

Evaluation of the Effect of Packaging Materials and Storage Temperatures on Quality Degradation of Extra Virgin Olive Oil from Olives Grown in Palestine

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Abstract The quality of extra virgin olive oil (EVOO) is intimately affected by packaging material and storage temperature. In this study, the influence of packaging materials and elevated temperature on EVOO quality was investigated during six months. At ambient temperatures, oil maintained EVOO when stored in glass, polyethylene terephthalate (PET), high density polyethylene (HDPE), cans and Pottery in terms of chemical tests (acidity, peroxide value, K_{232} , and K_{270}). Loss of phenols was the highest in pottery-stored oil and the lowest was found in glass-stored oil. Only PET-stored oil maintained the EVOO grade in terms of sensory evaluation when stored at room temperature. At elevated temperature, oil stored in all packaging materials lost extra virgin quality in terms of chemical tests. The loss of phenols was the largest in HDPE and smallest in cans-stored oil. Sensory evaluation, maintained glass-stored oil and PET-stored oil as EVOO. This study has reaffirmed that at both storage temperatures, the best container in maintaining the EVOO quality was glass and the worst was pottery. Grading of stored olive oil under investigation using sensory evaluation solely was not sufficient. Also it was clear that the absorption coefficient K_{270} was the most sensitive determinant chemical test that determines the quality of stored olive oil and could be used as a rapid indicator test.

Keywords: *Olea europaea L.*, olive oil, oil oxidation, stability indicators, storage conditions, packaging materials

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1. Introduction

Olive trees (*Olea europaea L.*) is an important trees internationally, produce high nutritional and health quality edible oil. The global production of olive oil in 2012 was around 2,903,680 tons, from which around 22,950 tons are produced in Palestine. As olive oil production fluctuates from year to year, the mean annual production of olive oil globally during the recent ten years (2003-2012) was 2,946,288 tons and the average annual contribution in Palestine was 17,045 tons [1]. The European Union (EU) is the leading producer of olive oil and within the EU, the Mediterranean members are the biggest producers, accounting for 95% of world production and 85% of world consumption of olive oil [2].

Virgin and extra virgin olive oil is a genuine fruit juice obtained from olive drupes, using exclusively mechanical procedures, without further treatments or chemical additions. Several clinical data have shown that consumption of olive oil can provide heart health benefits,

such as favorable effects on cholesterol regulation and LDL cholesterol oxidation, exerting anti-inflammatory, antithrombotic and antihypertensive effects [3]. Quality of olive oil is defined as the combination of its attributes that have significance in determining the degree of its acceptability by the consumer, and may be also defined from commercial, nutritional or organoleptic perspectives. The nutritional value of extra virgin olive oil (EVOO) originates from its high levels of oleic acid content and minor components, such as phenolic compounds that donate the oil its aroma [4]. Therefore, these quality parameters promote the consumption demands and price of olive oil in comparison with other edible oils ranking it superior among vegetable oils [5].

There is a need to develop reliable analytical methods to ensure compliance of olive oil quality with labeling, and to determine the genuineness of the product by the detection of eventual defects during adulterations, processing and storage conditions. Therefore, the International Olive Oil Council (IOOC) and European Communities Legislation (EC) define the identity characteristics of olive oil by specifying analytical methods and standard limit

values of the quality parameters such as peroxide value (PV), acidity, Ultra violet (UV) absorbance values (K_{232} and K_{270}) and organoleptic characteristics (odor, taste and color) for olive oils in order to improve product quality, expand international trade, and raise its consumption. The chemical tests and the organoleptic properties categorize olive oil into extra virgin, virgin, and lampant oil indicating its edible quality and marketable values. The extra virgin olive oil is the highest grade and must contain zero defects and greater than zero positive attributes as evaluated by a certified taste panel, and must have a free acidity of less than 0.8%, peroxide value doesn't exceed 20 milliequivalent $O_2 \text{ kg}^{-1}$ oil and should have clear flavor that reflect the fruit from which it is produced [6,7].

Quality of olive oil is potentially affected by different factors including genetic (tree variety), agronomic (ripening stage, fertilization, irrigation, and harvesting practices), health of the drupe [8], environmental (temperature, day length, and sunlight duration), geographical [9] factors, and finally the postharvest processing including packaging materials and storage conditions [6]. Furthermore, an important European regulation allows the Protected Denomination of Origin (PDO) labeling of some EU EVOOs and this designation guarantees that the geographical origin of the product is closely in conjunction with the quality of the product [11]. The complex interference of these factors make only 50% of the world's olive oil production is classified as extra virgin grade [12].

In order to fulfill the expectations of consumers, good quality control of olive oil should be assured in the course of production and storage line. The quality of olive oil decreases during storage, and is attributable to oxidation that lead to rancidity [5], and to hydrolytic degradations causing partial loss of healthy minor constituents [13]. Preserving the positive attributes of oil is a matter of great concern for the olive oil industry during the time elapsing from production to bottling, and up to purchasing and consumption [14,15], because the variation of storage conditions during olive oil storage and transportation affect its quality [8,16]. During shelf life of bottled extra virgin olive oil, the bottle must be adequately protective against autoxidation that cause rancidity [7]. Several types of plastic films or metal containers can be used, but glass bottles of different shape and color are the most common [14,17]. 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 oxidation [16]. Accordingly, oil producers need to pay a great deal of attention to the type of containers they place the oils in, 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 [17], while little information is known about the effect of high density polyethylene (HDPE), cans, and pottery jars. The effect of different packaging materials on the quality of olive oil is previously reported [7,14,17]. In the other hand, 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 illegally differ from those indicated on the label. Thus, an investigation of the type and magnitude of the

alterations in oil undergoes during its shelf life at elevated temperature may provide useful information about optimum practical storage or transport conditions that sustain high quality of olive oil for maximum storage period [7].

Although the effect of storage conditions, time and their consequences were studied for olive oils produced in many countries [9,18], there is no published studies - to our knowledge- corresponding to the effect of packaging materials and storage conditions on the quality of Palestinian olive oils except a recent investigation done by the research group of this investigation under different situations [15]. Therefore, the aim of this study is to evaluate different packaging materials (Glass bottles, PET plastic bottles, HDPE plastic bottles, tin plates, and pottery jars) in terms of their protective ability for quality indices of Palestinian extra virgin olive oil (acidity, peroxide value, K_{232} , K_{270} , phenolic compounds, sensory score 6.5) stored under different storage temperatures (18°C and 37°C) in a six months stability study. Additionally it is aimed to find the potential correlations between chemical quality indices with sensory evaluation test to optimize olive oil evaluation.

2. Materials and Methods

2.1. Experimental Design

Olive fruits of the cultivar 'Nabali Baladi' were handpicked in late October 2008 from an olives orchard located in Salfet district of a Mediterranean climatic region of Palestine. The fruits were selected with no defects and at an optimal stage of ripening (5.5 N detachment force, 3.8 pigmentation index, and 57.5% water content). Washed olives were processed using stone mill and hydraulic press. The initial whole oil sample was filled temporarily in two 20-liter HDPE containers and directly transported to the laboratories of Al-Quds University. Extra virgin quality of the extracted oil was proved (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, Table 1). The 40 liters extra virgin olive oil was distributed into subsamples (300-ml each) that were bottled in different packaging materials (amber glass bottles, polyethylene terephthalate (PET), high density polyethylene (HDPE), tin plate cans hermetically sealed, and pottery jars with covers), maintaining 2% head space in each bottle. Bottled oil was stored under different storage temperatures ($18 \pm 1^\circ\text{C}$ and $37 \pm 1^\circ\text{C}$); in thermostatic and ventilated incubators (with 100 Lux normal white light inside for around 10 hours daily simulating the condition on shelves). The samples were rearranged weakly to insure uniform spacial distribution of the bottles. The bottles (in four 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 temperature storage conditions) on the stability of the 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, ultraviolet extinction coefficients (K_{232} and K_{270}), total phenolic contents (expressed as mg of

gallic acid kg^{-1} oil), and sensory attributes (Panel test) during six months of the experimental period (0, 30, 60, 90, and 180 days of storage).

Table 1. Quality of olive oil sample initially used in the study

Quality parameter	Value	Unit
Acidity	0.38	g oleic acid per 100 g oil (%)
Peroxide value	10.49	equivalent O_2 per kg oil
Iodine value	82.63	ml I_2 per 100g oil
Saponification value	188	mg KOH per g oil
K_{232}	1.68	absorbance
K_{270}	0.158	absorbance
Density	0.919	g per ml oil
Refractive index	1.46675	-
Sensorial evaluation	0 defect, 4.7 fruity, 5 pungency, 4.5 bitterness	

2.2. Determination of Oil Quality Indicators

Acidity (g oleic acid 100 g^{-1} oil) and peroxide value (milliequivalent O_2 kg^{-1} oil) were determined according to the AOAC [19]. Ultraviolet light absorption indexes (K_{232} and K_{270} extinction coefficients) were determined using the methods described in IOOC [20]. Total Phenol compounds were extracted according to Georgios et al, 2006 [21] and analyzed according to AOAC [19], and their content (mg gallic acid kg^{-1} oil) was determined spectrophotometrically at 765 nm. Sensory evaluation was run by taster team for sensory analyses 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 [20]. The results obtained based on the ranking according to the median of notes from the tasters. Each bottle in each treatment was analyzed monthly for each mentioned chemical quality indicator up to six months. The sensory evaluation was inspected in three periods (0, 3, and 6 months).

2.3. Statistical Analysis

Four bottles of each treatment were independently analyzed in each sampling time. The results are expressed as mean \pm 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%.

Initially Pearson correlations were calculated to test the relation among quality indicators of stored olive oil at each storage condition separately and when data were pooled. The NOMISS option was used in order to obtain results consistent with subsequent multiple regression studies.

3. Results

3.1. Acidity

Our findings reveal that, acidity of EVOO increased dramatically with increasing storage time in all studied storage containers stored at elevated temperatures, except for that stored in pottery jars, where the highest acidity value was reached after 90 days, then was significantly reduced after 135 and 180 days of storage (Table 2). After 30 days of storage, glass containers retain the highest acidity values followed by pottery followed by cans followed by PET and the least was found in HDPE but the values were statistically not significant in both types of plastic containers. At the end of storage time, only glass and cans exceeded the limit for the extra virgin grade (0.8 % oleic acid), where they shared the highest acidity values in stored oil (0.81 and 0.82 for glass and cans respectively). At the end of storage period, the least acidity value was found in oil stored in pottery, while both types of plastic containers retained the same intermediate acidity values.

At room temperature, acidity of stored EVOO increased slightly but significantly with increasing time of storage. At the end of storage period, the least acidity value was reported in oil stored in pottery, while the other containers maintained similar values significantly. All storage containers protected stored EVOO in terms of acidity and maintained its extra virgin grade throughout storage period. Comparing the acidity in the same container type at the same storage time but different temperature treatments, acidity was higher under elevated temperature in all packaging materials.

Table 2. Acidity (% as oleic acid) at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	0.38 E, a (0.38 C), a	0.38 D, a (0.38 C), a	0.38 D, a (0.38 E), a	0.38 E, a (0.38 D), a	0.38 C, a (0.38 D), a
30	0.58* CD, a (0.42 CB), a	0.44 C, c (0.42 B), a	0.41* C, c (0.54 B), a	0.46* D, cb (0.40 C), a	0.50* B, b (0.39 C), a
45	0.57* D, a (0.42 CB), c	0.47* C, cb (0.42 B), c	0.50 B, b (0.50 C), a	0.59* C, a (0.47 CB), b	0.41 C, c (0.42 BC), c
90	0.63* C, b (0.43 B), c	0.53* B, c (0.49 A), ba	0.50 B, c (0.50 C), a	0.71* B, a (0.48 B), cb	0.53* A, c (0.45 CBA), ba
135	0.71* B, a (0.52 A), ba	0.54* B, b (0.50 A), b	0.57* A, b (0.43 D), a	0.73* B, a (0.53 BA), a	0.49 B, c (0.50 BA), b
180	0.81* A, a (0.53 A), a	0.58* A, b (0.51 A), a	0.57* A, b (0.58 A), a	0.82* A, a (0.56 A), a	0.49* B, c (0.51 A), b

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.2. Peroxide Value

Our results highlighted that, PV of stored EVOO at elevated temperature showed different responses in different packaging materials as a function of storage time (Table 3). It fluctuated in glass containers; where it decreased significantly after 30 days of storage in dramatic manner till 90 days of storage, then it increased and out-yielded the initial value but without significant difference. PV decreased drastically with time of storage in oil stored in both types of plastic containers, while increased in oil stored in pottery continuously with time of storage and overcame the limit of EVOO grade (20 milliequivalent O₂ kg⁻¹ oil) before 135 days of storage. All

storage containers except pottery retained the EVOO quality in terms of peroxide value during the experiment when stored at elevated temperature.

At ambient temperature, PV decreased significantly with time in oil stored in glass, PET and cans, while it didn't change significantly in oil stored in HDPE, and it was significantly elevated in oil stored in pottery. At the end of storage time, PV of oil stored at elevated temperature was found significantly higher than that stored at room temperature in glass and pottery, while the opposite was recorded for oil stored in cans. In the other hand, both types of plastic containers maintained peroxide values similar at both storage conditions.

Table 3. Peroxide value (as milliequivalent O₂ kg⁻¹ oil) at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	10.50 A, a (10.50 A), a	10.50 A, a (10.50 A), a	10.50 A, a (10.50 A), a	10.50 A, a (10.50 A), a	10.50 C, a (10.50 C), a
30	10.50 A, (9.90 A), a	8.10 A, a (8.27 B), a	10.87 A, a (8.70 A), a	10.53* A, a (8.20 B), a	10.8 7* C, a (8.37 D), a
45	7.87* B, cb (7.37 B), b	9.03* BA, cb (8.50 B), a	9.50* A, ba (8.63 A), a	7.30 B, c (8.37 B), a	11.10* C, a (8.13 D), a
90	6.23* C, d (8.23 B), b	8.13* B, c (8.63 B), b	8.83 A, b (9.37 A), b	5.90* B, d (8.17 B), b	16.53* B, a (11.63 CB), a
135	6.73* B, b (8.37 B), c	8.3 B, b (8.67 B), c	9.77* A, b (9.23 A), b	6.20* B, b (8.87 B), cb	22.73* A, a (12.57 B), a
180	10.87* A, b (8.43 B), c	8.43 B, c (8.87 B), c	9.77 A, b (10.03 A), b	6.17* B, d (7.70 B), d	21.83* A, a (14.10 A), a

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.3. Ultraviolet Extinction Coefficients

3.3.1. Extinction Coefficient at 232 nm (K₂₃₂)

The extinction coefficient K₂₃₂ of olive oil stored in HDPE at elevated temperature under study, increased continuously and significantly with extending time of storage (Table 4). The same response was recorded for oil stored in pottery in the first 135 days but this extinction coefficient was slightly and significantly decreased after 180 days compared to the previous measurement. In glass bottles, K₂₃₂ fluctuated during storage, where it decreased significantly after 45 days, then reached its peak after 135 days, where it was significantly higher than the initial measurement, and at the end of the experiment went back to a value similar to the initial one. In PET, K₂₃₂ showed a trend of increment during the experiment with a higher significant value at the end of the experiment compared with the baseline measurement. This quality index of oil stored in cans fluctuated during storage period; where the initial and final measurements were statistically similar. The extra virgin grade in terms of K₂₃₂ (<2.5) was

maintained in oil stored in glass, PET and cans even though they were stored for six months at elevated temperature. But oil stored in HDPE quitted this grade in terms of this quality index at the end of the experiment and that stored in pottery, exceeded 2.5 after 135 days and was marginal to the critical limit at the end of the storage period.

At ambient storage temperature, the extinction coefficient K₂₃₂, decreased slightly but significantly within the respective testing dates in oil samples stored in all packaging materials under study except for pottery jars, where a significant increase was reported after 90 days in pottery and the rate of increase was maintained till the end of the experiment. None of the samples stored in either packaging material at ambient temperature exceeded the higher limit of K₂₃₂ determining extra virgin quality of olive oil. Values of K₂₃₂ measured at each testing time for each packaging material was found significantly higher in oil stored at elevated temperature compared to oil stored at ambient temperature, and this was true for all packages under study.

Table 4. K₂₃₂ at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	2.02 B, a (2.02 A), a	2.02 C, a (2.02 A), a	2.02 D, a (2.02 BA), a	2.02 BC, a (2.02 A), a	2.02 E, a (2.02 D), a
30	2.04* B, b (1.77 D), b	2.03 C, b (2.03 A), a	2.09* D, ba (2.04 BA), a	2.15* A, a (2.02 A), a	2.09* D, ba (2.03 D), a
45	1.90* C, c (1.74 D), c	2.31* A, ba (2.01 BA), ba	2.26* C, b (2.08 A), a	1.91 D, c (1.84 B), cb	2.40* C, a (2.05 D), a
90	1.92* C, e (1.85 C), c	2.16* B, c (2.02 A), b	2.33* C, b (1.75 C), d	1.94* DC, d (1.88 B), c	2.37* C, a (2.23 B), a
135	2.34* A, c (1.90 CB), c	2.17* B, d (1.98 CB), b	2.49* B, b (1.95 BA), b	1.92 D, e (1.96 A), b	2.62* A, a (2.10 C), a
180	2.04* B, e (1.96 BA), b	2.31* A, c (1.96 C), b	2.60* A, a (1.87 CB), c	2.09* BA, d (1.83 B), c	2.48* B, b (2.36 A), a

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.3.2. Extinction Coefficients at 270 nm (K_{270})

Extinction coefficient measured at 270 nm (K_{270}) of stored olive oil at elevated temperature increased progressively in significant values with increasing time of storage in all studied packaging materials under study (Table 5). At the end of the experiment, the highest K_{270} value was found in oil stored in HDPE, followed by pottery without significant difference, followed by PET, followed by cans, and the least value was recorded in oil stored in glass bottles. All storage containers deteriorate stored olive oil and quitted from extra virgin grade in terms of K_{270} (< 0.2) when oil was stored at elevated temperature but at different storage periods. PET bottles retained stored oil as extra virgin in terms of K_{270} for less

than 135 days, and that stored in glass and cans for less than 90 days, and for that stored in HDPE and pottery for less than 45 days.

At ambient temperature, K_{270} was slightly increased in oil stored in glass, PET, and pottery, while it was not affected in oil stored in cans, but was significantly decreased in oil stored in HDPE. None of packaging materials under investigation elevated K_{270} of stored olive oil to the critical limit of extra virgin grade when oil stored at ambient temperature for six months. K_{270} values of oil stored at elevated temperature was higher than that stored at room temperature in all packaging materials under study in most storage periods.

Table 5. K_{270} at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	0.160 D, a (0.160 B), a	0.160 E, a (0.160 B), a	0.160 F, a (0.160 CB), a	0.160 D, a (0.160 A), a	0.160 D, a (0.160 C), a
30	0.187 CB, a (0.180 A), ba	0.193 DC, a (0.197 A), a	0.200* E, a (0.180 BA), ba	0.187* C, a (0.160 A), c	0.160* D, b (0.173 CB), cb
45	0.180* C, c (0.163 B), a	0.230* B, a (0.190 A), a	0.217* D, b (0.203 A), a	0.183* CB, c (0.163 A), a	0.213* C, b (0.197 A), a
90	0.203* BA, dc (0.160 B), bc	0.190* D, d (0.187 A), a	0.230* C, a (0.147 C), c	0.210* A, cb (0.167 A), ba	0.220* C, ba (0.187 BA), a
135	0.210* A, b (0.160 B), b	0.207* C, b (0.180 B), a	0.263* B, a (0.153 C), b	0.210* A, b (0.170 A), ba	0.247* B, a (0.167 C), ba
180	0.197 BCA, c (0.180 A), a	0.260* A, b (0.187 A), a	0.290* A, a (0.137 C), b	0.200* BA, c (0.170 A), a	0.280* A, a (0.190 BA), a

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.4. Total Phenolic Compounds

Storage at elevated temperature significantly reduced total phenolic compounds of EVOO stored in all packaging materials under study (Table 6). Total phenols were significantly and highly reduced at all consequent storage periods in oil stored in PET, HDPE, and pottery, while in glass and cans, the successive reduction of phenolic compounds were reported until 135 days of storage but were significantly elevated at the end of storage period. Comparing phenolic compounds contents of stored olive oil at the end of storage period related to

their initial contents in the same packaging material, the most reduced contents of phenolic compounds was found in HDPE followed by pottery followed by PET followed by glass and the least was recorded in oil stored in cans.

At room temperature storage condition, phenolic compounds were dramatically and significantly reduced with consecutive increase of storage period. At the end of storage period, the largest loss of phenolic compounds was found in pottery followed by HDPE, followed by cans and PET, and the least reduction of phenolic contents was recorded in glass.

Table 6. Total phenols at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	213.3 A, a (213.3 A), a	213.3 A, a (213.3 A), a			
30	194.3* B, b (203.0 B), b	213.7 A, a (206.3 BA), b	196.7* CB, b (207.7 B), ba	190.0* CB, bc (214.3 A), a	185.3 B, c (185.3 B), c
45	188.7* C, c (201.3 B), b	212.7* A, a (202.33 CB), b	197.7* B, b (201.7 C), b	184.7* DC, cd (213.7 A), a	182.7* B, d (178.6 CB), c
90	182.7* D, c (200.3 CB), a	202.7* B, a (199.7 CB), a	188.0* DC, b (183.3 E), bc	181.7 DC, c (195.3 B), ba	143.7* C, d (170.0 DC), c
135	180.3* D, b (196.3 DC), a	197.0* B, a (195.0 C), ba	183.0* D, b (191.7 D), b	179.3* D, b (194.7 B), ba	140.7* C, c (161.3 D), c
180	191.7* CB, ba (196.0 D), a	182.7* C, c (184.6 D), b	123.3* E, b (167.3 F), c	196.7* B, a (184.3 B), b	133.0* D, c (161.0 D), d
Reduction [‡] (%)	10.1 (8.1)	14.3 (13.5)	42.2 (21.6)	7.8 (13.6)	37.6 (24.5)

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

[‡]Total reduction of phenolic compounds at the end of storage period based on the initial contents of total phenols.

Total phenols contents in oil stored in all packaging materials under study was found less in oil stored at elevated temperature than that stored at ambient temperature when compared in the same packaging material and the same testing date after all consecutive storage periods except for oil stored in cans at the end of the storage period where the opposite was recorded.

3.5. Sensory Evaluations

Sensory evaluation was done for all samples subjected to storage conditions in three periods (before storage, after three months, and after six months of storage). Sensory evaluation (Table 7) reveals that, oil stored in glass

sustained the extra virgin grade under elevated temperature throughout the experiment, and also at room temperature till 90 days and then turned to virgin grade because of the appearance of sensory defects (Figure 1). The fruity of the glass-stored oil at both storage conditions decreased consequently with increasing time of storage. Caned oil responded the same at both storage conditions, and lost the extra virgin grade before 90 days of storage then remained in the virgin grade throughout the experiment, because fruity of oil was lost and sensory

defects appeared during storage. Oil stored in PET sustained the extra virgin grade for six months without sensory defects but with marginal loss in fruity. In HDPE, oil at both storage conditions became virgin after 90 days. Oil fruity decreased largely before 90 days of storage but the sensory defects appeared at the end of the experiment. Because of the complete loss of oil fruity, and the appearance of high level of sensory defects (>2.5), oil stored in pottery quitted from the virgin grade at both storage conditions before 90 days of storage.

Table 7. Olive oil grading according to the sensory evaluation for oil samples stored at elevated temperatures compared to room temperature bottled in different packaging materials

Container	days	Sensory Defects		Sensory Fruity		Olive oil grade	
		Elevated T	Room T	Elevated T	Room T	Elevated T	Room T
Glass	0	0.0	0.0	4.9	4.9	EVOO	EVOO
	90	0.0	0.0	1.9	2.0	EVOO	EVOO
	180	0.0	0.8	1.0	1.0	EVOO	VOO
Can	0	0.0	0.0	4.9	4.9	EVOO	EVOO
	90	1.5	1.5	0.0	0.0	VOO	VOO
	180	2.3	2.3	0.5	0.5	VOO	VOO
PET	0	0.0	0.0	4.9	4.9	EVOO	EVOO
	90	0.0	0.0	3.0	3.0	EVOO	EVOO
	180	0.0	0.0	2.6	2.6	EVOO	EVOO
HDPE	0	0.0	0.0	4.9	4.9	EVOO	EVOO
	90	0.0	0.0	1.2	1.2	EVOO	EVOO
	180	1.9	1.9	1.3	1.3	VOO	VOO
Pottery	0	0.0	0.0	4.9	4.9	EVOO	EVOO
	90	3.0	3.0	0.0	0.0	OVOO	OVOO
	180	3.5	3.5	0.0	0.0	OVOO	OVOO

EVOO is extra virgin olive oil, VOO is virgin olive oil, OVOO is ordinary virgin olive oil.

Table 8. Olive oil grading according to the sensory evaluation and other stability indices for oil samples stored at different temperatures bottled in different packaging materials

Container	days	Acidity	PV	K ₂₃₂	K ₂₇₀	Sensory evaluation		Olive oil grade
						Defect	Fruity	
Elevated temperature								
Glass	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.63	6.23	1.92	0.20	0.0	1.9	EVOO
	180	0.81	10.88	2.04	0.19	0.0	1.0	VOO
Can	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.71	7.30	1.94	0.21	1.5	0.0	VOO
	180	0.82	6.21	2.09	0.20	2.3	0.5	VOO
PET	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.53	8.13	2.16	0.19	0.0	3.0	EVOO
	180	0.58	8.44	2.31	0.26	0.0	2.6	OVOO
HDPE	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.50	8.82	2.33	0.23	0.0	1.2	VOO
	180	0.57	9.77	2.60	0.29	1.9	1.3	OVOO
Pottery	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.53	16.53	2.37	0.22	3.0	0.0	OVOO
	180	0.50	21.81	2.48	0.28	3.5	0.0	OVOO
Room temperature								
Glass	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.51	8.42	1.82	0.22	0.0	2.0	EVOO
	180	0.66	8.23	2.07	0.27	0.8	1.0	VOO
Can	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.47	8.17	1.88	0.17	1.5	0.0	VOO
	180	0.56	7.72	1.83	0.17	2.3	0.5	VOO
PET	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.43	7.99	2.03	0.24	0.0	3.0	VOO
	180	0.52	8.55	1.85	0.23	0.0	2.6	VOO
HDPE	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.49	9.42	1.73	0.18	0.0	1.2	EVOO
	180	0.56	10.84	1.80	0.21	1.9	1.3	VOO
Pottery	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
	90	0.51	11.63	2.23	0.19	3.0	0.0	OVOO
	180	0.42	14.12	2.36	0.19	3.5	0.0	OVOO

EVOO is extra virgin olive oil, VOO is virgin olive oil, OVOO is ordinary virgin olive oil.

3.6. Pearson Correlation with Oil Quality Parameters as Affected with Temperature Treatments

Pearson correlations between quality parameters of olive oil stored at room temperature (Table 9) show that peroxide value was positively and significantly correlated with K_{232} extinction coefficient but the correlation with phenolic contents was significantly negative, and also insignificantly correlated with K_{270} . K_{232} was significantly and positively correlated with K_{270} but was significantly and negatively correlated with phenolic contents. There was no significant correlation found between K_{270} and phenolic contents. At elevated temperatures, peroxide value was significantly and positively correlated with both extinction coefficients, and was also significantly and negatively correlated with phenolic contents. K_{232} was highly correlated with both K_{270} and phenolic compounds content but the correlation with the formers was positive while the correlation with the later was negative. K_{270} and phenolic contents was highly negatively correlated with each other. Pearson correlation between quality parameters of olive oil stored at both room and elevated temperature when all data was pooled (Table 10) shows that peroxide value was highly positively and significantly correlated with K_{232} . K_{270} was significantly and positively correlated with peroxide value and K_{232} . Phenolic contents showed highly negative and significant correlation with all quality parameters under study (peroxide value and K_{232} , and K_{270}).

Table 9. Pearson coefficients between quality parameters of oil stored at room temperature (above the diagonal) and at elevated temperature (below the diagonal)

	Peroxide	K_{232}	K_{270}	Phenols
Peroxide	-	0.608***	-0.055	-0.372***
K_{232}	0.559***	-	0.458***	-0.251*
K_{270}	0.311**	0.789***	-	-0.103
Phenols	-0.530***	-0.643***	-0.734***	-

Table 10. Pearson coefficients between quality parameters of olive oil stored at both room temperature and elevated temperature (pooled data)

	K_{232}	K_{270}	Phenols
Peroxide	0.550***	0.285***	-0.499***
K_{232}		0.779***	-0.546***
K_{270}			-0.584***

4. Discussion

The value of EVOO that determines its commercial and health quality originates from its high oleic acid contents and the presence phenolic compounds that donates it the special aroma and antioxidant activity [12,22]. Olive oil quality and stability are principally affected by lipid oxidation, generating off-flavor (rancidity) and reduction in oil nutritional value causing health risks and even toxicity for consumers. Lipid peroxidation produces toxic compounds which causes lung damage. In addition to this effect, reactions between peroxidized lipids and proteins have been shown to cause loss of enzyme activities, polymerization, accelerated formation of brown pigments and the destruction of essential amino acids such as histidine, lysine, tryptophan

and methionine. Aldehydes, ketones, hydrocarbons and furans, are known as the cleavage products of hydroperoxides, cause reduction in protein solubility, and reduction in nutritional value of proteins. As well, lipid oxidation provokes a decrease in nutritional values of some vitamins such as A, D, E and K. From the health point of view, lipid radicals and oxidation products contribute in aging, DNA damage, Parkinsonism, carcinogenesis, and coronary heart diseases [23].

As lipids oxidize, they form hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products, such as aldehydes, ketones, acids, and alcohols. In many cases, these compounds adversely affect flavor, aroma, taste, nutritional value, and overall quality. The oxidation process of triglycerides is complex because it always takes place by chain reactions either in dark involving free radicals, called autoxidation, or light-dependent reactions known as photooxidation [22]. Many catalytic systems such as light, temperature, enzymes (lipase), metals, and microorganisms, can accelerate lipids oxidation [24]. Variation during olive oil storage and transportation that enhance lipid oxidation is common, and may be attributed to natural or climatic condition and to extreme storage conditions [8,18]. In addition to storage conditions, the retention of oil quality for an extended period of time that allows its worldwide distribution is also highly affected by the type of packaging material [25]. Knowledge about packaging materials, and their interactions with the bottled oil, along with a deeper understanding of the oxidation pathways under various storage conditions provide necessary information for improving the quality of packaged olive oil during shelf life and transportation [17].

Therefore, in order to fulfill the consumer's requirements, good quality control of olive oil should be assured in the course of production and storage processes. The quality of olive oils is interpreted in terms of measurements of analytical parameters for which certain limit values are set. The most important quality requirements of olive oil in commercial transactions are: acidity, peroxide value, K_{232} , K_{270} , and total phenolic content in addition to the sensory evaluation. These parameters have been evaluated for the Palestinian olive oil samples under investigation as stability-indicators in terms of storage time in response to different packaging materials and storage temperatures.

4.1. Acidity

Acidity is mainly determined by titration using potassium hydroxide that measure the amount of free fatty acids (FFA's) present in the oil as oleic acid which is the major component in the triglycerides present in the olive oil, and should be less than 0.8% if the oil is extra virgin [26]. Although, acidity values are used as a basic criterion for classifying different categories of olive oil, it was not considered as the best criterion for evaluating olive oil quality by some investigators [27]. Acidity reflects oil stability and susceptibility to rancidity. The hydrolytic rancidity of oil due to presence of water and the catalytic action of the lipase (often derived by microorganisms) in oil as mentioned above, partially degrade triglycerides giving glycerol and free fatty acids, which increase acidity.

In agreement with our findings, acidity of EVOO stored in glass increased with increasing storage time but didn't

exceed the limits during storage at room temperature [28,29], but exceeded the limit at elevated temperatures [28,30]. While in contrary to our results, other reporters [30,31] found that acidity of oil stored in glass didn't change significantly during storage at room temperature. Several studies conducted on olive oil shelf life attested the glass as the best material for the storage [32], in terms of its acidity, especially when oil was stored in the dark with respect to other packages [33]. As acidity values of oil stored at room temperature in glass, PET, HDPE, and cans didn't differ significantly in our experiment, other investigators clearly indicated the glass as the best (less value) in terms of acidity in the following ranking Glass > HDPE > PET [34]. Metal containers have the same water resistant properties as glass and may protect the product from oxygen, light, and microorganisms that could increase the acidity of oil through increasing the rate of hydrolysis of triglycerides. But when oil was stored at elevated temperature, our results reported both glass and cans as the worst packaging materials in terms of acidity of stored oil which exceeded the extra virgin grade limit, while plastic materials (PET and HDPE) were found better and pottery was reported as the best. This can be explained by the high thermal conductivity of glass and cans compared to plastic ones, and for the cooling effect of pottery on stored oil.

4.2. Peroxide Value

Peroxide value (PV), a measure of total peroxides in olive oil (meq. O_2 kg^{-1} oil) is a major guide of oil quality. The official determination method is based on the titration of iodine liberated from potassium iodide by peroxides present in the oil. In other words, the peroxide value is a measure of the active oxygen bound by the oil which reflects the hydroperoxide value, and measures the degree of lipid peroxidation. The higher the number means the greater degradation due to oxidation with an upper limit of 20 meq. O_2 kg^{-1} oil, but levels higher than 10 may mean less stable oil with a shorter shelf life [35]. In lipid oxidation reactions, many free radicals and oxygen species, such as singlet oxygen are involved. The main substrates for these reactions are unsaturated fatty acids and oxygen. The free radical mechanism of lipid oxidation is usually described in a three stages chain reaction including initiation, propagation, and termination steps. Initiation starts with the abstraction of a hydrogen atom adjacent to a double bond in a fatty acid molecule, by the catalytic effect of light, heat, or metal ions to form a free radical, where direct reaction of fatty acid molecule with oxygen does not take place frequently, because of the high activation energy. The resultant free radical reacts with atmospheric oxygen to form an unstable peroxy free radical may in turn abstract a hydrogen atom from another unsaturated fatty acid to form a hydroperoxide. A new alkyl free radical initiates further oxidation and contributes to the chain reaction, and this chain reaction is called propagation stage of autoxidation. The chain reaction may be terminated by formation of nonradical products resulting from combination of two radical species. The propagation stage in autoxidation process includes an induction period when hydroperoxides formation is minimal. The rate of oxidation of fatty acids increases in relation to their degree of unsaturation,

therefore, oils that contain high proportions of polyunsaturated fatty acids may experience instability problems. The breakdown products of hydroperoxides, such as alcohols, aldehydes, ketones, furans, esters, lactones and hydrocarbons, generally cause off-flavors, and may also interact with other food components and change their functional and nutritional properties [36].

In accordance with our results, other investigators [5,30,37] found that PV of oils bottled in glass and PET stored at room temperature fluctuated during storage time and did not exceed the official limit during six months of storage. In the other hand, a linear increase in PV with storage time at room temperature in oil stored in glass bottles [9,10] and in tin plates [38] was reported. In the same line with our results in oil stored in glass, PET, and cans at room temperature and elevated temperature, PV decreased significantly with increasing storage time [7]. But in contrast with our findings except for oil stored in pottery, other scientists [22] reported an increase in the PV of oil samples stored under elevated temperature. In accordance with our results, fluctuation in the PV of oil samples stored at elevated temperature [10], and at shelf [15] was reported. The decrease in the PV with increasing time in many testing dates observed in our results in different packaging materials and at both storage temperatures, can be explained by the degradation of primary oxidation products (peroxides) to form secondary oxidation products which can be detected by K_{232} values. The results of PV was correlated with that obtained by K_{232} (Table 9, Table 10) and agreed with other reporters [7,28,39]. 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 temperature which 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 cans and glass containers and less in those packed in plastic and pottery. The oil samples packed in pottery and stored at both room and elevated temperatures have higher peroxide values compared to those stored in other containers. These results may point to the probable intrusion of oxygen and water through pottery, although it is impermeable to light with low thermal conductivity that retain primary oxidation products for longer time and delay their destruction to produce secondary oxidation products.

4.3 Ultraviolet Extinction Coefficients

Determination of the absorption coefficients in the ultraviolet region (232 nm and 270 nm) reflects the stage of oxidation for olive oil during storage [40], in which the shelf-life of virgin olive oil is determined by the increase in the K_{232} absorption coefficient [41], or by means of the time required to reach the upper legal limit of K_{270} absorption coefficient [7, 42]. Primary oxidation products in olive oil (fatty acid hydroperoxides and oxidized triacylglycerols) are measured as peroxide value and K_{232} absorption coefficient (measure the conjugated dienes), while secondary oxidation products (aldehydes, alcohols, ketones and hydrocarbons) are detected by K_{270} absorption coefficient [40,42]. Hydroperoxides are the initial

products of oxidation -very sensitive and comparatively unstable- and used as indicator of the early stages of oxidative deterioration in the oxidation process [17,43], 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 [42].

For instance, an increase in K_{232} and K_{270} values is very common between extraction of olive oil and its consumption as affected by storage time and conditions [22]. It is documented that heat affects olive oil quality by increasing the trienes formation, measured by K_{270} [9], more than the dienes measured as K_{232} [5]. In agreement with our findings, K_{270} values were affected by the heat exposure conditions more than that of K_{232} , with higher values reported in the samples stored at elevated temperature than in those kept at ambient temperature [44]. Such a response is due to the degradation of primary oxidation products (peroxides) to form secondary oxidation products, as K_{232} representing the amount of conjugated dienes of the primary oxidation products [7,28,39] and are transferred to trienes measured by K_{270} [45].

Our findings are in agreement with previously reported results [37] which found that K_{270} of oil stored in glass bottles and PET containers at elevated temperatures, exceeded the limit of extra virgin grade after two and three months of storage for glass and PET respectively. Also in the same line with our results, other investigators [30] reported an increase in K_{270} of oil samples stored in glass and PET at room temperature throughout the storage, but in contrary with our findings, they found that K_{270} values exceeded the limit (0.2) after two months of storage. The increase in K_{232} with increasing time of oil -in contrary with our findings- was reported [30,37] when oil bottled in glass and PET container stored at room temperature but the values did not exceed the official limit, and values in glass overcame that in PET. Because of the significant variation of K_{270} values during olive oil storage as a response to oil oxidation, this parameter may be of capital importance to control the quality of stored extra virgin olive oils in terms of determining the time at which they will lose their "extra" category [7].

4.4. Total Phenolic Compounds

Extra virgin olive oil, is one of the few oils being consumed without any chemical treatment. It has high resistance to oxidative deterioration mainly due to its fatty acid composition -high monounsaturated to polyunsaturated ratio- and to the presence of natural antioxidants, especially phenolic compounds, carotenoids, and tocopherols, therefore delay the oxidation of lipids and the production of the undesirable volatile compounds [8,22]. During oil storage, the hydrolysis, esterification and oxidation deplete the minor constituents, because of the action of phenolic compounds as antioxidants mainly at the initial stage of autoxidation [46] by scavenging free radicals and chelating metals. Accordingly, the determination of the minor constituents in olive oil is essential for the analytical assessment of its quality and self protection potential.

In agreement with previous reports [7,28,47], our findings showed that total polyphenolic contents of extra virgin olive oil under investigation decreased during

storage in all means of packaging materials and storage conditions (Table 6); due to degradation of these compounds that was well fitted to first order kinetics. At the end of storage period, the phenolic compounds of samples stored at elevated temperature showed significantly higher reduction than those stored at ambient temperature [5,17,30,32] in all types of packaging materials except those were stored in cans. Some reporters [30] found that total phenols of oils bottled in glass and PET container didn't show significant decrease during storage, while others reported an increase in phenolic compounds contents with increasing time of storage [10,28], a situation found in our findings when oil was stored in glass and cans after 180 days of storage compared to the previous sampling date (135 days), which could be due to hydrolysis of secoiridoid derivatives in oil. As phenolic compounds act as natural antioxidants in oil and inhibit autoxidation of lipids (RH) by trapping intermediate peroxy radicals [48], their reduction during storage is a result of oil oxidation [38].

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, temperature and above all light exposure, therefore, the level of degradation of an oil results from a balance of all these factors [14]. The phenolic compounds act by giving an electron so that they can interrupt the radical reaction occurring with oxidation. The carotenoids act as electron acceptors, quenching the singlet oxygen. Finally, tocopherols act both as electron donors, slowing down the oxidative reaction, and as electron acceptors, determining the singlet oxygen quenching or scavenging, with consequent inhibition of the oxidation of lipids [49]. At the beginning of storage time, olive oil under this study contained $214 \pm 1.5 \text{ mg kg}^{-1}$ oil of total phenolic compounds, and this value was in consistent with the data ($121\text{-}410 \text{ mg kg}^{-1}$) reported previously [15]. 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 elevated temperature storage condition. Table 6 showed that the lowest difference between the initial and final antiradical activity (percentage loss of total phenols) at ambient temperature was in glass bottles (8.1%), followed by PET (13.5%) and cans (13.6%), followed by HDPE (21.6%), and the highest reduction was found in pottery (24.5%) stored at room temperature. But concerning the reduction of phenolic compounds in glass and cans was more pronounced after 135 day of storage in a reduction percentage similar to each other and to PET (15.5% and 15.9% for glass and cans respectively). It was previously reported that glass bottles kept more phenolic compounds than that stored in PET containers [15]. The reduction of antioxidants in plastic containers could be due to their permeability to oxygen and the migration of active compounds between oil and packaging material. The large reduction found in oil stored in pottery could be due to the penetration of both oxygen and moisture which both accelerate the hydrolysis of fatty acids, formation of radicals and the depletion of antioxidants.

At elevated temperature, the highest reduction in phenol compounds was found in HDPE (42.2%), followed by pottery (37.6%), followed by PET (14.3%), followed by glass bottles (10.1%), and the least reduction was found in

cans (7.8%). Although cans and glass bottles have the highest thermal conductivity, they showed the least reduction in phenolic compounds. This can be discussed by the effect of oxygen penetration on the oxidation of oil and the consequent reduction of antioxidant compounds including total phenols and the more oxygen penetration through PET, HDPE, and pottery stated clearly that phenolic compound loss intensity during storage is directly proportional to the attitude and degree of oxidation occurred in the presence of oxygen.

4.5. Correlation among PV, K_{232} , K_{270} , and Phenolic Compounds

As the oxidation process of olive oil triglycerides occur as a consecutive chain reaction, and each stage in this oxidation pathway could be monitored by quality indicator(s), olive oil quality indices are correlated to each others. PV is correlated with the K_{232} value not only at time zero but also during storage. The significant correlation between K_{232} and peroxide value is expected as both parameters reflect primary oxidation products of the oil and therefore positive correlation was observed and was previously reported [44]. Therefore, for safety issues, PV determination could be excluded from the routine control of olive oil and replaced by K_{232} determination and the use of unwanted chemicals used in PV analysis could be avoided. No significant correlation was found between K_{270} and peroxide value as K_{270} reflects the secondary oxidation products of the oil. Regarding the negative correlation between peroxide value and phenolic content, this correlation is expected because when phenolic content decreases (by oxidation), the peroxide value increases and this explain why a negative correlation was observed. K_{232} and K_{270} are positively correlated which implies that there is a direct relationship between primary oxidation products and secondary oxidation products i.e. as primary oxidation products increases, secondary oxidation products increases too [44].

A close look at Pearson coefficients of quality indicators of oil stored at elevated temperature as compared to ambient temperature (Table 9) reveals that the correlation was stronger at the former storage condition as compared to the later, indicating that, the deterioration rate at elevated temperature is higher. Moreover the correlation between PV and K_{232} -which both indicate the primary oxidation products- are similar at both temperature treatments while the correlation between both mentioned indicators and K_{270} which indicates secondary oxidation products was higher at elevated temperature (there was no correlation between PV and K_{270} at room temperature). This highlight that the rate of transfer from primary oxidation products to secondary oxidation products is higher at elevated temperature as compared to that at room temperature [9]. This was also clearly observed in the presence of high negative Pearson coefficients at elevated temperature between total phenols and K_{270} (secondary oxidation products) compared to insignificant correlation between both indicators at room temperature. Also the correlation between the phenols in one hand and both PV an K_{232} (primary oxidation products) in the other hand was more negative at elevated temperature as compared to that at room temperature. This indicate that the formation of both

primary and secondary oxidation products contribute to the depletion of phenolic compounds at higher temperature while the main contributor in the depletion of phenolic compounds under room temperature was the presence of primary oxidation products proving the importance of phenolic compounds as antioxidants in early stages of autoxidation [46].

4.6. Sensory Evaluation

The consumer expresses his judgment on olive oil quality considering some sensory characteristics, such as the pungent taste, fruity and mild flavor. A wide range of preferences within this context can be found, because the sensory quality may match cultural aspects or simple dietary habits. Characteristic aroma and in particular green and fruity features of olive oil originates from many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase pathway which takes place during the oil extraction process [50,51]. Beside volatile compounds, non-volatile compounds such as phenolic compounds also stimulate the tasting perception of bitterness and pungency. The concentrations of volatile compounds depend on the enzymatic activity [52], and though, the external parameters (e.g. climate, soil, harvesting and extraction conditions) may alter the inherent olive oil sensory profile [53]. The aroma of olive oil is attributed to aldehydes (hexanal, trans-2-hexenal, acetaldehyde), alcohols (methanol, hexan-1-ol, 3-methylbutan-1-ol), esters (methyl acetate, ethyl acetate, hexyl acetate), hydrocarbons (2-methylbutane, hexane, nonane), ketones (2-butanone, 3-methyl-2-butanone, 3-pentanone), furans and other undefined volatile compounds. The major volatiles in virgin olive oils are C6 and C5 volatile compounds [50,54].

Evaluating the quality of stored olive oil in terms of its grade of virginity as influenced by different packaging materials using both chemical and sensory tests is shown in Table 8. At room temperature, the best type of container was shared by glass and HDPE (sustained EVOO grade for more that 90 days and was found VOO after six months of storage), followed by cans and PET (was found VOO after 90 days and 180 days), and the worst container was pottery which was found ordinary virgin olive oil (OVOO) after 90 days of storage. At elevated temperature, glass containers were superior and pottery was inferior while the other types of containers were intermediate.

Considering both chemical and sensory tests (Table 8), results reveal that, the quality of olive oil stored at room temperature deviated from the extra virgin grade because of the absorption coefficient K_{270} (which was the only determinant chemical test) along with the sensory evaluation parameters (presence of sensory defect and/or absence of sensory fruity, Figure 1). At elevated temperature (Table 8), the most relevant chemical test contributed in the loss of oil quality was K_{270} followed by sensory evaluation parameters, followed by acidity and both PV and K_{232} were the least contributors. Table 8 revealed that, grading of stored olive oil under investigation using sensory evaluation without chemical analysis is not sufficient. Also it is clear that the absorption coefficient K_{270} was the most sensitive determinant chemical test that determines the quality of stored olive oil.

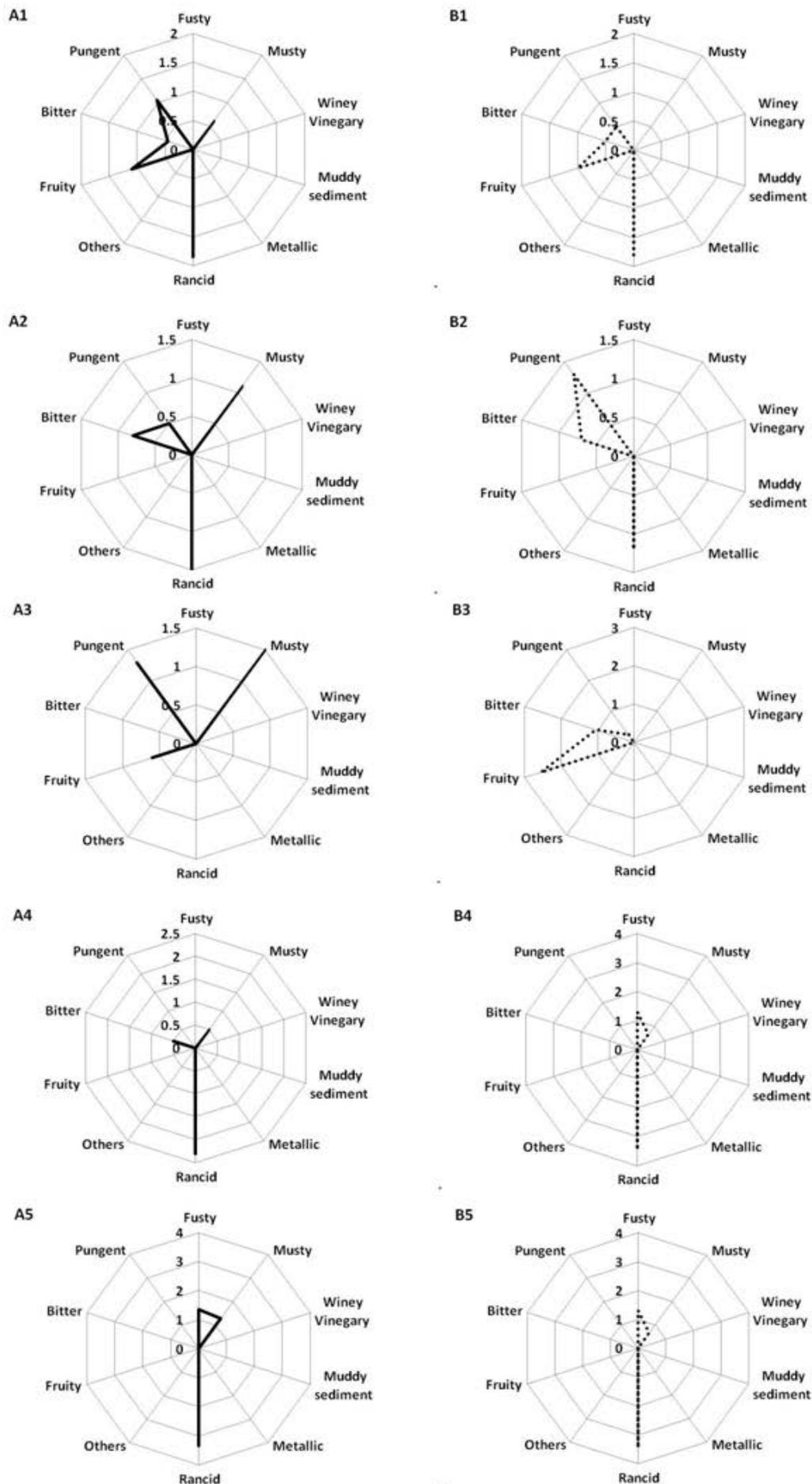


Figure 1. Evaluation of sensory attributes for EVOO stored at elevated temperature (A), and at room temperature (B) in glass bottles (1), PET (2), HDPE (3), cans (4), and pottery (5)

It was found that EVOO stored in glass bottles at low temperature maintained the extra virgin quality, whereas for that stored at elevated temperature (30°C) presented a sharp decrease in sensory score and lost its extra quality after less than two months of storage and become lambent due to loss of the positive attributes (fruity apple, green) and appearance of the negative ones (winy, muddy, rancid) [22]. A group of researchers [28] found a decrease in fruitiness during one year of oil storage and the rancid defect appear after 10-12 months at room temperature. Other investigators [55] found that the bitterness and pungency of virgin olive oil stored in glass bottles at increasing temperatures for 12-18 months decreased during storage time and the intensity of depletion was positively correlated with the increase in temperature of storage. Another research team [47] found that storage of olive oil in amber glass at low temperature results in lower amount of hexanal (off-flavor), but at ambient temperatures, positive attributes decrease throughout storage time.

5. Conclusions

As final statements and as a consequence of the results reported herein, olive oil storage and packaging are final steps of the production process and are as important as the other steps. The packaging material should ensure protection from storage conditions in order to maintain the olive oil quality. This study has reaffirmed that at ambient storage temperature, the best container in maintain the quality of stored oil is glass followed by HDPE, followed by both cans and PET, and the worst was pottery. At elevated temperature, glass was found the best primary packaging material, followed by PET, followed cans, followed by HDPE, and the worst container was pottery.

Deterioration agents can decrease the quality of olive oil during storage, so a correct control and monitoring of some quality indicators can be useful to predict the olive oil shelf life. The quality of olive oils is interpreted in terms of measurements of analytical parameters for which certain limit values are set. It was concluded that, grading of stored olive oil under investigation using sensory evaluation without chemical analysis is not sufficient. Also it is clear that the absorption coefficient K_{270} was the most sensitive determinant chemical test that determines the quality of stored olive oil.

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Abbreviations

EVOO: Extra virgin olive oil; **PET:** Polyethylene terephthalate; **HDPE:** High density polyethylene; **VOO:** Virgin olive oil; **EU:** European union; **LDL:** Low density lipoprotein; **IOOC:** International olive oil council; **PV:** Peroxide value; **EC:** European communities; **UV:** Ultra

violet; **CRD:** Complete randomized design; **AOAC:** Association of official analytical communities; **FFA:** Free fatty acid.

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