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Effect of bag-in-box packaging material on quality characteristics of extra virgin olive oil stored under household and abuse temperature conditions

A. Lolis, A.V. Badeka, M.G. Kontominas*

Laboratory of Food Chemistry, Department of Chemistry, University of Ioannina, Ioannina, 45110, Greece

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ABSTRACT

The effect of bag-in-box (B) packaging material on quality characteristics of extra virgin olive oil (EVOO) was studied as a function of storage time (0–120 days) and temperature (22 °C and 37 °C). Olive oil packaged in tin-plated steel (S) containers served as control. Olive oil sampling was carried out every 20 days, assessing quality deterioration by monitoring multiple quality parameters: acidity, PV, K_{232}, K_{270}, ΔK, color, total phenolic content (TPC), FA composition and volatile compounds' profile. Based mainly on acidity, PV, K_{232}, and K_{270} values, samples packaged in S could no longer rank as EVOO after 80 days of storage at 22 °C, and after 60 days at 37 °C. Samples stored in B retained EVOO specifications throughout storage at 22 °C and had a 100 days shelf life at 37 °C. Conclusively, results showed that B packaging proved to be more suitable for all olive oil samples even for those exposed to abuse temperatures.

1. Introduction

The olive tree (*Olea europaea L.*) is mainly cultivated in the Mediterranean basin and according to recent statistics published by the (International Olive Commission (IOC), the European Union is the leading producer of olive oil worldwide. Greece in particular comes third in terms of olive oil production (474,600 t in 2015/16) and consumption (140,000 t in 2015/16) among European countries (http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures). Olive oil is extracted directly from the fresh fruit of the olive tree using only mechanical means (EEC 2568/91, 1991, 2019EEC 2568/91/, 2019EEC 2568/91, 1991, 2019) so that its natural components are kept intact (Altieri, Genovese, Auriello, & Di Renzo, 2015; Angerosa, 2002).

Consumers show their preference to olive oil not only for its excellent and appreciated taste and aroma, due to volatile and non-volatile compounds, fatty acids (mainly oleic acid) and natural antioxidants (Pouliarekou et al., 2011; López-Cortés, Salazar-García, Velázquez-Martí, & Salazar, 2013; Dais & Hatzakis, 2013; Rodrigues, Dias, Veloso, Pereira, & Peres, 2016) but also for the fact that consumers of high quality olive oil enjoy numerous health related benefits including the reduction of major cardiovascular incidents, the protection of LDL from oxidation, the increase in HDL cholesterol levels etc. (Estruch et al., 2013; Oliveras-López, Berná, Jurado-Ruiz, López-García de la Serrana, & Martín, 2014; Marrugat et al., 2004).

Agricultural practices, extraction process, climate, soil and environmental conditions may significantly affect olive oil quality (Angerosa, Mostallino, Basti, & Vito, 2001; Bustan et al., 2014; Ouni et al., 2011; Taticchi et al., 2013) but only up to the point it is packaged and stored. Subsequently, olive oil quality is affected by storage conditions (Abbadi et al., 2014; Ayyad et al., 2015; Caponio et al., 2013; Escudero, Ramos, La Rubia, & Pacheco, 2016), packaging material (Abbadi et al., 2014; Pristouri, Badeka, & Kontominas, 2010) and exposure to oxygen and/or light (Ayyad et al., 2015; Escudero et al., 2016; Pristouri et al., 2010) which raises the question of extra virgin olive oil commercial shelf life.

Reactions involved during olive oil storage include hydrolysis and oxidation in the form of auto-oxidation and photo-oxidation depending on the presence of light, triplet or singlet oxygen, pro-oxidants, etc. (Frankel, 1980; Pristouri et al., 2010; Cecchi, Passamonti, & Cecchi, 2010). On the other hand phenolic compounds, the main contributors to oil stability, protect olive oil from both auto-oxidation and photo-oxidation during storage (Gutiérrez & Fernández, 2002; Abbadi et al., 2014).

Inevitably, degradation of olive oil components during storage may lead a particular olive oil classified in a specific quality grade when bottled, to an inferior quality grade when purchased and consumed. Thus, packaging material properties play a key role in terms of quality retention and an adequate product shelf life. Materials used for the packaging of olive oil include dark-colored glass, PET, tinplate,

* Corresponding author.
E-mail address: mkontomi@cc.uoi.gr (M.G. Kontominas).

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aluminum, plastic-coated paperboard (tetra brik type packages) and multilayer pouches (bag-in-box type packages) (Kanavouras, Munoz, & Coutelieris, 2006; Pristouri et al., 2010; Kontominas, 2017).

Numerous studies have evaluated packaging material performance in contact with olive oil under various temperature and lighting conditions. More specifically, Abbadi et al. (2014), suggested that, at ambient storage temperature, the best container to maintain the quality of stored olive oil is glass followed by HDPE, followed by both tin cans and polyethylene terephthalate (PET), while the worst was pottery in terms of physico-chemical quality parameter values (acidity, peroxide value, $K232$, and $K270$). At elevated temperatures olive oil stored in all above packaging materials lost EVOO quality within six months. According to Dabbou et al. (2011), exposure of olive oil samples stored at room temperature to light, caused substantial deterioration of product quality parameters and significant loss in phenolic content with tin cans being the best packaging material followed by glass. Clear PET and glass jars proved to be unsuitable packaging materials.

Moreover, Gutiérrez and Fernández (2002), showed higher oxidative deterioration and polyphenol loss in glass bottles stored at 30 °C and exposed to light than those stored at 2 °C in the dark whereas Gargouri, Zribi, and Bouaziz (2015), tested olive oil of the Chemlali cultivar and reported that quality was retained almost intact when packaged in tin containers and dark glass bottles for up to 6 months at ambient temperature. Furthermore, Pristouri et al. (2010), showed that materials with high oxygen permeability [Polypropylene (PP) and PE)] are not suitable for olive oil packaging and that preferably dark colored glass stored in the dark and at temperatures less than 22 °C should be used to maintain EVOO's quality for six months.

To the best of our knowledge there are no reports in the literature on the effect of multilayer bag-in-box type packaging on the quality of stored extra virgin olive oil. Thus, the objective of the present study was to investigate the effect of this packaging material on EVOO quality for a four month period under household and abuse storage temperature conditions.

2. Materials and methods

2.1. Samples

EVOO samples were donated by the Union of Agricultural Cooperatives of Peza located in Heraklion, Crete. The olive oil originated from olives of the "Koroneiki" variety cultivated mainly on the isle of Crete. Olive samples were collected during the 2016 harvesting period (October to December) from the greater Heraklion area in plastic crates of 25 kg capacity and used to produce olive oil within 24 h of harvesting. Olives were processed using an Alfa Laval commercial two-phase olive oil processing line composed of two, type 10 hammer mill crushers (2800 rpm), a four station atmosphera type kneader (malaxation time: 35 min, malaxation temperature: 28 °C to avoid extreme loss of volatile compounds, 20 rpm), a single Y- 10 decanter (3500 rpm) and two UVPX type vertical centrifuge separators (6500 rpm).

2.2. Experimental procedure set up

To study the effect of packaging material on quality parameters and shelf life of EVOO, the oil was commercially packaged in the Peza Cooperative plant, within 48 h of production, in bag-in-box (3 L) multilayer pouches placed inside parallelepiped, corrugated paperboard boxes. The composition of multilayer pouches (obtained from VLACHOS S.A., Athens, Greece) was: PET(outside)/adhesive/metallized PET/adhesive/LDPE (inside). The oxygen permeability of the multilayer pouch was < 0.5 mL $O_2/m^2 \cdot 24$ h $\cdot$atm according to the manufacturer. Similarly, parallelepiped tinplated steel containers (3 L) (obtained from ELSA S.A., Athens, Greece) were used as control samples. Tin can internal varnish was epoxy-phenolic derivative. Tin can headspace volume was 300 ml/3 L (10% of total tin can volume); respective headspace volume of the bag-in-box pouch was 60 mL (2% of total pouch volume) being filled with Nitrogen during product filling into the pouch. A sample of ≈300 ml of oil (remaining head space after first oil removal was 600 ml for the tin can and 360 mL for the multilayer pouch) was collected from each of the containers at time zero (0) for initial analysis. All containers were stored either at 22 ± 1 °C or at 37 ± 1 °C in a controlled temperature environmental chamber (Memmert, Binder model WTB, Germany). The above two temperatures were selected so as to simulate normal home storage temperatures and abuse temperature conditions respectively). Additional 300 ml samples were collected from the same container for analysis every 20 days for a period of 4 months. The total number of containers tested was: 2 types of containers x 2 replicates x 2 temperatures = 8 containers ($n = 2 \times 2 = 4$/type of container).

2.3. Methods

2.3.1. Determination of olive oil quality parameters

The determination of the physico-chemical parameters namely: free acidity, peroxide value and absorption coefficients (K_{232}, K_{270}, ΔK) was carried out following the methods described by the EEC/2568/91 regulation of the European Union Commission.

2.3.2. Semi-quantitative determination of volatile compounds using SPME-GC/MS

4.5 g of olive oil with 20 µL of an internal standard solution (4-methyl-2-pentanol, 160.4 mg in 100 mL methanol) and a micro-stirring bar were placed in a 20 mL glass vial sealed with an aluminum crimp cap provided with a needle pierceable septum. Solid-phase microextraction (SPME) was performed with a 30/50 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellafonte, USA). The sample was placed in a 40 °C water bath and stirred. After allowing 10 min for the sample to equilibrate, the needle of the SPME device was inserted into the vial and the fiber was exposed to the headspace of the sample for 45 min. The fiber was then retracted from the vial headspace and inserted into the gas chromatograph injector. A SHIMADZU GC-2010 Plus series gas chromatograph was used for the analysis of volatile compounds adsorbed onto the SPME fiber. The column used was an Agilent DB-5MS, 60m-0.250mm-1.00 µm. The flow rate of the helium carrier gas was 1.2 mL/min. The injector was operated in split mode (2:1 split ratio) at 260 °C. The SPME fiber remained in the injector for 10 min. The column was maintained at 40°C held for 5 min, heated to 160 °C at a rate of 3 °C/min, then heated to 240 °C at a rate of 10 °C/min and held to 240 °C for 5 min. The parameter values presented above were the result of a preliminary optimization procedure. MS conditions were as follows: source temperature: 200°C; quadrupole temperature: 150°C; transfer line temperature: 270°C; acquisition mode electron impact (EI 70 eV) by 3 scans s^{-1} and mass range m/z: 29–350. Peak identification was performed by the comparison of retention times and mass spectra of eluting compounds to those of the NIST- 11 Mass Spectral Library.

2.3.3. Determination of fatty acid composition and total phenolic compounds

The fatty acid composition was determined according to the official method described by the EEC/2568/91 regulation of the European Union Commission. Total phenolic compounds were determined as described by Kosma, Badeka, Vatavali, Kontakos, and Kontominas (2015).

2.3.4. Color measurement

The olive oil surface color was measured using a HunterLab, model D25 L optical sensor (Hunter Associates Reston, VA, USA). The colorimeter was calibrated with a white and a black standard plate. Sixty mL of olive oil were placed into a cylindrical (base diameter 11.3 cm and height 2 cm) optical cell. Reflectance values were obtained using a

Table 1
Conventional EVOO quality parameters as a function of storage time and temperature (S = tinplated steel; B = bag-in-box).

		S		B	
		22 °C	37 °C	22 °C	37 °C
Acidity (% oleic acid)					
Days	0	0.70 ± 0.00^{A}			
	20	0.73 ± 0.03^{aA}	$0.77 \pm 0.01^{\alpha B}$	0.72 ± 0.02^{aAB}	0.73 ± 0.03^{aAB}
	40	0.74 ± 0.01^{aB}	0.78 ± 0.01^{bB}	0.74 ± 0.01^{aB}	0.74 ± 0.02^{aB}
	60	0.76 ± 0.02^{aB}	0.79 ± 0.01^{aB}	0.76 ± 0.03^{aB}	0.77 ± 0.02^{aB}
	80	0.79 ± 0.01^{aC}	0.82 ± 0.02^{bBC}	0.76 ± 0.02^{aB}	0.79 ± 0.00^{aC}
	100	0.84 ± 0.01^{bD}	0.89 ± 0.01^{cD}	0.79 ± 0.00^{aC}	0.80 ± 0.01^{aC}
	120	0.84 ± 0.00^{bD}	0.90 ± 0.00^{cD}	0.79 ± 0.00^{aC}	0.84 ± 0.01^{bD}
PV (meq O_2/kg)					
Days	0	12.31 ± 0.09^{A}			
	20	13.55 ± 0.10^{bB}	15.36 ± 0.07^{cB}	12.72 ± 0.13^{aB}	12.81 ± 0.14^{aB}
	40	13.92 ± 0.07^{cC}	15.72 ± 0.18^{dC}	12.80 ± 0.04^{aB}	13.08 ± 0.16^{bB}
	60	14.32 ± 0.22^{bD}	16.38 ± 0.06^{cD}	12.87 ± 0.24^{aBC}	13.06 ± 0.52^{aBC}
	80	14.96 ± 0.09^{bE}	18.80 ± 0.32^{cE}	13.25 ± 0.32^{aC}	13.56 ± 0.06^{aC}
	100	15.58 ± 0.13^{bF}	20.43 ± 0.24^{cF}	13.45 ± 0.41^{aCD}	13.99 ± 0.17^{aD}
	120	16.01 ± 0.13^{cG}	22.56 ± 0.10^{dG}	13.73 ± 0.20^{aD}	14.11 ± 0.17^{bD}
K_{270}					
Days	0	0.15 ± 0.03^{A}			
	20	0.16 ± 0.01^{aA}	0.16 ± 0.01^{aA}	0.17 ± 0.01^{aA}	0.17 ± 0.02^{aA}
	40	0.17 ± 0.04^{aA}	0.17 ± 0.01^{aA}	0.17 ± 0.01^{aA}	0.17 ± 0.02^{aA}
	60	$0.17 \pm 0{,}04^{aA}$	0.18 ± 0.02^{aA}	0.16 ± 0.05^{aA}	0.16 ± 0.03^{aA}
	80	0.17 ± 0.09^{aA}	0.21 ± 0.04^{aAB}	0.15 ± 0.04^{aA}	0.19 ± 0.04^{aA}
	100	0.18 ± 0.08^{aA}	0.22 ± 0.03^{bB}	0.19 ± 0.02^{aA}	0.20 ± 0.06^{aA}
	120	0.18 ± 0.02^{aA}	0.24 ± 0.03^{bB}	0.18 ± 0.02^{aA}	0.20 ± 0.02^{aA}
K_{232}					
Days	0	1.16 ± 0.08^{A}			
	20	1.24 ± 0.05^{aA}	1.58 ± 0.16^{bB}	1.25 ± 0.10^{aA}	1.32 ± 0.18^{aA}
	40	1.38 ± 0.15^{aB}	1.89 ± 0.20^{bB}	1.38 ± 0.14^{aA}	1.63 ± 0.10^{bB}
	60	1.78 ± 0.10^{aC}	2.35 ± 0.11^{bC}	1.74 ± 0.08^{aB}	1.91 ± 0.14^{aC}
	80	1.88 ± 0.12^{aC}	2.66 ± 0.06^{cD}	1.78 ± 0.05^{aB}	2.13 ± 0.10^{bCD}
	100	1.90 ± 0.11^{aC}	2.88 ± 0.14^{bE}	1.87 ± 0.20^{aBC}	2.20 ± 0.19^{aCD}
	120	1.96 ± 0.10^{aC}	3.09 ± 0.07^{cE}	1.88 ± 0.05^{aC}	2.34 ± 0.08^{bD}

*SD.
$^{a, b, c, d}$are used to compare means of samples for both materials and temperatures on the same day. Means that do not bear a common superscript differ significantly.
$^{A, B, C, D, E, F, G}$are used to compare means of samples at each temperature and material throughout storage time. Means that do not bear a common superscript differ significantly.

45 mm viewing aperture.

2.3.5. Statistical analysis

Data were subjected to statistical two way analysis of variance using SPSS 21 program (IBM Corporation, USA). Where statistical differences were noted, differences among packages were determined using the least significant difference (LSD) test. Significance was defined at $p < 0.05$.

3. Results and discussion

Olive oil quality parameter values are given as a function of storage time and temperature in Table 1.

Results show that olive oil stored in tinplated steel containers at 22 °C reached the upper limit of acidity (0.8%) after 80 days of storage, while olive oil stored in bag-in-box containers at the same temperature did not exceed this limit throughout storage. Respectively at 37 °C, the acidity of samples stored in tin plated containers reached the upper limit of 0.8% after 60 days of storage while samples stored in bag-in-box containers at the same temperature reached the same limit after 100 days of storage. Thus, in terms of % acidity of EVOO, the bag-in-box container was superior to the tin plated can. These results are in general agreement with those of Abbadi et al. (2014); Mendéz and Falqué (2007) and Gargouri et al. (2015) regarding storage of olive oil in tin plated steel cans at room and elevated temperatures.

Results regarding the peroxide value showed that all samples remained below 20 meq O_2/kg with the exception of those stored in tinplated steel containers stored at 37 °C which exceeded the respective limit after 100 days of storage. This may be attributed to the fact that the olive oil is protected from auto-oxidation by its polyphenol content in addition to its tocopherol and carotenoid content. After 100 days the total phenolic content of the samples stored in tinplated steel containers at 37 °C appeared to have the greatest % reduction compared to the other treatments (data and discussion are shown below).

Regarding $K_{232,}$ all samples remained below the limit of 2.5 except for those stored in tinplated steel containers at 37 °C which exceeded this limit after 80 days of storage. Finally, regarding the absorption coefficient K_{270}, only tinplated steel containers at 37 °C exceeded the limit of 0.22 after 120 days of storage. It is obvious that at abuse temperatures (37 °C) the bag-in-box container proved superior to the tin can as documented by all four olive oil quality parameter values. At 22 °C both packaging materials proved adequate for maintaining olive oil quality with the bag-in-box container recording consistently lower values for all four quality parameters compared to the tin can. ΔK values (data not shown) were lower than or equal to 0.01 for all samples in both packaging materials and at both temperatures.

Abuse storage temperatures and the excessive presence of oxygen, as a result of the headspace created in the tin plated container after each sampling, resulted in a more rapid deterioration of oil quality for the samples stored in tinplated steel containers as the presence of oxygen enhances oxidation. Similar findings were reported by Pristouri et al. (2010); Gargouri et al. (2015) and Di Giovacchino, Mucciarella, Costantini, Ferrante, and Surricchio (2002).

In agreement with Vekiari, Papadopoulou, and Kiritsakis (2007), oil

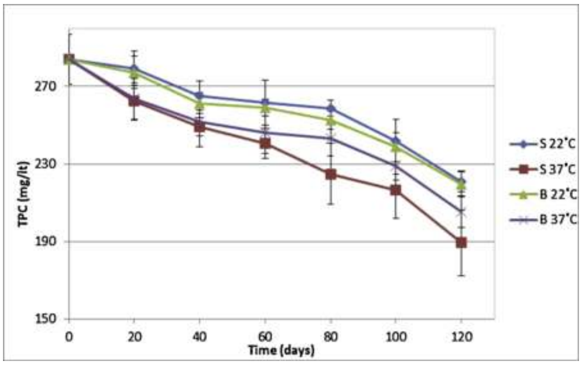

Fig. 1. Changes in EVOO total phenolic content as a function of storage time and temperature (S = tinplated steel; B = bag-in-box).

samples stored in the dark contain mainly primary oxidation products reflected by K_{232} values. Mendéz and Falqué (2007) found that absorption coefficients increase during a six month storage period for olive oil samples stored in the dark and under light in different packaging materials (clear PET bottles, PET bottles covered with aluminum foil, glass bottles, tin plated and Tetra-brik). Finally, Gomez-Alonso, Mancebo-Campos, Desamparados Salvador, and Fregapane (2007), reported that K_{232} was the first quality index to reach the upper limit for the EVOO category, for samples stored at 4 °C in amber glass bottles in the dark after 33–63 weeks and could be used as a marker to identify the level of deterioration of the stored olive oil. In the present study, both K_{232} and K_{270} indices (reflecting primary and secondary oxidation products respectively) exceeded respective limits for EVOO samples stored at 37 °C only in tinplated steel containers indicating that bag-in-box containers provided a better protection to the oil against auto-oxidation even at abuse temperatures due to the absence of oxygen in the head space in the latter case. Since all samples were filled and sealed on the same day, originating from the same olive oil batch, they were expected to have the same amount of dissolved oxygen and thus this parameter was not considered in the study.

Based on all above quality parameter values, the actual shelf life of EVOO stored at 22°C was at least 120 days packaged in the bag-in-box pouches and only 80 days packaged in the tin plated steel cans. Shelf life of EVOO at 37 °C was 100 and 60 days for samples packaged in the bag-in-box pouch and tinplated steel respectively.

Fig. 1, shows the Total Phenolic content (TPC) evolution as a function of storage time and temperature. Initial TPC content was 286 mg/L. Statistically non-significant changes ($p > 0.05$) in TPC were noted during storage at 22°C. At the end of storage, samples stored at 22 °C suffered a TPC loss ranging from 22.7% and 23.5% for bag-in-box and tinplated steel containers respectively. At 37 °C bag-in-box samples recorded a TPC loss of 27.7% after 120 days of storage while the greatest loss in TPC, 34% ($p < 0.05$) was noted for tinplated steel containers. Present findings are in agreement with the studies of Esti, Contini, Moneta, and Sinesio (2009) and Dabbou et al. (2011) showing that during storage, phenols undergo qualitative and quantitative modifications due to decomposition and oxidation reactions. Vacca, Del Caro, Poiana, and Piga (2006) reported that TPC was reduced for all tested olive oil samples sealed in colorless transparent glass bottles and stored at room temperature, but those stored in the dark kept their TPC at higher levels than those kept under light for an 18 month storage period. According to Psomiadou, Karakostas, Blekas, Tsimidou, and Boskou (2003) the determination of total polar phenols, total chlorophylls and α-tocopherol, may be used for a better evaluation of VOO quality.

Statistical analysis showed that samples packaged both in tinplated steel and in “bag-in-box” containers were affected by storage temperature ($p < 0.05$) but those stored in “bag-in-box” containers were less affected. Gargouri et al. (2015) also showed that packaging material light transmittance has a significant effect on olive oil TPC since all of the tested samples lost part of their phenolic content with samples stored in PE and clear glass bottles suffering a greater loss in their phenolic concentration than those stored in tin containers and dark

Table 2
Color parameter values of EVOO as a function of storage time and temperature (S = tinplated steel; B = bag-in-box).

		Color parameters				
		S		B		
		22 °C	37 °C	22 °C	37 °C	
Days	0	$66.17 \pm 0.12^{*A}$				L*
	20	66.11 ± 0.03^{aA}	66.54 ± 0.07^{bB}	66.20 ± 0.13^{aA}	66.51 ± 0.08^{bB}	
	40	65.99 ± 0.11^{aA}	66.42 ± 0.07^{bB}	66.14 ± 0.05^{aA}	66.39 ± 0.09^{bB}	
	60	66.21 ± 0.01^{aB}	66.56 ± 0.12^{bB}	66.16 ± 0.17^{aA}	66.44 ± 0.14^{abB}	
	80	66.84 ± 0.08^{bC}	66.87 ± 0.06^{bC}	66.42 ± 0.04^{aB}	66.88 ± 0.07^{bC}	
	100	66.53 ± 0.30^{abC}	66.87 ± 0.05^{bC}	66.85 ± 0.25^{abC}	66.71 ± 0.09^{bC}	
	120	66.82 ± 0.07^{bC}	67.10 ± 0.05^{cD}	66.61 ± 0.11^{aC}	67.05 ± 0.07^{cD}	
Days	0	-5.26 ± 0.28^{A}				a*
	20	-5.43 ± 0.02^{aA}	-5.77 ± 0.06^{bB}	-5.66 ± 0.27^{abA}	-5.87 ± 0.10^{bB}	
	40	-6.28 ± 0.21^{aB}	-6.12 ± 0.05^{aC}	-6.22 ± 0.06^{aB}	-5.98 ± 0.20^{aB}	
	60	$- 6.60 \pm 0.03^{aC}$	-6.50 ± 0.10^{aD}	-6.62 ± 0.11^{aC}	-6.58 ± 0.05^{aC}	
	80	-6.73 ± 0.18^{aC}	-6.71 ± 0.18^{aD}	-6.85 ± 0.18^{aC}	-6.92 ± 0.17^{aD}	
	100	-7.03 ± 0.31^{aCD}	-7.24 ± 0.07^{aE}	-7.08 ± 0.15^{aD}	-7.20 ± 0.05^{aE}	
	120	-7.42 ± 0.21^{aD}	-7.53 ± 0.25^{aE}	-7.37 ± 0.18^{aD}	-7.42 ± 0.21^{aE}	
Days	0	96.23 ± 0.14^{A}				b*
	20	96.56 ± 0.28^{aA}	96.32 ± 0.10^{aA}	96.21 ± 0.12^{aA}	96.50 ± 0.40^{aA}	
	40	94.72 ± 0.12^{aB}	94.93 ± 0.05^{bB}	95.09 ± 0.36^{abB}	94.60 ± 0.09^{aB}	
	60	94.82 ± 0.15^{bB}	94.04 ± 0.36^{aC}	95.13 ± 0.07^{cB}	94.24 ± 0.30^{aB}	
	80	94.31 ± 0.18^{bC}	93.26 ± 0.15^{aD}	95.26 ± 0.22^{cB}	94.47 ± 0.33^{bB}	
	100	94.16 ± 0.23^{bC}	92.94 ± 0.06^{aE}	95.21 ± 0.23^{cB}	94.25 ± 0.16^{bB}	
	120	94.24 ± 0.13^{cC}	91.52 ± 0.18^{aF}	94.42 ± 0.24^{cC}	93.64 ± 0.22^{bC}	

[a, b, c] are used to compare means of samples for both materials and temperatures on the same day. Means that do not bear a common superscript differ significantly.
[A, B, C, D, E, F] are used to compare means of samples at each temperature and material throughout storage time. Means that do not bear a common superscript differ significantly.
* SD.

Table 3
Semi-quantified volatile compounds (mg/L) in EVOO as a function of storage period.

Compound	Days					
	0	40				80
		22 °C		37 °C		22 °C
		B	S	B	S	B
Alcohols						
Ethanol	0.31 ± 0.02^{*a}	0.28 ± 0.03^{a}	0.29 ± 0.01^{a}	0.28 ± 0.02^{a}	0.28 ± 0.04^{a}	0.29 ± 0.03^{a}
1-Penten-3-ol	0.34 ± 0.06^{a}	0.25 ± 0.03^{a}	0.27 ± 0.05^{a}	0.27 ± 0.03^{a}	0.27 ± 0.06^{a}	0.23 ± 0.06^{a}
2-Penten-1-ol, (Z)-	0.24 ± 0.04^{a}	0.19 ± 0.02^{a}	0.26 ± 0.06^{a}	0.19 ± 0.09^{a}	0.26 ± 0.08^{a}	0.16 ± 0.05^{a}
2-Hexen-1-ol, (E)-	1.13 ± 0.17^{ab}	1.06 ± 0.12^{ab}	1.04 ± 0.08^{a}	1.12 ± 0.09^{a}	1.01 ± 0.08^{ab}	1.13 ± 0.14^{a}
1-Hexanol	0.67 ± 0.11^{a}	0.60 ± 0.06^{a}	0.58 ± 0.16^{ab}	0.62 ± 0.07^{a}	0.44 ± 0.06^{bc}	0.61 ± 0.09^{a}
1-Octanol	0.80 ± 0.22^{a}	0.70 ± 0.26^{a}	0.68 ± 0.16^{a}	0.62 ± 0.12^{a}	0.56 ± 0.06^{a}	0.57 ± 0.08^{a}
1-Nonanol	0.41 ± 0.11^{a}	0.35 ± 0.05^{a}	0.39 ± 0.06^{a}	0.33 ± 0.08^{a}	0.37 ± 0.12^{a}	0.36 ± 0.05^{a}
Total	3.90 ± 0.44	3.44 ± 0.30	3.29 ± 0.28	3.33 ± 0.44	3.12 ± 0.11	3.35 ± 0.19
Aldehydes						
Pentanal	0.22 ± 0.08^{a}	0.24 ± 0.03^{a}	0.28 ± 0.05^{a}	0.26 ± 0.03^{a}	0.28 ± 0.04^{a}	0.23 ± 0.02^{a}
2-Pentenal, (E)-	0.14 ± 0.07^{a}	0.14 ± 0.08^{a}	0.16 ± 0.02^{a}	0.15 ± 0.06^{a}	0.15 ± 0.06^{a}	0.16 ± 0.05^{a}
Hexanal	1.01 ± 0.28^{ab}	0.97 ± 0.12^{a}	1.00 ± 0.08^{a}	1.44 ± 0.18^{b}	1.42 ± 0.22^{b}	1.21 ± 0.09^{ab}
(E) 2-Hexenal	24.26 ± 2.46^{a}	23.66 ± 2.91^{a}	24.49 ± 1.51^{a}	24.74 ± 1.78^{a}	24.49 ± 3.12^{a}	24.02 ± 1.99^{a}
Heptanal	0.20 ± 0.03^{a}	0.21 ± 0.07^{a}	0.25 ± 0.04^{a}	0.25 ± 0.03^{a}	0.26 ± 0.04^{a}	0.39 ± 0.06^{b}
2-Heptenal, (E)-	0.79 ± 0.24^{a}	0.75 ± 0.09^{a}	0.78 ± 0.09^{a}	1.03 ± 0.12^{a}	1.20 ± 0.16^{ab}	0.86 ± 0.09^{a}
Benzaldehyde	0.20 ± 0.09^{a}	0.18 ± 0.05^{a}	0.17 ± 0.02^{a}	0.11 ± 0.03^{a}	0.12 ± 0.04^{a}	0.16 ± 0.06^{a}
2,4-Hexadienal, (E,E)-	0.09 ± 0.04^{a}	0.06 ± 0.02^{a}	0.07 ± 0.01^{a}	0.09 ± 0.02^{a}	0.12 ± 0.04^{a}	0.06 ± 0.04^{a}
Octanal	1.38 ± 0.18^{a}	1.78 ± 0.50^{ab}	1.57 ± 0.49^{ab}	1.70 ± 0.22^{ab}	1.68 ± 0.19^{ab}	1.80 ± 0.33^{ab}
Nonanal	0.77 ± 0.08^{a}	0.79 ± 0.12^{a}	0.82 ± 0.10^{a}	0.94 ± 0.10^{ab}	1.05 ± 0.09^{b}	0.83 ± 0.09^{a}
2-Nonenal, (E)-	0.46 ± 0.05^{a}	0.49 ± 0.14^{a}	0.39 ± 0.19^{a}	0.48 ± 0.09^{a}	0.48 ± 0.12^{a}	0.51 ± 0.07^{a}
Decanal	0.35 ± 0.09^{a}	0.37 ± 0.19^{a}	0.36 ± 0.17^{a}	0.49 ± 0.09^{a}	0.48 ± 0.07^{a}	0.41 ± 0.04^{a}
2-Decenal, (Z)-	3.58 ± 0.47^{a}	3.75 ± 1.14^{a}	3.58 ± 1.21^{a}	3.76 ± 0.45^{a}	4.18 ± 0.38^{a}	4.08 ± 0.87^{a}
2-Dodecenal, (E)-	0.38 ± 0.07^{a}	0.35 ± 0.12^{a}	0.35 ± 0.11^{a}	0.32 ± 0.07^{a}	0.36 ± 0.04^{a}	0.37 ± 0.11^{a}
Total	34.98 ± 3.45	33.55 ± 2.98	34.03 ± 2.19	35.66 ± 3.70	35.88 ± 2.77	34.85 ± 3.09
Esters						
Ethyl Acetate	0.06 ± 0.01^{a}	0.04 ± 0.03^{a}	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}	0.04 ± 0.02^{a}	0.07 ± 0.02^{a}
3-Hexen-1-ol, acetate, (Z)-	7.92 ± 0.44^{a}	7.90 ± 0.88^{a}	7.78 ± 1.00^{a}	7.79 ± 0.68^{a}	7.90 ± 0.49^{a}	7.72 ± 0.39^{a}
Acetic acid, hexyl ester	2.20 ± 0.41^{a}	2.18 ± 0.24^{a}	2.22 ± 0.46^{a}	2.25 ± 0.19^{a}	2.15 ± 0.33^{a}	2.10 ± 0.22^{a}
Total	10.19 ± 2.19	10.12 ± 1.93	9.96 ± 1.82	10.09 ± 2.53	10.09 ± 1.98	9.89 ± 2.12
Ketones						
1-Penten-3-one	0.32 ± 0.08^{a}	0.27 ± 0.09^{a}	0.28 ± 0.11^{a}	0.26 ± 0.07^{a}	0.30 ± 0.10^{a}	0.25 ± 0.11^{a}
3-Pentanone	0.20 ± 0.04^{a}	0.18 ± 0.06^{a}	0.17 ± 0.05^{a}	0.22 ± 0.05^{a}	0.16 ± 0.08^{a}	0.18 ± 0.06^{a}
5-Hepten-2-one, 6-methyl-	0.27 ± 0.07^{a}	0.30 ± 0.04^{a}	0.27 ± 0.07^{a}	0.35 ± 0.05^{a}	0.34 ± 0.06^{a}	0.29 ± 0.04^{a}
Total	0.89 ± 0.18	0.68 ± 0.19	0.72 ± 0.09	0.83 ± 0.22	0.80 ± 0.27	0.65 ± 0.32
Hydrocarbons						
1,3-Pentadiene, (E)-	0.44 ± 0.11^{a}	0.37 ± 0.10^{a}	0.32 ± 0.11^{a}	0.38 ± 0.13^{a}	0.36 ± 0.08^{a}	0.35 ± 0.13^{a}
3-Ethyl-1,5-octadiene	0.58 ± 0.15^{a}	0.64 ± 0.08^{a}	0.67 ± 0.18^{a}	0.81 ± 0.22^{a}	0.78 ± 0.20^{a}	0.67 ± 0.13^{a}
.beta.-Ocimene	0.18 ± 0.02^{a}	0.19 ± 0.03^{a}	0.22 ± 0.07^{a}	0.22 ± 0.04^{a}	0.23 ± 0.06^{a}	0.20 ± 0.06^{a}
2-Dodecene, (E)-	4.24 ± 0.41^{a}	4.59 ± 1.14^{a}	4.62 ± 0.70^{a}	4.93 ± 0.32^{a}	4.74 ± 0.50^{a}	4.73 ± 0.33^{a}
Copaene	0.18 ± 0.05^{a}	0.14 ± 0.09^{a}	0.16 ± 0.07^{a}	0.24 ± 0.12^{a}	0.13 ± 0.07^{a}	0.22 ± 0.08^{a}
alpha.-Farnesene	0.73 ± 0.03^{a}	0.61 ± 0.12^{a}	0.62 ± 0.12^{a}	0.63 ± 0.22^{a}	0.63 ± 0.09^{a}	0.66 ± 0.11^{a}
Total	6.35 ± 0.18	6.54 ± 0.22	6.61 ± 0.12	7.21 ± 0.27	6.87 ± 0.20	6.83 ± 0.19

(continued on next page)

Table 3 (*continued*)

Compound	Days						
	80			120			
	22 °C	37 °C		22 °C		37 °C	
	S	B	ST	B	S	B	S
Alcohols							
Ethanol	0.28 ± 0.06^{a}	0.28 ± 0.01^{a}	0.26 ± 0.05^{a}	0.26 ± 0.03^{a}	0.29 ± 0.03^{a}	0.25 ± 0.04^{a}	0.30 ± 0.03^{a}
1-Penten-3-ol	0.24 ± 0.04^{a}	0.26 ± 0.02^{a}	0.27 ± 0.03^{a}	0.23 ± 0.07^{a}	0.23 ± 0.05^{a}	0.24 ± 0.05^{a}	0.22 ± 0.06^{a}
2-Penten-1-ol, (Z)-	0.21 ± 0.04^{a}	0.22 ± 0.05^{a}	0.18 ± 0.07^{a}	0.18 ± 0.03^{a}	0.18 ± 0.06^{a}	0.14 ± 0.06^{a}	0.20 ± 0.02^{a}
2-Hexen-1-ol, (E)-	1.04 ± 0.11^{ab}	1.06 ± 0.07^{a}	0.88 ± 0.09^{b}	1.08 ± 0.02^{a}	1.05 ± 0.07^{a}	0.96 ± 0.09^{ab}	0.87 ± 0.06^{b}
1-Hexanol	0.53 ± 0.10^{ab}	0.54 ± 0.04^{ab}	0.39 ± 0.09^{bc}	0.58 ± 0.08^{a}	0.45 ± 0.07^{bc}	0.42 ± 0.09^{bc}	0.33 ± 0.07^{c}
1-Octanol	0.64 ± 0.09^{a}	0.63 ± 0.17^{a}	0.58 ± 0.07^{a}	0.55 ± 0.09^{a}	0.65 ± 0.17^{a}	0.62 ± 0.09^{a}	0.54 ± 0.08^{a}
1-Nonanol	0.41 ± 0.11^{a}	0.34 ± 0.06^{a}	0.39 ± 0.12^{a}	0.34 ± 0.10^{a}	0.34 ± 0.04^{a}	0.30 ± 0.08^{a}	0.32 ± 0.05^{a}
Total	3.72 ± 0.22	3.71 ± 0.38	2.87 ± 0.21	3.22 ± 0.13	3.59 ± 0.31	2.90 ± 0.16	2.97 ± 0.26
Aldehydes							
Pentanal	0.26 ± 0.07^{a}	0.22 ± 0.11^{a}	0.27 ± 0.05^{a}	0.26 ± 0.03^{a}	0.28 ± 0.08^{a}	0.24 ± 0.05^{a}	0.29 ± 0.10^{a}
2-Pentenal, (E)-	0.16 ± 0.08^{a}	0.18 ± 0.08^{a}	0.26 ± 0.06^{a}	0.14 ± 0.07^{a}	0.16 ± 0.05^{a}	0.18 ± 0.05^{a}	0.27 ± 0.06^{a}
Hexanal	1.38 ± 0.11^{b}	1.99 ± 0.15^{c}	1.97 ± 0.11^{c}	1.43 ± 0.22^{b}	1.75 ± 0.18^{bc}	2.10 ± 0.13^{c}	2.53 ± 0.19^{d}
(E) 2-Hexenal	25.11 ± 3.67^{a}	25.58 ± 2.44^{a}	25.83 ± 2.19^{a}	24.10 ± 2.87^{a}	24.82 ± 1.90^{a}	26.19 ± 2.72^{a}	26.25 ± 3.83^{a}
Heptanal	0.39 ± 0.04^{b}	0.39 ± 0.01^{b}	0.41 ± 0.10^{b}	0.41 ± 0.09^{b}	0.40 ± 0.09^{b}	0.42 ± 0.03^{b}	0.45 ± 0.07^{b}
2-Heptenal, (E)-	1.06 ± 0.08^{a}	1.25 ± 0.09^{b}	1.64 ± 0.16^{c}	1.10 ± 0.12^{ab}	1.13 ± 0.18^{ab}	1.39 ± 0.09^{b}	2.31 ± 0.21^{d}
Benzaldehyde	0.12 ± 0.06^{a}	0.15 ± 0.02^{a}	0.16 ± 0.04^{a}	0.13 ± 0.07^{a}	0.14 ± 0.02^{a}	0.09 ± 0.07^{a}	0.12 ± 0.02^{a}
2,4-Hexadienal, (E,E)-	0.07 ± 0.03^{a}	0.11 ± 0.04^{a}	0.11 ± 0.03^{a}	0.09 ± 0.03^{a}	0.09 ± 0.02^{a}	0.16 ± 0.08^{a}	0.14 ± 0.06^{a}
Octanal	2.00 ± 0.34^{b}	1.72 ± 0.26^{ab}	1.77 ± 0.31^{ab}	2.00 ± 0.19^{b}	2.16 ± 0.27^{b}	2.18 ± 0.12^{b}	2.15 ± 0.22^{b}
Nonanal	0.93 ± 0.07^{ab}	1.09 ± 0.10^{b}	1.20 ± 0.17^{bc}	0.93 ± 0.10^{ab}	1.03 ± 0.07^{b}	1.16 ± 0.05^{b}	1.43 ± 0.12^{c}
2-Nonenal, (E)-	0.44 ± 0.12^{a}	0.48 ± 0.10^{a}	0.49 ± 0.08^{a}	0.55 ± 0.09^{a}	0.59 ± 0.11^{a}	0.61 ± 0.14^{a}	0.62 ± 0.18^{a}
Decanal	0.45 ± 0.08^{a}	0.48 ± 0.10^{a}	0.49 ± 0.19^{a}	0.45 ± 0.04^{a}	0.54 ± 0.16^{a}	0.52 ± 0.04^{a}	0.51 ± 0.08^{a}
2-Decenal, (Z)-	3.82 ± 0.51^{a}	4.25 ± 0.78^{a}	4.45 ± 0.70^{a}	4.10 ± 0.33^{a}	4.28 ± 0.69^{a}	4.50 ± 0.90^{a}	4.58 ± 0.64^{a}
2-Dodecenal, (E)-	0.37 ± 0.09^{a}	0.32 ± 0.04^{a}	0.41 ± 0.07^{a}	0.41 ± 0.05^{a}	0.38 ± 0.11^{a}	0.44 ± 0.08^{a}	0.41 ± 0.12^{a}
Total	36.09 ± 2.65	38.57 ± 4.27	38.82 ± 3.73	35.84 ± 2.69	37.33 ± 2.81	39.63 ± 3.54	41.81 ± 2.93
Esters							
Ethyl Acetate	0.07 ± 0.03^{a}	0.06 ± 0.01^{a}	0.08 ± 0.02^{a}	0.07 ± 0.01^{a}	0.08 ± 0.02^{a}	0.05 ± 0.02^{a}	0.07 ± 0.03^{a}
3-Hexen-1-ol, acetate, (Z)-	7.70 ± 0.87^{a}	7.48 ± 0.22^{a}	7.45 ± 0.60^{a}	7.63 ± 0.91^{a}	7.63 ± 0.28^{a}	7.44 ± 0.37^{a}	7.25 ± 0.39^{a}
Acetic acid, hexyl ester	2.10 ± 0.10^{a}	2.11 ± 0.33^{a}	2.27 ± 0.18^{a}	2.04 ± 0.22^{a}	2.26 ± 0.17^{a}	2.12 ± 0.11^{a}	2.15 ± 0.20^{a}
Total	10.05 ± 2.02	9.55 ± 2.49	9.80 ± 1.76	10.10 ± 1.80	10.07 ± 2.16	9.61 ± 2.09	9.57 ± 1.78
Ketones							
1-Penten-3-one	0.28 ± 0.09^{a}	0.27 ± 0.04^{a}	0.28 ± 0.05^{a}	0.25 ± 0.08^{a}	0.28 ± 0.12^{a}	0.28 ± 0.06^{a}	0.29 ± 0.14^{a}
3-Pentanone	0.17 ± 0.04^{a}	0.22 ± 0.05^{a}	0.16 ± 0.07^{a}	0.18 ± 0.04^{a}	0.18 ± 0.06^{a}	0.19 ± 0.07^{a}	0.18 ± 0.07^{a}
5-Hepten-2-one, 6-methyl-	0.29 ± 0.05^{a}	0.36 ± 0.05^{a}	0.38 ± 0.08^{a}	0.33 ± 0.06^{a}	0.30 ± 0.04^{a}	0.38 ± 0.04^{a}	0.48 ± 0.06^{a}
Total	0.69 ± 0.19	0.85 ± 0.14	0.82 ± 0.17	0.66 ± 0.20	0.76 ± 0.11	0.85 ± 0.07	0.95 ± 0.12
Hydrocarbons							
1,3-Pentadiene, (E)-	0.31 ± 0.16^{a}	0.35 ± 0.08^{a}	0.40 ± 0.15^{a}	0.30 ± 0.09^{a}	0.32 ± 0.16^{a}	0.33 ± 0.18^{a}	0.35 ± 0.17^{a}
3-Ethyl-1,5-octadiene	0.70 ± 0.09^{a}	0.82 ± 0.11^{a}	0.88 ± 0.17^{a}	0.63 ± 0.16^{a}	0.64 ± 0.19^{a}	0.89 ± 0.21^{a}	0.89 ± 0.18^{a}
.beta.-Ocimene	0.19 ± 0.04^{a}	0.22 ± 0.07^{a}	0.19 ± 0.03^{a}	0.18 ± 0.04^{a}	0.16 ± 0.06^{a}	0.21 ± 0.03^{a}	0.22 ± 0.03^{a}
2-Dodecene, (E)-	4.78 ± 0.24^{a}	4.98 ± 0.65^{a}	4.89 ± 0.28^{a}	4.72 ± 0.71^{a}	4.79 ± 0.44^{a}	5.07 ± 0.45^{a}	4.99 ± 0.49^{a}
Copaene	0.18 ± 0.05^{a}	0.19 ± 0.03^{a}	0.16 ± 0.08^{a}	0.20 ± 0.06^{a}	0.16 ± 0.03^{a}	0.14 ± 0.08^{a}	0.14 ± 0.04^{a}
alpha.-Farnesene	0.59 ± 0.17^{a}	0.70 ± 0.09^{a}	0.64 ± 0.06^{a}	0.57 ± 0.09^{a}	0.57 ± 0.11^{a}	0.62 ± 0.06^{a}	0.56 ± 0.13^{a}
Total	6.75 ± 0.17	7.26 ± 0.18	7.16 ± 0.26	6.60 ± 0.11	6.64 ± 0.11	7.26 ± 0.16	7.15 ± 0.13

^{a,b,c,d}are used to compare means of samples in both materials and temperatures at different times. Means that do not bear a common superscript differ significantly.

* SD.

glass bottles.

Table 2, shows changes in color parameters of EVOO as a function of storage time and temperature. Non-significant changes ($p > 0.05$) were observed in luminosity parameter L* values of all samples at all sampling times. Color parameter a* decreased significantly ($p < 0.05$) with time in samples packaged in both containers at both temperatures indicating a change in color from less green to more green. A small but statistically significant ($p < 0.05$) decrease was observed in color parameter b*, which corresponds to the yellow zone, during storage at 37 °C for both packaging materials beginning with day 80 of storage indicating a decrease in yellowness of EVOO. Such a change may be due to the partial loss of carotenoids during storage. At this point it should be mentioned that visual macroscopic differences in olive oil color between different container type, storage time and temperature were not evident.

Morelo, Motilva, Tovar, and Romero (2004), found no change in parameter b* and an increase in parameter L*, as a consequence of loss of carotenoids and chlorophyll, after a 12 months storage of fresh olive oil kept in the dark at ambient temperature. Sikorska et al. (2007) reported that for olive oil packaged in clear glass, color remained stable when stored in the dark while for samples stored under diffused light, L* and a* increased while b* decreased. Finally, Mendéz and Falqué (2007) reported a change in color to more brownish after 3 months of storage for olive oil samples stored in clear PET, PET covered with aluminum foil and glass bottles at room temperature, all with the same surface exposure to air and light. The phenomenon was not observed in tin and Tetra brick containers under the same storage conditions.

With regard to the fatty acid profile, results indicate that there were no significant changes ($p > 0.05$) in monounsaturated and polyunsaturated fatty acids of EVOO packaged in both containers at both temperatures tested throughout the four month storage period (results not shown). The initial content of fatty acids of olive oil samples was within the established limits. The predominant fatty acid was oleic (≈ 78%) followed by palmitic (≈ 11%) and linoleic (≈ 6%). The unsaturated fatty acids (linoleic and linolenic acids) are very important regarding the stability of oils and their positive contribution to health issues such as cardioprotective effects and provision of mechanisms aiding to the prevention and treatment of numerous diseases.

Present results are in general agreement with those of Gargouri et al. (2015), and Gutiérrez and Fernández (2002) who reported minor changes in olive oil fatty acids stored at room temperature under diffused light and at 2 °C (in the dark) and 30 °C (under illumination) respectively. Non-significant changes were also reported by Gomez-Alonso et al. (2007) for low temperature storage conditions in the dark. The same trend was observed in both unsaturated and saturated fatty acids. Similar results are also reported by Mendéz and Falqué (2007). Likewise, Rastrelli, Passi, Ippolito, Vacca, and De Simone (2002) reported no change in polyunsaturated fatty acids for olive oil stored for an 8 month period in colorless glass (full and half full bottles) in the dark.

Table 3, shows the initial ($t = 0$) volatile compounds profile of EVOO and its changes at $t = 20$, $t = 80$ and $t = 120$ days. It is a typical profile for the Koroneiki variety as also reported by Pouliarekou et al. (2011) and Kosma et al. (2015). (E)-2-Hexenal was the predominant compound of the volatile fraction which along with other C5 and C6 alcohols, aldehydes and ketones originating from linoleic and linolenic acid through the lipoxygenase (LOX) pathway give EVOO its characteristic aroma described as “green”, “leaf-like”, “green-apple”, “cut grass”, “bitter almonds”, “fruity” etc. (Aparicio & Luna, 2002; Morales, Luna, & Aparicio, 2005). In addition, terpenoid hydrocarbons such as alpha-Farnesene, Copaene and beta-Ocimene were present, responsible for “herbal”, “woody”, “sweet”, etc. aromas (Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006).

Within changes in volatile compounds that occurred throughout the 4 month storage period we focused on LOX (pentanal, 2-pentenal (E), hexanal, 2-hexenal (E), 2-hexen-1-ol (E), 1-hexanol, 3-hexen-1-ol acetate (Z) and acetic acid hexyl ester) as well as basic oxidation products which are responsible for the rancid defect and “fatty”, “bitter” aroma (heptanal, 2-heptenal (Z), octanal, nonanal and 2-Decenal (Z)) of olive oil. According to Morales et al. (2005) the odor threshold (μg/kg) of these aldehydes is very low making their presence crucial in the development of the EVOO aroma defects.

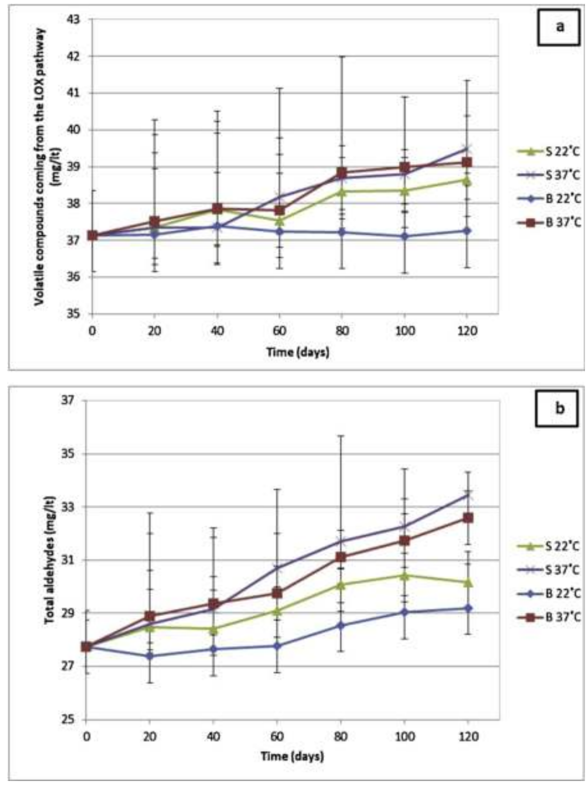

Fig. 2. Changes in (a) total volatile compounds and (b) total aldehydes as a function of storage time and temperature (S = tinplated steel; B = bag-in-box).

Fig. 2 shows a general trend of the changes in the amount of volatile compounds throughout the storage period.

In Fig. 2a, volatile compounds originating from the LOX pathway generally increased up to 120 days of storage in all samples. Overall, these changes for both packaging materials and temperatures were non-significant ($p > 0.05$) as the initial volatiles’ content was 37.1 mg/L reaching a total of 39.4 mg/L for samples stored in tinplated steel containers at 37°C.

More specifically, Fig. 2b, shows that total aldehydes originating from the LOX and/or secondary oxidation pathways increased with time. As expected, the aldehyde content was lower ($p < 0.05$) at 22 °C compared to that at 37 °C for both packaging materials. Temperature significantly ($p < 0.05$) affected aldehyde production leading to a major increase in aldehyde content at 37 °C. Similar results were also reported by Esposto et al. (2017) linking the phenolic content of the sample to the production rate of aldehydes. The same link appeared in the present study as well. As mentioned above, samples at 37 °C showed the greatest loss in TPC and also the greatest increase in aldehyde content. Di Giovacchino et al. (2002) also reported an increase in olive oil volatile compounds up to 10 and 15 months of storage mainly owed to aldehyde formation such as hexanal and *trans*-2-hexenal especially at extreme storage temperatures (40 °C). Moreover, Brkić, Koprivnjak, Sladonja, and Belobrajić (2014) suggested that storage temperatures lower than 20–22 °C aid extra virgin olive oil in maintaining its favorable volatile profile.

C5 alcohols, ketones and aldehydes originating from the lipoxygenase pathway were slightly reduced on day 120 ($p > 0.05$). Hexanal significantly increased ($p < 0.05$) on day 120, but there were

Table 4A
Pearson coefficients between quality parameters of oil stored at tinplated steel at 22 °C (above the diagonal) and at 37 °C (below the diagonal).

Correlations	acidity	PV	TPC	K_{232}	K_{270}	L*	a*	b*
acidity	–	.942**	−.942**	.931**	.953**	.353	−.945**	−.835*
PV	.984**	–	−.872*	.937**	.890**	.429	−.921**	−.856*
TPC	−.947**	−.982**	–	−.872*	−.924**	−.246	.954**	.820*
K_{232}	.951**	.963**	−.970**	–	.873*	.242	−.953**	−.888**
K_{270}	.952**	.982**	−.968**	.966**	–	.155	−.953**	−.889**
L*	.463	.471	−.419	.329	.353	–	−.123	.098
a*	−.977**	−.981**	.975**	−.990**	−.974**	−.366	–	.941**
b*	−.910**	−.944**	.965**	−.970**	−.974**	−.197	.968**	–

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

Table 4B
Pearson coefficients between quality parameters of oil stored at bag-in-box at 22 °C (above the diagonal) and at 37 °C (below the diagonal).

Correlations	acidity	PV	TPC	K_{232}	K_{270}	L*	a*	b*
acidity	–	.976**	−.946**	.944**	.567	.126	−.967**	−.011
PV	.961**	–	−.968**	.898**	.484	.204	−.946**	−.010
TPC	−.982**	−.958**	–	−.888**	−.466	.010	.942**	.087
K_{232}	.970**	.951**	−.922**	–	.334	−.032	−.976**	−.160
K_{270}	.878**	.959**	−.884**	.851*	–	.090	−.413	.241
L*	.309	.343	−.377	.155	.511	–	.012	.401
a*	−.996**	−.944**	.970**	−.970**	−.857*	−.324	–	.237
b*	−.791*	−.712	.751	−.850*	−.523	.183	.805*	–

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

no significant differences between materials at a given temperature. 2-Hexenal (E) remained stable for samples stored at both temperatures and for both materials. 2-hexenal (E) and 1-hexanol have been suggested as quality marker compounds by Kalua, Bedgood, Andrea, Bishop, and Prenzler (2006) for storage of olive oil in the dark. The loss of these compounds could lead to a less favorable olive oil aroma. In the present study a significant loss ($p < 0.05$) in 1-hexanol was recorded with time.

Likewise, 2-Hexen-1-ol (E), 3-Hexen-1-ol, acetate (Z) and acetic acid, hexyl ester decreased for both materials and temperatures ($p > 0.05$).

A significant increase ($p < 0.05$) was recorded in heptanal, 2-heptenal (E), octanal and nonanal concentration on day 120 as compared to day 0. In most cases higher values for these aldehydes were recorded at 37 °C as compared to 22 °C. In the case of 2-heptenal (E) and nonanal at 37 °C, the bag-in-box packaging material managed to maintain these compounds at significantly ($p < 0.05$) lower concentrations than tinplated steel. According to Morales et al. (2005) 2-heptenal (E) is characterized by an oxidized, tallowy and pungent aroma while nonanal by a fatty, waxy, pungent aroma which renders them unpleasant for consumers. Nonanal may be detected in fresh oils as well, but 2-heptenal (E) is mainly produced during storage and may be used as a marker of oxidation as suggested by Kanavouras, Hernandez-Münoz, and Coutelieris (2004) for samples stored under various temperature (15 °C, 30 °C and 40 °C) and packaging material (glass, PET, PVC) conditions under light or in the dark.

Finally, Pearson correlations between quality parameters of samples packaged in tinplated steel containers and stored at 22 °C and 37 °C are presented in Table 4A. Results (for 22 °C, Table 4A) show that peroxide value was positively and significantly correlated to acidity and K_{232} extinction coefficient. The correlation with phenolic content was significantly negative, and also non-significantly correlated to K_{270}. K_{232} was significantly and positively correlated to acidity and peroxide value but was significantly and negatively correlated to phenolic content. There was no significant correlation found between K_{270} and phenolic content. At 37 °C, peroxide value was significantly and positively correlated to acidity and both extinction coefficients, and also significantly and negatively correlated to phenolic content. K_{232} was positively correlated to both K_{270} and phenolic content. The correlation of K_{232} to K_{270} was positive while its correlation to the phenolic content was negative. K_{270} and phenolic content were significantly and negatively correlated to each other. The same results apply to samples packaged in bag-in-box containers and stored at both 22 °C and 37 °C as presented in Table 4B.

4. Conclusions

Based on data presented in the present study, it may be concluded that EVOO packaged in bag-in-box containers intended for household use will retain its original high quality longer than that packaged in tin plated steel containers both at 22 °C and 37 °C. Indeed, EVOO packaged in bag-in-box containers at room temperature retained its high quality throughout the 120 day storage period unlike EVOO packaged in stainless steel containers whose shelf life was limited to 80 days. In addition, EVOO packaged in stainless steel containers should always be kept at temperatures below or equal to ambient otherwise its shelf life will be restricted to less than 60 days.

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