberate free fatty acids. At the end of storage period of six months, none of the samples stored at these conditions exceeded the critical limit of extra virgin olive oil category in terms of acidity (0.8%) according to the international standards. Our results are in accordance with what was reported previously [37, 38] which found that acidity did not increase significantly with increasing time when samples were stored in dark.

In agreement with our findings, it was previously documented that free acidity was higher in oil stored in light compared to that stored in dark because light negatively affects olive oil quality with increasing storage time [8, 39-41]. The increase in acidity throughout storage time as affected by light can be explained by its effect on the activation of triglycerides hydrolysis resulting in the liberation of free fatty acids [10, 41-44] and the subsequent development of oil rancidity [41, 42].

The increase of acidity of oil stored in glass in the presence of light (at shelf and extended illumination) is because the transparency of glass to light, therefore negatively affecting olive oil quality in terms of acidity as a stability indicator [39]. A significant increase in acidity was also observed in oil samples stored in plastic packages (both polyethylene terephthalate (PET)

and high density polyethylene (HDPE)) as time of storage increased. This can be explained by their diffusivity to oxygen which negatively affects olive oil quality by enhancing the oxidative deterioration of oil. Acidity of oil stored in plastic containers (PET and HDPE) was maintained in lower levels compared to that stored in glass, which can be attributed to the fact that plastic has barrier properties to light more than glass [23]. The increase of acidity as increasing storage time found in our study is reported by previous investigators [39, 45-47].

Comparing previously mentioned packaging material in terms of their influence on acidity of olive oil subjected to artificial illumination; our findings showed that glass was the most affected by light followed by HDPE followed PET. PET was found to be more protective in terms of light diffusion where it prevents wave length less than 300 nm to pass through it compared to glass [23]. HDPE bottles gave better results (less increase in acidity %) since these packages are colored and relatively prevent light form passage to the oil more than transparent PET bottles. Our finding are in agreement with the results of many researchers [8, 45] who found that acidity was affected by illumination and increased within time of storage in glass and plastic containers.

4.2 Peroxide Value

In agreement with our results, the peroxide values of oil stored at shelf in all studied packaging materials underwent an initial increase at the beginning of storage period, and then it marginally decreased with increasing storage period [8, 39, 47]. This because the newly formed oxidation products (we left a bottle headspace) are further converted to secondary products [39]. Oil samples stored in the dark showed higher peroxide values compared to that subjected to light (shelf or extended lighting) at each respective storage time [8, 26, 39, 47] which indicated greater primary oxidation, while the samples exposed to light exhibited a lower peroxide value, which could be ascribed to evolution from primary to secondary oxidation [14]. The lesser formation of secondary products in samples stored in the dark may explain the higher peroxide values obtained for oil stored at this condition in this study [8]. In the same line with our findings, peroxide values of oils stored in glass at illumination showed a linear decrease with storage time [10]. The decrease in the PV with increasing time can be explained by the degradation of primary oxidation products (peroxides) to form secondary oxidation products which can be detected by K_{270} value. The results of PV was correlated with that obtained by K_{232} which was observed to be decreased or stay stable during the storage period [10, 40, 44, 47]. The oil samples packed in HDPE and exposed to light presented higher peroxide values compared to those packed in glass containers. These results are similar with other findings and point to the probable intrusion of oxygen through HDPE as a consequence of its permeability to oxygen and its less light penetration ability. Peroxide values in oil stored in PET was similar to that stored in glass as affected to increasing storage time at extended illumination due to the combined effects of the permeability of PET to oxygen and at the same time its transmittance to light [48].

Generally, during the beginning of storage, PV in different packaging materials increased as a consequence of the action of both, diluted and headspace oxygen in the containers and additionally, the light induce a rapid deterioration of oil in terms of PV. After a period of storage, the PV progressively decrease because of the degradation of primary products into secondary products, which is more obvious in the samples packed in glass containers and less in those packed in plastic bottles. This could be explained as the evolution of photo oxidation [49].

4.3 Extinction Coefficients

It was documented that the shelf-life of virgin olive oil is determined by the increase in the K_{232} absorption coefficient as a quality parameter [50], or by means of

the time required to reach the upper legal limit of K_{270} absorption coefficient [10, 51]. Primary oxidation products in olive oil (fatty acid hydroperoxides and oxidized triacylglycerols) are measured as peroxide value (PV) and K_{232} absorption coefficient, while secondary oxidation products (fatty acid hydroperoxides decomposition products such as aldehydes, alcohols, ketones and hydrocarbons) are detected by K_{270} absorption coefficient [51]. Hydroperoxides are the initial products of oxidation—very sensitive and comparatively unstable—and used as indicator of the early stages of oxidative deterioration at the beginning of the oxidation process [39, 49, 52], while the K_{270} index is used to study the behavior of the secondary oxidation products by the formation of dimers and polymers of triacylglycerides [51]. Because of the significant variation of the K_{270} value during olive oil storage as a response to oil oxidation, and is easily measured, this parameter may be of capital importance to control the quality of stored virgin olive oils in terms of determining the time at which they will lose their "extra" category [10].

It is well known that light affects olive oil quality, making possible an increase in the triene formation, measured by K_{270} [29, 53], more than in the diene measured as K_{232} [8]. In agreement with our findings, K_{270} values were affected by the exposure conditions, with higher values reported in the samples stored in the light than in those kept in the dark [8, 38, 54] probably because of the presence of chlorophylls in the oil acts as an antioxidant in the dark [47], while pigments of the olive oil (chlorophylls and pheophytins) in presence of light have an oxidizing effect through acceleration of photo oxidation [8] increasing triene containing secondary products of oxidation and thus K_{270} increased more than K_{232}. In contrary with our findings, one researcher reported higher values of K_{232} in the samples stored in dark compared to those kept in light because of conjugate dienes as the oxidation products present in greater amounts in dark [26], while concerning our results, the opposite was found and may be discussed by the high rate of production of primary and secondary oxidation products as affected by light, this indicates that the rate of secondary oxidation is not higher than that of primary oxidation. The value of K_{270} remained almost unchanged at dark condition. By contrast, in the samples exposed to light both K_{232} and K_{270} were significantly higher than the values found in oils kept in the dark. This indicated that in the light, degradation of primary oxidation compounds was facilitated and peroxides underwent breakdown reactions more rapidly. Our findings are in agreement with other researchers [23]. Furthermore, after six months of storage, the value of K_{270} of the oils exposed to light exceeded the limits for virgin olive oils and agreed with results of other researchers [14]. In this investigation, K_{232} values were maintained under the limit of 2.5 units for oil stored in light (at shelf and at extended lighting) and dark in all packaging materials under study while K_{270} values exceeded the limit of 0.20 units during the six months of storage in both light intensities (at shelf and extended illumination) in all packaging means and the same was previously reported [39].

Our findings are in agreement with results previously [55] which found that oil samples stored in PET and glass under light were associated mainly with secondary oxidation products. It was found that for oil samples stored in glass bottles under illumination, K_{232} increased while the samples stored in dark K_{232} remain constant, while K_{270} showed a sharp increase in samples stored under illumination and exceed the limit value for EVOO after three months of storage [10]. The action of light on olive oil samples stored in plastic bottles resulted from the effect of light through enhancing photo-oxidation and the permeability of plastic packaging material to oxygen and humidity. A group of investigators showed that for samples stored in glass in dark K_{232} increased from 1.96 to 2.015 after 9 months [38] while others [10] showed that for oil samples stored in glass bottle in dark K_{232} and K_{270}

remain constant throughout the storage period. In contrary, other findings showed that both UV absorption coefficients for olive oil samples stored in glass in dark increased throughout the storage time and exceed the established limit by legislation [26, 56, 57]. Glass acts as a barrier to oxygen, avoiding the loss of certain components that deteriorate under oxygen presence but glass allows the direct action of light on the stored olive oil and this could promote oxidative rancidity as a consequence of its sensibility to photo-oxidation [39]

4.4 Total Phenols

In agreement with previous reports [10], total polyphenol (TP) contents of extra virgin olive oil decreased during storage in all means of storage conditions and packaging materials under study; due to degradation of these compounds that was well fitted to first order kinetics. Although, phenolic compounds (Tables 1, 3 and 5) constantly decreased during storage; samples stored in the dark revealed a significantly higher values than those stored in the light [8, 14, 32]. Phenolic compounds act as natural antioxidants in oil and their reduction during storage is a result of oil oxidation [41, 58, 59], where phenolic antioxidants inhibit autoxidation of lipids (RH) by trapping intermediate peroxyl radicals [60]. The loss of phenolic compounds of olive oil during storage is mainly due to the action of photo oxidation as a result of light that initiate oxidation process which occur by photochemical hemolytic cleavage of RH bond to produce free radicals [61]. Photo-oxidation processes occurred in parallel with auto-oxidation [14] and consequently reduce phenol contents in stored oil.

Compared with other vegetable oils, virgin olive oil is more stable against oxidation due to multiple factors such as the relatively low content of polyunsaturated fatty acids, the high level of monounsaturated fatty acids (mainly oleic acid) and the presence of some natural antioxidants (tocopherols, carotenes and phenolic aglycons, based on the molecules of tyrosol and hydroxytyrosol, deriving from phenolic glycosides). The stability of virgin olive oil also depends on the presence of pro-oxidant substances as well as on factors linked to the storage conditions, namely the presence of oxygen, the temperature and above all light exposure, therefore, the level of degradation of an oil results from a balance of all these factors [14].

The different trend observed in terms of the reduction of phenolic substances in different lighting conditions may be attributed to their specific mechanisms of action as antioxidants. The phenolic compounds act by giving an electron so that they can interrupt the radical reaction occurring with oxidation [62]. The carotenoids act as electron acceptors, quenching the singlet oxygen [63]. Finally, tocopherols act both as electron donors, slowing down the oxidative reaction, and as electron acceptors, determining the quenching or the scavenging of singlet oxygen, with consequent inhibition of the photooxidation of lipids [27]. Nonetheless, the singlet oxygen formed in the photo-oxidative reaction (in presence of light) is 1,000-1,500 times more reactive than the triplet oxygen taking part in the reaction of auto-oxidation which take place in dark [62]. This means that photooxidation takes place faster than auto-oxidation and implies a greater decrease in tocopherols in the samples exposed to light. This suggests that in presence of light oil is protected from oxidation mainly by tocopherols and carotenoids, and those phenolic substances have a secondary role, in the dark, instead, the main reaction is auto-oxidation and the phenolic substances seem to be involved more than the other antioxidants in the protection of the oil from oxidation [14].

At the beginning of storage time, olive oil contained 214 mg kg^{-1} ± 1.46 mg kg^{-1} oil of total phenolic compounds, and this value was in consistent with the data (121-410 mg kg^{-1}) reported previously [62]. Afterwards, the total content of phenols decreased as a function of time, with various degree of reduction among the storage containers, and the decrease was

more pronounced under light conditions. Fig. 1 showed that the lowest range between the initial and final antiradical activity (percentage loss of total phenols) at dark condition was in glass bottle (6.5%), then PET and HDPE (13.5% and 22%, respectively) showing the low ability of plastic containers to keep the quality of olive oil through maintaining its activity to scavenge the free radicals when stored in dark [44]. In addition, at dark condition, glass containers kept more phenolic compounds than plastic containers (PET more than HDPE), which agreed with that reported previously [42] where olive oils samples exhibited insignificant loss of their total phenols during storage at condition away from light in glass bottles. The reduction of antioxidants in plastic containers could be due to its permeability to oxygen and the migration of active compounds between oil and packaging material [45]. In the presence of light (at shelf and extended lighting), the opposite response was found. Both plastic containers retained phenolic compounds (PET more than HDPE) more than glass containers. The loss of phenolic compounds at shelf was highest in oil stored in glass (27.6%) followed by PET (22.4%) followed by HDPE (14.5%), the same response was found under extended illumination but the loss of total phenols was larger in oil stored in glass and PET bottles (20.1%, 19.2% and 15.9% for glass, PET, and HDPE, respectively). This can be discussed by the effect of light on the photo-oxidation of oil and the consequent reduction of antioxidant compounds including total phenols and the more light transparency of glass than PET followed by HDPE in light of the stated above it was cleat that phenolic compound loss intensity during storage is directly propotional to the attitude and degree of oxidation occurred.

4.5 Sensory Analysis

The descriptive sensory analysis of olive oil stored at the three types of packaging materials during storage in different lighting conditions is shown in Tables 2, 4 and 6. It can be seen that samples stored at dark condition had the lowest changes in sensory values in all studied packaging materials maintaining the stored oil in its extra quality during the period of the experiment. In the presence of light (at shelf, and extended lighting), HDPE was found the best in maintaining the stored oil with the lowest defects at the end of the storage period followed by PET, and the worst was glass containers where oil lost its virginity before 90 days of storage at extended lighting condition and before 180 days at shelf. In contrary with our findings, it was reported that samples stored in the glass container at shelf had the lowest changes in positive sensory attributes, and was considered the best material followed by plastic bottle [41]. This was due to the argument that EVOO samples in glass containers had the highest values of color, taste, flavor, and odor retention followed by those in plastic containers. The reduction of sensory attributes could be due to that the physical characteristics of the packaging material may affect the final quality of the oil, depending on the extent of the deteriorative interactions [64].

The pigments content in olive oil correlate with the shelf life of stored oil and, in particular, its resistance to oxidation. The green color of olive oil faded off as the oil ages, which might be caused by the conversion of chlorophyll to alternative yellow and brown pigments, i.e., pheophytins (PP) and pyropheophytins (PPP). The rancid flavor development in olive oil could be due to oxidation; the decomposition of the hydroperoxides formed and the consequent formation of newly generated volatile compounds [64]. The volatile aldehydes and vinyl ketones are known to be mainly responsible for potent off-flavors, because their odor threshold levels are very low [59, 65] demonstrated that as free fatty acids concentration increased, undesirable sensory properties occurred. It was demonstrated that the negative sensory attributes in olive oil can be associated with volatile compounds, which are mainly formed by chemical oxidation of oil [21, 66]. Our results show that EVOO placed in the glass container had the highest acidity followed by

those in the plastic containers when they were stored at shelf and extended lighting conditions, and as time increased from 0 to 180 days the total phenolic compounds decreased, which could be caused by oil oxidation during storage. In addition, the oil in the glass containers kept less phenolic compounds than that in the plastic container when they were subjected to light (at shelf and extended light) [59].

5. Conclusions

Finally, as a consequence of the results reported herein, the packaging material should ensure protection from storage conditions in order to maintain the olive oil quality, especially when the oil is stored under the studied commercial conditions in terms of different lighting conditions. This study has reaffirmed that HDPE bottles, stored at shelf and at extended illumination conserve the oil much better providing higher protection from oxidation compared to PET and glass containers. At both normal and extended lighting storage conditions, glass bottles were not able to protect stored EVOO, and the oil quit from extra virgin grade in the former and from edible compliance in the later during six months of storage. In the other hand glass bottles showed superiority over plastic containers in conserving oil when they were stored at dark condition but the three types of packaging material conserve oil and maintained the extra virgin quality during six months. The extinction coefficient K_{270} is the quality index that was showed tight correlation with the sensory evaluation test more than acidity, peroxide value and K_{232}. Therefore, the storage of extra virgin olive oil in HDPE bottle, could be suggested the most appropriate mean for maintaining the quality of the extra virgin olive oil.

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