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Impact of packaging material and storage time on olive oil quality

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Olive oil is very appreciated for its characteristic flavor and its biological and nutritional value which are strongly related to the quality. The effect of packaging materials (stainless, jar, clear polyethylenene terephthalate (PET), clear glass and dark glass bottles) on quality attributes of extra virgin olive oil (EVOO) was studied as a function of storage time (0 to 12 months). The results made it possible to highlight a light influence of time as well as type of container on the acidic composition of oils, although oleic acid slightly increased at the end of the analytical period. Indeed, the least stable oils were those stored in the jars with a progressive increase in quality attributes and the palmitic acid level. A clear reduction in the contents of antioxidants (carotenes, chlorophylls and total phenols) was observed in the oils stored in the earthenware jars and PET. Quality indexes were strongly influenced by the type of packaging material and the time of storage. Overall, the results revealed that the storage of oils in stainless and dark glass appears most adequate, thus supporting the conservation of primarily contents antioxidants with indices of quality indicating an unrefined olive oil lasting storage.

Key words: Olive oil quality, storage, type of recipient, fatty acids, phenols, pigments.

INTRODUCTION

Virgin olive oil (VOO) constitutes the principal source of dietary fat in the Mediterranean basin. Unlike vegetable oils, VOO is a fresh squeezed juice from olives; maintains its properties including natural antioxidant components such as phenolic compounds, tocopherols and pigments, as well as volatile compounds (Angerosa, 2002; Tsimidou et al., 2003) that will keep the olive oil longer than the other vegetable oils without a refined processing (Bosque-Sendra et al., 2011). Furthermore, the composition differs from that of other dietary fats in that olive oil is rich in monounsaturated fatty acids, which contribute to beneficial health effects (Ozyilkan et al., 2005).

The importance of olive oil is due to the increasingly consumption around the world, because of its nutritional and sensory properties (mainly aroma) which represent the result of a complex mixture of volatile compounds (Bosque-Sendra et al., 2011). However, like other vegetable oils, it is susceptible to oxidation, which has

been recognised as the predominant cause of oil deterioration during storage (Morello et al., 2004). Moreover, during storage of olive oil, hydrolysis, esterifi-cation and oxidative reactions also originate changes, especially a partial loss in the minor constituents, considered primarily responsible for its beneficial health effects (Ligor and Buszewski, 2008). The minor components in VOO may act either as anti-oxidants or pro-oxidants, and processing and storage of the oil influence the composition of these minor constituents and hence the oil's stability. This is why VOOs, with identical fatty acid compositions, can show differences in stability (Kalua et al., 2007).

To assess the role of the different modes of storage on the quality of olive oil, literature results concerning the analytical definition of the quality and composition of oils stored were critically reviewed. Different papers have been published in relation to olive oil stability where they proved that packaging can directly influence olive oil quality by protecting the product from both oxygen and light (Kanavouras et al., 2004; Kiritsakis 1998). In fact, many authors (Del Nobile et al., 2003; Zanoni et al., 2005; Kanavouras et al., 2006) with three different types

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of container such as glass, PET (Polyethylene tetraphthalate) and PVC (polyvinylchoride) showed that predictive modelling using advanced statistical techniques has also been used to evaluate olive oil stability. A comparison between a modified active oxygen method and long term storage was evaluated (Kaya et al., 1993). The effect of the storage at different temperatures on the quality parameters of VOO was monitored (Pasini et al., 2009). Furthermore, the quality parameters of VOO were studied in different types of containers during six months at 20 to 22°C (Méndez and Falqué, 2007). Secoiridoid and tocopherol contents as well as antioxidant activity were studied for eight months of storage in the dark at 25 and 40°C (Roca et al., 2003). Chemical changes (quality parameters, fatty acid composition, oxidative stability index, phenol and tocopherol content) produced in an extra virgin olive oil in the presence and absence of its phenolic fraction during an accelerated storage treatment at 60°C up to seven weeks were undertaken (Lerma-García et al., 2009). Moreover, the evolution of the phenolic fraction of olive oil in amber glass bottles was studied over a period of 12 months at room temperature (Sicari et al., 2010). Pigment composition was analysed monthly during one year at 15°C to predict a model, using discriminated criterion, for olive variety classification (Lavelli et al., 2006). Another investigation was done on olive oil oxidation, during 24 months at 18 to 28 °C where it analysed some chemical parameters such as, chlorophyll, carotenoïd, total polar compounds, squalene and α-tocopherol content (Psomiadou and Tsimidou, 2002a, 2002b). There are also research works which studied the stability of olive oils, in relation to lighting room and ambient temperature, using quality parameters. tocopherols, fatty acids and sterols (Gutiérrez and Fernández, 2002). A work conducted by Esti et al. (2009) on phenolic compounds and temporal perception of bitterness and pungency was evaluated in oils stored in full filled dark bottles, up to 18 months at temperatures of 10 and 28 °C.

The stability of extra virgin olive is primarily due to its fatty acid composition and the antioxidant activity of its polyphenols and tocopherols. The principal degradative aspects of storage involve oxidation of fats, especially in the presence of trace metals. Reactive oxygen radicals attack double bonds of unsaturated fatty acids with the initial formation of lipid peroxide. The susceptibility to oxidation is greatly increased by polyunsaturation (Harwood and Yaqoob, 2002; Conde et al., 2008). Furthermore, storage influences oil colour, which is one of the basic quality characteristics of VOOs. The greenyellowish colour is due to various pigments, such as chlorophylls, pheophytins and carotenoïds. Such natural pigments can also affect considerably the preservation of the product as prooxidant, in synergy with metals eventually present (Cichelli and Pertesana, 2004). In particular, the chlorophylls and the pheophytins in the presence of the light act as catalysts in the formation of singlet state oxygen (Rahmani and Csallany, 1998) and

therefore they promote the first phases of the autoxidation process. Moreover, some researches underline the delaying role of the carotenoïds in the photooxidation process (Chen and Liu, 1998).

So far as we know, no previous studies have been conducted on the traditional recipient for storage oil with a special emphasis on the evaluation of quality and physicochemical and antioxidant attributes of the stored oil. This work was undertaken to investigate the applications of selected recipient for the effect of time storage and to evaluate the nutritive quality of the oil obtained. The physicochemical and antioxidant characteristics of the oils, produced by different treatments, were studied and compared with those of the control oils.

MATERIALS AND METHODS

Olive sampling and oil extraction

The olive oil samples were obtained from a Tunisian quality-assured industrial oil mills during the crop season 2008/2009. The stability of the samples was analysed under the following storage conditions: diffused light and room temperature. The samples were stored in the established conditions and in the following pack aging materials (stainless, jar, clear PET, clear glass and dark glass bottles). Then, they were analysed every three months throughout the duration of the study. The study was programmed for 12 months after the extraction of oils.

Physical and chemical parameters of oil

Regulated physicochemical quality parameters such as free fatty acids (FFA), peroxide value (PV) and the absorption values at 232 and 270 nm of the oils were assessed following the analytical methods described by the Regulation EEC/2568/91 and EEC/1429/92 of the Commission of the European Union (EUC, 1991).

Fatty acids analysis

In order to determine fatty acid composition (%), the methyl-esters were prepared by vigorous shaking of a solution of oil in hexane (0.1 g in 2 ml) with 0.2 ml of 2 N methanolic potassium hydroxide solution and analysed by GC with a Hewlett–Packard (HP 5890) chromatograph equipped with a FID detector. A fused silica column, HP-Innowax (30 m length \times 0.25 mm i.d \times 0.25 μm film thickness), was used. Nitrogen was employed as a carrier gas, with a flow through the column of 1 ml/min. The temperatures of the injector and detector were set at 250 and 270 °C. An injection volume of 1 μl was used. The operating conditions were as follows: oven temperature was held at 180 °C for 1 min and then increased by 10 °C min $^{-1}$ to 220 °C, held for 1 min at 220 °C, increased again to 240 °C at 2 °C min $^{-1}$ and finally isotherm at 240 °C for 1 min. Results were expressed as percent of relative area (Dabbou et al., 2009).

Pigments

Oil (7.5 g) was accurately weighed, dissolved in cyclohexane and taken to a final volume of 25 ml. Carotenes and chlorophylls pigments were determined by measuring the absorbance at 470 and 670 nm, respectively. The results were expressed as mg of pheophytin " α " and lutein per kg of oil, respectively (Minguez-Mosquera et al., 1991).

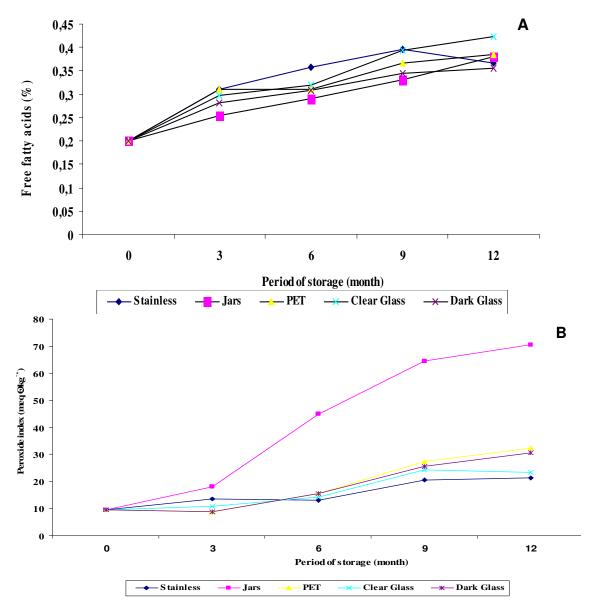


Figure 1. Effect of storage on quality parameters of olive oil. A, Effect on free fatty acids contents; B, effect on peroxide value formation; C, effect on K232; D, effect on K270

Total phenols

Total phenol compounds were isolated by extraction of a solution of oil in methanol/water mixture (80:20) 2% between 20, two times. Folin-Ciocalteau reagent and sodium carbonate were added to a suitable aliquot of the combined extracts and the absorbances of the solution, at 765 nm, were measured. Values were given as mg of hydroxytyrosol per kg of oil (Montedoro et al., 1993).

Statistical analysis

All the experiments were performed in triplicate and the statistical analysis of the data was done by analysis of variance (ANOVA) using a SPSS programme release 11.0 for Windows (SPSS, Chicago, IL, USA). A probability value at p < 0.05 was considered statistically significant. Data were expressed as mean values \pm

standard deviation derived from triplicate determinations.

RESULTS AND DISCUSSION

Quality characteristic in fresh oils

The quality characteristics of the oils in the fruits before been stored were determined. The values of FFA, PV and coefficients of specific extinction (K232 and K270) are shown in Fig. 1A, B, C and D. All values were lower than the limits set by IOC (2009) for extra virgin olive oil. The lower values of FFA and PV found in fresh oils (0. 2% and 9.60 meg O2 kg-1 of oil, respectively) suggest

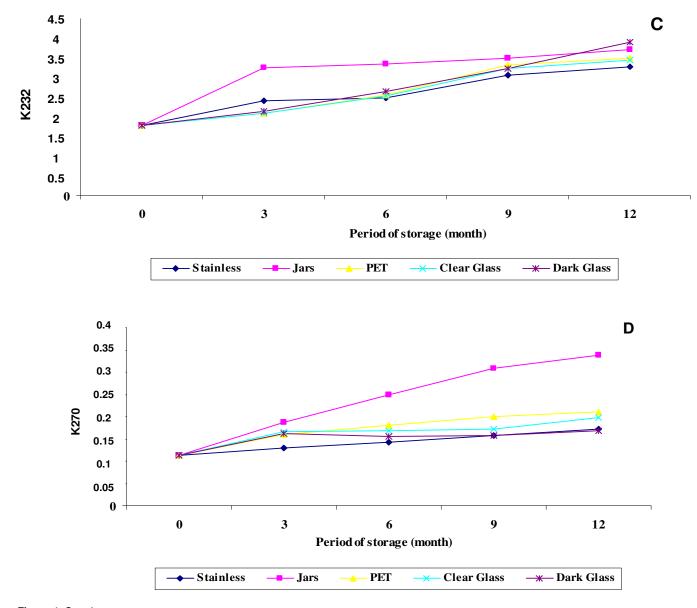


Figure 1. Contd.

that the oil can be stored for a long period without deterioration (Nehdi, 2011). In fact, these values confirmed the high quality of the olive oils studied.

Physico-chemical properties of stored oils

The quality parameters of the stored oils behaved differently during storage.

Changes in free fatty acids

Figure 1A shows that there was a minimum increment in the free fatty acids level in all materials analyzed, but it was within the limits. These results agreed with those of Pristouri et al. (2010). In jars material, this parameter showed the lowest trend whereas in the stainless, this increase was always higher.

Changes in peroxide value

Hydroperoxide formation in a crude oil can serve as an indicator of the oxidative processes and in turn, of the oil quality. Thus, a rapid hydroperoxide formation evidences the initiation of the oxidative reactions that precede rancidity (Elez-Martinez et al., 2007). The evaluation of PV is shown in Figure 1B. In jars material, there was an uptrend of this parameter. In fact, in this material, PV showed a data increase which was emphasized in the sixth month. PV increased at about seven times higher

than the control at the end of storage. Stainless, PET, clear and dark glass materials presented similar trends than jars material but with short variability and an up to limit after the ninth month. For these stored oil samples, an increase of about two to three times was detected. Kiritsakis and Dugan (1984) also reported that peroxide values were higher for olive oil packaged in plastic containers as compared to those packaged in glass bottles in the dark. In fact, the oil can be oxidized easily when it is displayed in stores in diffused light. During that time, oxygen may enter into the plastic containers due to the permeability of some plastic bottles and initiate the oxidation mechanism. In addition, the presence of light will facilitate oxidation.

In clear glass, PV increased up to a maximum (reached after 9 months of storage) and then decreased. According to Baiano et al. (2005), this behaviour can be explained if it is accepted that there was an initial increase in hydroperoxides (odorless, flavorless compounds, produced during the primary step of oxidation) and that they successively broke down into aldehydes and ketones. These latter compounds are responsible for off-flavors (secondary oxidation). Non-volatile compounds such as oligopolymers and cyclic compounds are also produced by breakdown of hydroperoxides. The aforementioned results are in general agreement with those of Min (1998) who reported higher losses in olive oil quality stored under light as compared to those stored in the dark.

Changes in K270 and K232 parameters

The specific extinctions at 232 and 270 nm, which revealed the oxidative deterioration and purity of the oils, are shown in Figure 1C. In jars material, K232, an indicator of formation of hydroperoxide and conjugated dienes, increased in value starting from the 3rd month. K270 nm, a good indicator of the secondary phase of oxidation because it is related to the presence of final products such as trienes or unsaturated carbonyl compounds, which account for the characteristic flavor of an oxidized oil, showed the same trends (Gertz and Klostermann, 2000). During the 12 months of storage, K270 of VOO increased very slowly, while the increase of K232 was much faster, although without exceeding the extra VOO maximum limit. However, this increase is not so pronounced like FFA and PV levels (Figure 1C). This indicates accumulation of primary oxidation products and negligible formation of secondary products, characteristic of the initial phase of oxidative degradation (Koprivnjak et al., 2010). Therefore, the changes in K270 parameter showed formation of ethylenic diketones (Bosque-Sendra et al., 2011). All the other materials presented a similar behaviour among the parameters: K270 and K232 parameters seem to be stable and they did not show tendency in their data. Therefore, there was no evidence

of formation of hydroperoxide or secondary oxidation products. On the other hand, the K232 trend started in the 3rd month.

Then, the elevated operational time might affect the oil quality, particularly, the oxidation state of the oils. Consequently and as previously reported, glass containers are generally preferred to plastic for bottling VOO due to the fact that glass containers prevent the permeation of oxygen molecules into the bottle; slow down the rate at which the autoxidation reaction of unsaturated fatty acids proceeds as compared to their plastic counterparts (Del Nobile et al., 2003).

Chemical composition of fresh oils

As illustrated in Table 1, fresh olive oil showed high contents of linolenic (1.3%) and linoleic (17.2%) acids as well as palmitic acid (18.4%), but a moderate level of oleic acid (57.23%). The greater percentage of unsaturated fatty acids present in this oil could lead to faster rate of rancidity in the oil during extended storage and exposure to higher storage temperature (Terigar et al., 2010). Furthermore, as shown in Figures 2 and 3, the fresh oil seems to be very stable thanks to its richness in the minor constituents (49.3, 18.31 and 725 mg kg⁻¹ of oil, for chlorophylls, carotenes and phenols, respectively).

Changes in the olive oil components

Changes in Fatty acids composition

Lipid oxidation is a major deteriorative reaction affecting edible oils and fats and consequently of primary concern to processors and consumers. Unsaturated lipids are particularly susceptible to oxidation during processing and storage via autoxidation and photosensitized oxidation (Marfil et al., 2008). Olive oil fatty acid composition for stainless, jar, PET, clear glass and dark glass bottles are given in Table 1 as a function of storage time. The fatty acid contents fell within the intervals required by the EU (European Union) regulations in all the analysed olive oil samples. Storage of olive oil for 12 months in relation to the type of container had no significant effect on the fatty acid composition between the studied storage conditions since significant differences could not be found between the storage times under the same storage conditions. In fact, although ANOVA test and the Duncan test applied on olive oil samples did not evidence any statistically significant difference for all fatty acids analysed, fatty acid profiles were slightly modified throughout the storage period. As regards the content of palmitic acid, the significantly highest values were found for the samples of oils stored in jars, PET and clear glass. For palmitoleic, margaric and behenic acids, the highest values were also found in

Table 1. Fatty acid composition (%) of virgin olive oils from the different storage conditions.

Parameter -	Fatty acid composition (%)									
	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1				
Stainless										
Fresh	18.41(0.15)**	2.94(0.01)*	0.08(0.01)*	0.18(0.03)*	1.13(0.03)*,**	57.23(0.06)*				
3 M	18.67(0.01)a,b,*,**	2.96(0.03)a,b,*	0.07(0.01)b,*	0.15(0.03)a,*	1.08(0.10)c,**	56.85(0.74)a,*				
6 M	18.85(0.28)e,f,*	2.94(0.04)f,*	0.08(0.01)f,g,*	0.17(0.01)e,*	1.20(0.04)f,*	57.08(0.05)g,*				
9 M	18.69(0.13) I,*,**	2.96(0.09)1,*	0.07(0.04)k,l,*	0.19(0.04)k,*	1.17(0.01)I,*,**	57.03(0.47)1,*				
12 M	18.50(0.05)x,**	2.95(0.02)w,*	0.07(0.01)w,x,*	0.14(0.01)x,*	1.11(0.06)x,*,**	57.33(0.10)x,*				
Jars										
Fresh	18.41(0.15)###	2.94(0.01)#	0.08(0.01)#	0.18(0.03)#	1.13(0.03) ###	57.23(0.06) ###				
3 M	18.85(0.04)a,b,c,##	2.89(0.01)c,##	0.09(0.01)a,#	0.17(0.01)a,#,##	1.29(0.01)a,#	57.07(0.02)a,###				
6 M	18.82(0.04)e,f,##	2.91(0.01)f,#,##	0.08(0.01)g,#	0.17(0.01)e,#,##	1.21(0.01)f,##	57.58(0.01)e,##				
9 M	19.02(0.03)k,#	2.89(0.03)1,#,##	0.04(0.01)1,###	0.16(0.01)k,#,##	1.24(0.02)k,#,##	57.80(0.16)k,#				
12 M	19.07(0.05)w,#	2.92(0.05)w,#,##	0.06(0.02)x,##	0.15(0.01)w,x,##	1.24(0.06)w,#,##	57.88(0.13)w,#				
PET										
Fresh	18.41(0.15)§	2.94(0.01)§	0.08(0.01)§,§§	0.18(0.03)§	1.13(0.03)§	57.23(0.06)§,§§				
3 M	19.60(0.04)b,c,§	2.91(0.01)b,c,§	0.08(0.01)b,§§	0.17(0.04)a,§	1.19(0.05)b,§	57.10(0.08)a,§§,§§§				
6 M	18.52(0.03)f,§	2.99(0.03)e,§	0.09(0.01)e,§	0.20(0.02)e,§	1.20(0.04)f,§	57.03(0.29)g,§§,§§§				
9 M	18.56(0.18) l,m,§	2.96(0.07)1,§	0.08(0.01)k,§,§§	0.16(0.02)k,§	1.18(0.07)k,l,§	57.46(0.06)k,l,§				
12 M	18.58(0.10)x,§	2.98(0.05)w,§	0.08(0.01)w,§§	0.17(0.03)w,§	1.12(0.10)x,§	56.83(0.31)y,§§§				
C. Glass										
Fresh	18.41(0.15)♦♦	2.94(0.01)♦♦	0.08(0.01) +, ++	0.18(0.03) +, ++	1.13(0.03) ****	57.23(0.06)♦				
3 M	18.93(0.27)a,◆	2.87(0.04)c,◆◆	0.07(0.01)b, **, ***	0.15(0.01)a, **, ***	1.20(0.02)b, ◆◆◆	57.15(0.23)a,◆				
6 M	19.17(0.31)e,◆	2.73(0.02)h, ◆◆◆	0.09(0.01)e,f,◆	0.19(0.02)e,◆	1.31(0.01)e,◆	57.42(0.23)e,f,◆				
9 M	18.39(0.02) m, ♦♦	3.13(0.13) k,◆	0.07(0.01)k,l, ◆◆◆	0.14(0.01)1,	0.98(0.01)m,****	56.45(0.27)m, ♦ ♦				
12 M	18.92(0.01)w, ♦	2.84(0.03)x, ♦♦ , ♦♦♦	0.08(0.01)w, ◆ , ◆ ◆	0.15(0.01)w,x, ** , **	1.25(0.01)w, ◆◆	57.24(0.10)x, ♦				
D. Glass										
Fresh	18.41(0.15)†††	2.94(0.01)††	0.08(0.01)†	0.18(0.03)†	1.13(0.03)††	57.23(0.06)†				
3 M	18.43(0.14)c,††,†††	3.00(0.05)a,†	0.07(0.01)b,††	0.14(0.01)a,†	1.08(0.02)c,††	57.21(0.06)a,†				
6 M	18.75(0.05)f,†	2.87(0.01)g,†††	0.04(0.01)h,†††	0.18(0,03)e,†	1.25(0.07)e,f,†	57.21(0.01)f,g,†				
9 M	18.68(0.03)1,†,††	2.87(0.01) l,†††	0.04(0.01)I,†††	0.18(0.02)k,†	1.24(0.02)k,†	57.40(0.06)k,l,†				
12 M	18.50(0.22)x,†,††,†††	2.94(0.04)w,††	0.07(0.01)w,x,††	0.14(0.01)x,†	1.12(0.01)x,††	57.21(0.19)x,†				

Table 1 Contd. Fatty acid composition (%) of virgin olive oils from the different storage conditions.

D	Fatty acid composition (%)										
Parameter	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0				
Stainless											
Fresh	17.22(0.01)***	1.29(0.12)*,**	0.58(0.01)*	0.33(0.01)*	0.02(0.01)*	0.20(0.01)*	0.40(0.02)*				
3 M	17.67(0.63)a,*	1.17(0.05)a*+	0.58(0.01)a*	0.32(0.01)a*	0.02(0.01)b*	0.17(0.01)b**	0.29(0.01)c**				
6 M	17.01(0.14)e**	1.30(0.08)ef**	0.58(0.01)fg*	0.32(0.01)ef*	0.02(0.01)f*	0.16(0.01)g+	0.30(0.01)f**				
9 M	17.32(0.16)I**+	1.44(0.24)k*	0.59(0.03)kl*	0.34(0.03)k*	0.02(0.01)kl*	0.03(0.01)I***	0.15(0.02)m***				
12 M	17.26(0.21)wx**	1.14(0.03)y**	0.57(0.01)wx*	0.32(0.01)w*	0.02(0.01)x*	0.20(0.01)w*	0.39(0.04)w*				
Jars											
Fresh	17.22(0.01)#	1.29(0.12)#	0.58(0.01)#	0.33(0.01)#	0.02(0.01)##	0.20(0.01)#	0.40(0.02)#,##				
3 M	16.94(0.01)b##	1.17(0.05)a,##	0.55(0.01)d,###	0.29(0.02)b,##	0.02(0.01)a,b,##	0.22(0.01)a,b,#	0.46(0.01)a,#				
6 M	16.72(0.01)f###	1.15(0.01)g,##	0.57(0.01)f,##	0.31(0.01)e,f,#,##	0.02(0.01)f##	0.17(0.01)f,g,#	0.30(0.01)f,##,###				
9 M	16.47(0.03)n###	1.13(0.01) ##	0.57(0.01)1,#,##	0.30(0.01)1,##	0.05(0.03)kl#	0.09(0.08)1,##	0.23(0.08)1,###				
12 M	16.41(0.07)y####	1.10(0.02)z##	0.56(0.01)xy##	0.31(0.01)xy###	0.02(0.01)x##	0.02(0.01)x,###	0.26(0.11)w#####				
PET											
Fresh	17.22(0.01)§	1.29(0.12)§§§	0.58(0.01)§	0.33(0.01)§	0.02(0.01)§§	0.20(0.01)§	0.40(0.02)§§				
3 M	17.10(0.10)b§	1.23(0.03)a§§§	0.57(0.01)b§	0.31(0.01)a§	0.03(0.01)a§	0.23(0.01)a§	0.49(0.04)a§§§				
6 M	17.18(0.11)e§	1.38(0.14)e§	0.58(0.02)f§	0.32(0.02)e§	0.02(0.01)f§§	0.16(0.01)fg§§	0.33(0.01)ef§				
9 M	17.03(0.20)m§	1.19(0.04)I§§	0.57(0.01)I§	0.32(0.01)kl§	0.02(0.01)kl§§	0.17(0.01)k,§§	0.30(0.02)kl§				
12 M	17.51(0.51)w§	1.25(0.04)w§§§	0.58(0.01)w§	0.31(0.01)wx§	0.02(0.01)x§§	0.21(0.05)w§§§	0.36(0.07)w§§§				
C. Glass											
Fresh	17.22(0.01)♦♦	1.29(0.12)♦	0.58(0.01) ***	0.33(0.01)♦	0.02(0.01) ****	0.20(0.01)	0.40(0.02)				
3 M	17.10(0.13)b ♦ ♦	1.20(0.02)a, ♦ , ♦♦	0.56(0.01)c, ****	0.32(0.01)ab◆◆	0.02(0.01)ab◆◆	0.08(0.07)c♦♦	0.37(0.06)b◆				
6 M	16.34(0.13)g ◆◆◆ ◆	1.17(0.03)f,g,♦♦	0.62(0.01)e◆	0.32(0.01)♦	0.02(0.01)f++++	0.21(0.02)e◆	0.41(0.09)e◆				
9 M	18.26(0.16)k ♦	1.13(0.10)I♦♦	0.60(0.01)k ◆ ◆	0.31(0.01)k◆	0.01(0.01) +++	0.17(0.02)k◆	0.33(0.04)k♦				
12 M	16.86(0.03)xy ◆◆◆	1.17(0.03)xy ◆◆	0.56(0.01)y++++	0.33(0.01)y ◆◆	0.04(0.01)w◆	0.20(0.01)w◆	0.40(0.09)w◆				
D. Glass											
Fresh	17.22(0.01)††	1.29(0.12)†	0.58(0.01)†	0.33(0.01)†	0.02(0.01)†	0.20(0.01)†	0.40(0.02)†††				
3 M	17.44(0.14)ab†	1.20(0.01)a†	0.56(0.01)c††††	0.30(0.01)a†††	0.02(0.01)b†	0.18(0.03)ab†	0.35(0.05)b†				
6 M	17.02(0.02)e†††	1.20(0.02)f,g,†	0.55(0.01)g,†††	0.31(0.01)f,††	0.05(0.03)e†	0.19(0.04)ef†	0.39(0.06)e†				
9 M	16.96(0.02)m†††	1.21(0.01)1,†	0.57(0.01)1,††	0.30(0.02)1,††	0.06(0.04)k†	0.19(0.02)k†	0.32(0.01)k++				
12 M	17.32(0.08)wx†††	1.20(0.01)x†	0.56(0.01)y++++	0.31(0.01)wx†††	0.02(0.01)x†	0.21(0.01)w†	0.39(0.01)w†				

Values are the means of the three different VOO samples (n=3) \pm standard deviations. Different letters within a column indicate significant differences (p < 0.05) with respect to packaging material in each sampling period. The same symbols within a column indicate significant differences (p < 0.05) with respect to packaging material for different periods. M; months

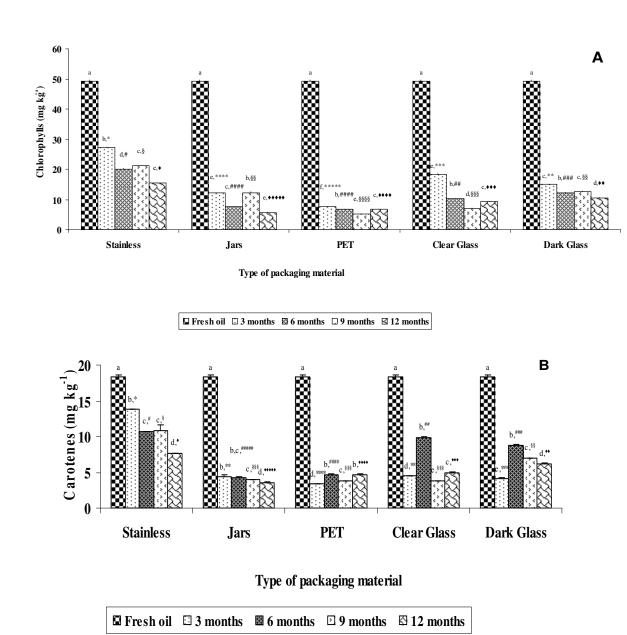


Figure 2. Effect of storage on pigment contents. A Effect on chlorophylls contents, B. Effect on carotenes content. Values are the means of the three different VOO samples $(n=3) \pm \text{standard deviations}$. Different letters within histogram indicate significant differences (p < 0.05) with respect to packaging material in each sampling period. The same symbols within a histogram indicate significant differences (p < 0.05) with respect to packaging material for different periods.

the PET, clear and dark glass olive oil samples, with significant differences, as regards time of storage. The jars olive oil samples stood out for their high contents of margaroleic and stearic acids, but the differences were not significant with respect to time of storage. The oil samples obtained from the different packaging materials did not show significant differences during the different storage periods, as regard oleic acid, linoleic and linolenic acids, except for oils stored in jars where we observed losses in polyunsaturated fatty acids (Stefanoudaki et al., 2010). In fact, the low content of oleic acid and high content of linoleic acid shown by the

olive oil sample may contribute to the lower oxidative stability (Table 1), as this causes a low ratio of mono/polyunsaturated acids (Beltrán et al., 2000). No significant differences with respect to time of storage for the content of oleic acid were found although it slightly increased at the end of the analytical period. These results are in accordance with those of a previous report (Guil-Guerrero and Urda-Romacho, 2009).

Clear glass showed the highest values for arachidic acid and jars the lowest for gadoleic acid, with no significant differences among the different periods of storage for these fatty acids. The lignoceric acid content,

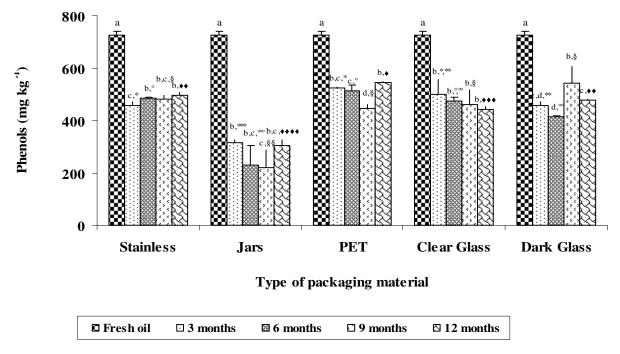


Figure 3. Effect of storage on total phenolic compounds contents. Values are the means of the three different VOO samples $(n=3) \pm \text{standard}$ deviations. Different letters within histogram indicate significant differences (p < 0.05) with respect to packaging material in each sampling period. The same symbols within a histogram indicate significant differences (p < 0.05) with respect to packaging material for different periods.

was not useful for discriminating among the evaluated packaging materials. The contents of arachidic and mainly, gadoleic acid, were very useful for this discrimination.

Changes in Chlorophylls and carotenes contents

Changes in the concentrations of chlorophylls and carotenes in the olive oil samples during storage for 3, 6, 9 and 12 months in stainless, jars, PET, clear and dark glass bottles are shown in Figure 2; concentration of chlorophylls decreased from 48 kg⁻¹ to 8 to 28 mg kg-1, whereas that of carotenes decreased from 18 mg kg-1 to 4 to 13 kg⁻¹ during the first three months of storage, and then decreased thereafter. The intensity of the chlorophyll and carotenes levels decreased in all of the stored samples, to the extent which depended on the storage conditions (time and type of container). However, chlorophylls losses were more noticeable than those related to carotenoid, which reaffirmed the findings of Guil-Guerrero and Urda-Romacho (2009). The lowest chlorophyll concentrations were observed in oil stored in PET and jars bottles. In fact, the oil stored in these bottles exhibited a reduced intensity of the chlorophyll pigments after 12 months of the experiment, with a very small reduction in the oil stored in darkness. The destruction of these two pigments was greater in illuminated samples than in those stored in darkness which confirmed previous researches (Psomiadou and Tsimidou, 2002a, 2002b).

Changes in total phenols contents

Recent interest in olive phenols has greatly increased because of their antioxidant and free radical scavenging abilities, associated with the potential benefits for human health, and the high oxidative stability they confer to the resulting olive oil during storage (Conde et al., 2008). Total phenols as affected by packaging material and storage period are present in Figure 3. The phenols decreased with the storage time, with the rates depending on the storage conditions. The total phenols content decrease lead to the typical bitter taste and pungent note of fresh EVOO decrease in intensity. This finding proved other works showing that during storage, phenols undergo qualitative and quantitative modifycations due to decomposition and oxidation reactions (Morellò et al., 2004; Esti et al., 2009). During the first three months of storage, total phenols decreased from about 750 to 300 mg kg⁻¹. On the other hand, the degradation of phenols significantly happened in early stage when oil was stored in clear glass bottles; however, in all the other materials, it decreased during the first months then increased at 9 and 12 months storage. Comparing the influence of different packaging materials on olive oil samples, jars seem to be the less protective

again phenol degradation.

Contrary to pigments contents, the decrease in the phenols substances was much less pronounced in all the storage conditions. These results confirmed previous works where it had been shown that the oils exposed to light showed chlorophyll and carotenoid contents lower than the same oils kept in darkness, while the decrease in phenolic substances was much less pronounced in all the storage conditions (Sikorska et al., 2007).

Conclusion

Overall, from this study, it can be concluded that, in the storage conditions of light and room temperature, the critical physical-chemical parameters of the olive oil increased significantly after 12 months. The storage material and time had an influence on the chemical composition of the oil at room temperature storage. For whole olive oils stored, there was little to no change in overall lipid content between early and late storage. The oil quality parameters were affected slightly in stainless, PET, clear and dark glass bottles as compared to jars but the antioxidant constituents, and fatty acid profiles were fairly improved by packaging material and therefore induced a direct and indirect improvement on the quality of the oil. There was also a significant (p<0.05) decreement in phenol compounds, carotenes and chlorophylls. This study has reaffirmed that glass bottles provide better protection from oxidation for olive oil than do polyethylene plastic bottles. According to the results, the best packaging material for olive oil packaging was stainless followed by glass. PET and jars proved to be unsuitable for such an application. Exposure of olive oil samples to light and high storage temperatures (room temperature) caused substantial deterioration in product quality parameters. The relative contribution of parameters studied to the retention of olive oil quality was firstly packaging material and then time. Thus, olive oil should be stored in bottles which are not transparent to light or permeable to oxygen in order to minimize oxidative deterioration during storage.

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