Chemical Stability of Extra-Virgin Olive Oil Added with Oregano Essential Oil

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Abstract: Extra virgin olive oil is highly consumed and well known for its nutritional and health benefits. However, it is fatty food highly susceptible to lipid oxidation. The objective of this study was to evaluate the preserving effect of oregano (*Origanum vulgare* L. spp *vulgare* called "oregano compacto") essential oil on physical and chemical properties in extra virgin olive oil during storage. Oregano essential oil composition was analyzed by GC-MS. This essential oil was added into extra virgin olive oil at 0.05%. The samples were stored in 3 different conditions: darkness, light exposure, and temperature (60 °C). Chemical indicators of lipid oxidation (peroxide value, p-anisidine value, conjugated dienes, free fatty acidity, and carotenoid and chlorophyll contents) were measured. High content in carvomenthol (22.52%), terpinolene (19.77%), thymol (13.51%), and γ-terpinene (10.30%) were detected in oregano essential oil. Olive oil samples without oregano essential oil stored at 60 °C and exposure at artificial light had the highest peroxide values during storage. Higher p-anisidine and K232 values after day 7 of storage were detected in temperature, darkness, and light exposure treatments. Light treatment was the main factor that degraded chlorophyll causing loss of color. The highest chlorophyll content (3.87 mg/kg) was observed in olive oil with essential oil at the end of storage. In general, olive oil samples added with oregano essential oil had lower peroxide, conjugated dienes, and p-anisidine values and higher chlorophyll and carotenoid contents during storage. Oregano essential oil retards lipid oxidation process in olive oil prolonging its shelf life.

Keywords: antioxidant, olive, oregano, oxidation, stability

Practical Application: Oregano essential oil was and is used with the purpose of flavoring and aromatizing food. This essential oil due to its composition has shown antioxidant activity. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are thought to be promoters of carcinogenesis. Extra virgin olive oil is widely consumed because of its nutritional benefits and sensory properties which are very important to be preserved in the product. In this study, the oregano essential oil showed remarkable antioxidant activity in olive oil. Therefore, this essential oil could be considered for the industry as natural antioxidant not only to be used in olive oil but also in other fatty food products to substitute synthetic ones.

Introduction

Origanum vulgare L. spp *vulgare* is the most important aromatic herb cultivated in Argentina, not only for the surface (80%), but also for the population demand (Di Fabio 2000). The main areas of farming are located in Mendoza province (250 ha) followed by Córdoba (113 ha) and San Juan (56 ha) provinces.

The farms in which oregano is cultivated are very small. The size of 56% farms is 5 ha and almost 75% of them have less than 10 ha. In this production area, it has been identified 4 different sub species named "Cordobés," "Criollo," "Mendocino," and "Compacto." Leaves and flowers are used as seasoning and flavoring of fresh and preserve foods, sauces, and liquors due to oregano's bitter-stimulating properties (Torres and others 2010). This aromatic herb also has therapeutic benefits (diaphoretic, antiseptic, antispasmodic, and tonic) and it is used in alternative or homeopathic medicine or naturopathy medicine in many countries. It was also widely used in pharmaceutical industries and cosmetology (Souza and others 2007). Nowadays, there is a nonsatisfied national demand of products obtained from Oregano, specially its essential oil, which is stimulating the development of new edapho-climatic areas for expanding the agronomic production and increasing the harvest volume.

First, the essential oils (EO) from aromatic plants were used only with the purpose of flavoring and aromatizing food. Their compounds have proved to be natural agents for food preservation. Their effectiveness against microorganisms has been repeatedly demonstrated (Botsoglou and others 2003; Burt 2004). A few years ago, EO have shown antioxidant properties retarding the process of lipid oxidation in oils and fatty foods (Karpinska and others 2001). Moreover, they were proposed as potential substitutes of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) that are thought to be promoters of carcinogenesis (Namiki 1990; Pokorny 1991). Shahidi and others (1992) reported that the antioxidant effect of

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EO is due to the presence of hydroxy groups in phenolic compounds. Adam and others (1998) and Juliano and others (2000) studied the antioxidant activity of EO and they found that oregano essential oil, rich in thymol and carvacrol, showed a considerable antioxidant effect on the process of lard oxidation.

Extra virgin olive oil is widely consumed because of its sensory properties and nutritional benefits, which are primarily related to the fatty acid composition, mainly due to the high content of oleic acid and also to the balanced ratio of saturated and polyunsaturated fatty acids. For these reasons, olive oil is considered a functional food important in the prevention of many diseases such as cancer and atherosclerosis among others (Mancini and Giaco 1993; Antoun and Tsimidou 1997; Grati-Kamoun 2007). Therefore, it is very important to preserve the quality of this product during its shelf life.

The objective of this study was to evaluate the preserving effect of oregano essential oil on physical and chemical properties in extra virgin olive oil during storage.

Materials and Methods

Leaves and flowers of *Origanum vulgare* L. spp. *Vulgare* known as oregano type "Compacto" was used in this study. Plants were collected from the Experimental Area of Facultad Ciencias Agropecuarias in Capilla de los Remedios, Córdoba, Argentina.

Extra virgin olive oil was purchased in Finca di Fieno (Camino Las Rosas, 3.3 km. Dpto. Cruz del Eje. Córdoba, Argentina).

Essential oil extraction

Samples of leaves and flowers were hydrodistilled for 2 h in a Clevenger-type apparatus with a separated extraction chamber. The resulting EO were dried over anhydrous sodium sulfate and kept in dark flask at –18 °C in freezer until the essential oil samples were analyzed by gas–liquid chromatography and mass spectrometry (GC-MS) or used in the storage study.

GC-MS analysis

A Perkin–Elmer® Q 700 GC-MS (Shelton, Conn., U.S.A.) coupled with an ion trap mass detector equipped with a capillary column DB-5 (30 m long, 0.25 mm i.d., and 0.25 mm coating thickness) was used for the separation of the components. Helium was the carrier gas with a flow rate of 0.9 mL/min. Ionization was performed by electron impact at 70 eV. Mass spectral data were acquired in the scan mode in the m/z range 35 to 450.

The temperature program was 60 °C for 5 min and from 60 to 250 with a rate of 5 °C/min. The injector and detector were maintained at 260 and 280 °C, respectively. The compounds were identified by comparing their retention time and mass spectra with published data (Adams 1995) and libraries NIST. The main components were further identified by coinjection of authentic standards (Sigma® St. Louis, Mo., U.S.A.). Fenchone was used as internal standard at a concentration of 0.1 mg/mL dichloromethane. The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to equal 1.

Radical scavenging activity determination

Antioxidant activity was measured on the basis of scavenging of the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) using a modified version of a previously described method (Choi and others 2008). The free radical scavenging activity was expressed as follows:

$$\text{DPPH scavenging activity (\%)} = ((\text{Ac} - \text{As})/\text{Ac}).100$$

where, "Ac" is the absorbance of the control sample, and "As" is the absorbance of the test sample.

Storage study

Oregano essential oil was added at 0.05% w/w to olive oil. Total of 3 different treatments were carried out: light (24 h exposure for 28 d), darkness, and temperature (60 °C) in samples with and without oregano essential oil. Light and darkness treatments were at room temperature (20 to 23 °C). The samples were:

(1) Light: Olive oil exposed to light at room temperature (CL) and olive added with oregano essential oil exposed to light at room temperature (OL).

(2) Darkness: Olive oil exposed to darkness at room temperature (CD) and olive added with oregano essential oil exposed to darkness at room temperature (OD).

(3) Temperature: Olive oil in darkness at 60 °C (CT) and olive added with oregano essential oil in darkness at 60 °C (OT).

All samples were storage for 28 d and were removed every 7 d to be chemically analyzed.

Chemical analysis of stored samples

Peroxide value (PV), p-anisidine value (AV), specific extintion K232, and free fatty acidity, were performed on samples coming from the storage.

PV was evaluated following the AOAC method (AOAC 1995). The PV was expressed as miliequivalents of active oxygen per kilogram of oil (meqO2/kg).

Free fatty acid (FFA) was determined by the titration method (AOAC 1995). The FFA content was calculated as percentage of oleic acid according.

Table 1– Terpenoid composition in *Origanum vulgare* L. spp. *Vulgare* essential oil according to their elution order in the GC-MS analysis.

Retention index (s)	Compounds	Percentage (%)
1052	α-thujene	0.453
10819	1Rα-pinene	0.423
1142	Camphene	0.131
1205	β-Phellandrene	2.5700
12431	Carbinol	0.1450
12617	3-Octanone	0.3340
1278	β-pinene	0.5440
1387	Terpinolene	4.2690
14205	o-cymene	8.9660
1432	α-limonene	0.6160
1440	β-phellandrene	0.4320
1542	γ-terpinene	10.3010
1584	terpinolene	19.7720
1947	Borneol	0.7300
1982	Carvomenthol	22.5230
2017	α-terpineol	2.2460
2023	Trans-pipertol	0.8510
2118	thymol methyl ether	6.6430
3809	thymol	13.5110
2605	β-bourbonene	0.3170
2709	caryophyllene	0.9790
2733	germacrene	0.0812
2808	α-caryophyllene	0.1020
2878	Naphtalene	1.1590
2916	γ-elemene	0.7540
2927	α-himachalene	0.1920
3132	spathulenol	0.8690

AV was evaluated following the IUPAC method (IUPAC 1987). The AV was given by the formula:

$$AV = 25 \times (1.2As - Ab) \times (m - 1)$$

where, As is absorbance of the fat solution after reaction with the p-anisidine reagent, Ab is the absorbance of the fat solution, and m is the mass of the olive oil in grams.

Specific extinction values (K232) absorbance was measured at 232 nm in a spectrophotometer (UV-V Diode Array Spectrophotometer Hewlett Packard™ HP 8452 A, Hewlett Packard™, Palo Alto CA, U.S.A.). The results were reported as the sample extinction coefficient E (1%, 1cm) (COI 2001).

Chlorophyll and carotenoid contents were measured following the procedures described by Mosquera and others (1991). A sample of olive oil (7.5 g) was placed in a Falcon tube and filled until 25 mL with cycle-hexane. The chlorophyll fraction was measured in a spectrophotometer at 670 nm and the carotenoids fraction at 470 nm. The concentration of pigments was expressed using the following equations:

$$\text{Chlorophylls (mg/kg)} = (\text{Abs}670' \, 106)/(613\text{x}\, 100\text{x density})$$

$$\text{Carotenoids (mg/kg)} = (\text{Abs}470\text{x}106)/(2000' \, 100\text{x desinty})$$

Statistical analysis

The experiment was replicated 3 times. The data were analyzed using the InfoStat software, version 2010p (Facultad de Ciencias Agropecuarias, Univ. Nacional de Córdoba). Means and standard deviations were calculated. Analysis of variance (ANOVA, $\alpha = 0.05$) and least significant difference (LSD) Fisher's multiple range test were performed to find significant differences among means. The simple regression equations of the chemical variables from the storage study of olive oil (PV, conjugated dienes, FFAs, chlorophyll, and carotenoid content) were calculated. Principal component analysis (PCA) (Johnson and Wichern 1998) was performed on the correlation matrix of the standardized data from chemical indicators. The purpose of the PCA was to explore associations between different studied treatments and chemical indicators of deterioration in extra virgin olive oil.

Results and Discussion

Essential oil composition

The essential oil composition of *Origanum vulgare* L. spp. *Vulgare* is shown in Table 1. Only those components with concentrations greater than 0.05% are reported. The compounds that showed higher content were carvomenthol (22.52%) followed by terpinolene (19.77%), thymol (13.51%), and γ-terpinene (10.30%). Dambolena and others (2010) reported that the major components in the essential oil of this oregano species were trans-sabinene hydrate (32.47%), thymol (20.5%), and γ-terpinene (15.47%). Although, other researchers like Skoula and others (1999), Essen and others (2007), found that the EO in *Origanum vulgare* ssp. *vulgare* and ssp. *virens* were rich in acyclic compounds and sesquiterpenoids, oregano samples from Argentina were rich in sabinyl compounds (Dambolena and others 2010).

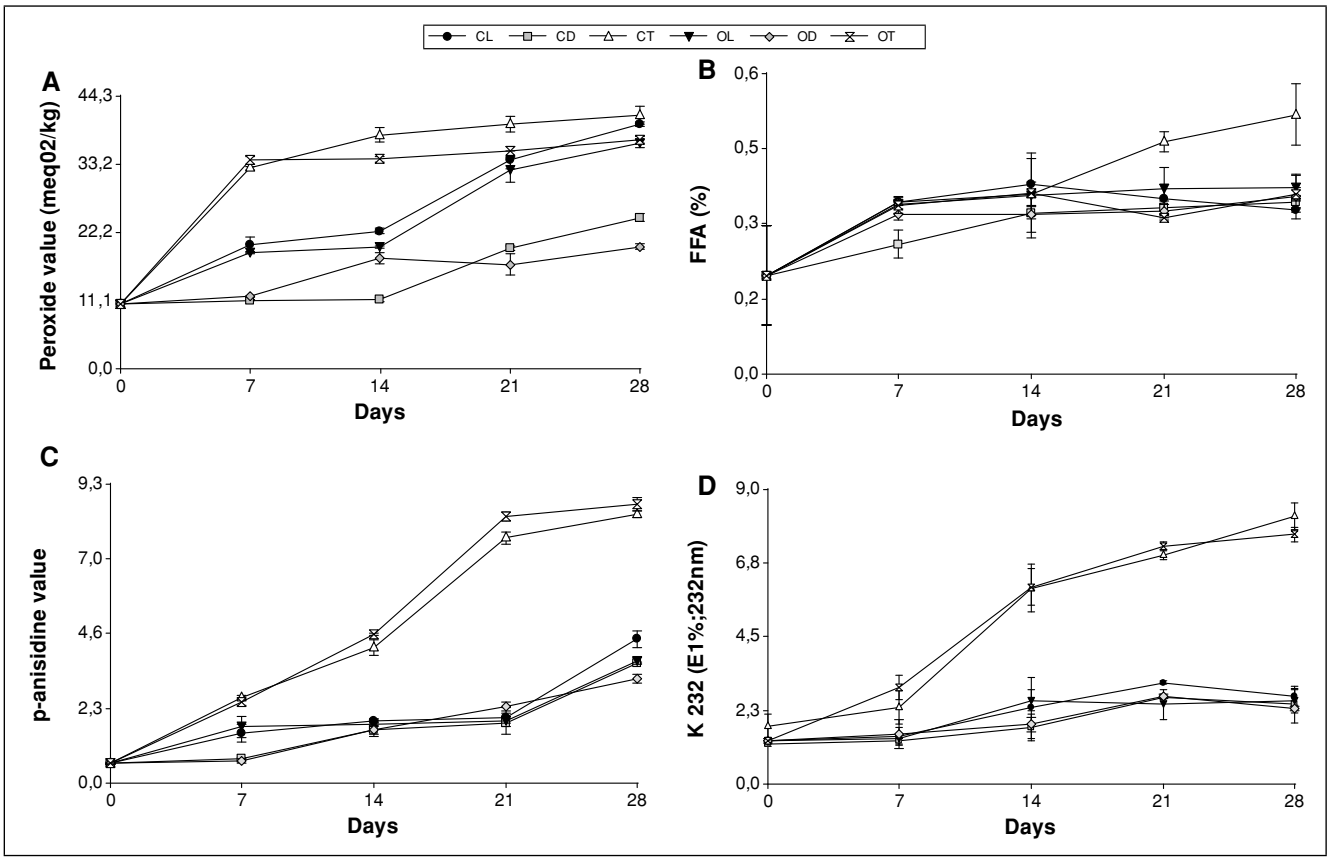

Figure 1–PV (a), FFA (b), AV (c), and conjugated dienes (d) in samples of olive oil under different treatments: olive oil added with oregano at light (OL), olive oil added with oregano at darkness (OD), olive oil added with oregano at 60 °C (OT), olive oil control sample at light (CL), olive oil control sample at darkness (CD), and olive oil control sample at 60 °C (CT).

The oregano essential oil had a DPPH scavenging capacity of 65.93%. The highest DPPH scavenging capacity of the tested oregano samples was to quench 75.3% DPPH, which was observed in the same oregano species (Dambolena and others 2010). DPPH results indicate that oregano essential oil could have a potential antioxidant activity. The antioxidant effect of this essential oil was related to the presence of thymol and carvacrol (Yanishlieva and others 1999)

Storage study for stability evaluation

Physical–chemical changes of olive oil added with oregano essential oil after 28 d of storage are presented in Figure 1. Many researchers have related the PV, product of primary lipid oxidation, with the quality of edible oils and with changes related to high temperature exposures (Melton and others 1994; Frankel 2005). In this study, olive oil treatments in darkness with and without oregano essential oil (OD and CD) showed lower PV exhibiting OD the lowest PV at day 28. Olive oil samples without oregano essential oil stored at 60 °C (CT) and exposure at artificial light (CL) experienced the highest PVs at day 28 of storage. In the same treatment (light, darkness, and temperature), all olive oil samples added with oregano essential oil showed lower PV than the olive oil samples without essential oil at storage day 28 and, in general, during storage. These results indicate that oregano essential oil has an antioxidant effect in the olive oil protecting it against primary lipid oxidation.

Hydrolysis process in fats and oils results in the formation of FFAs, mono- and di-glycerides, and glycerol. This hydrolysis is a deterioration process of lipid and it can be estimated measuring the FFAs (Gertz 1996). FFA in the different olive oil treatments showed an increase during storage being higher in the sample CT (olive oil without oregano essential oil in temperature treatment) after day 21. The highest FFA value was detected in CT (0.54%) at day 28. The FFA increase in every treatment was lower in the olive oil samples added with oregano essential oil. Ayadi and others (2009) reported that the addition of grounded leaves and flowers of aromatic plant (rosemary, lavender, sage, lemon, and thyme) in olive oil caused a slight increase in FFA. In the present study, the oregano essential oil addition in olive oil for all treatments did not increase the FFA.

AV depends on, in advanced stages of lipid oxidation, the organic peroxides decomposition into secondary products, including alcohols, carboxylic acids, aldehydes, and ketones (Ancin and Martinez 1991; Frankel 2005). Higher AVs after day 7 of storage were detected in temperature treatments at 60 °C (CT and OT). Darkness treatments (CD and OD) and light exposure

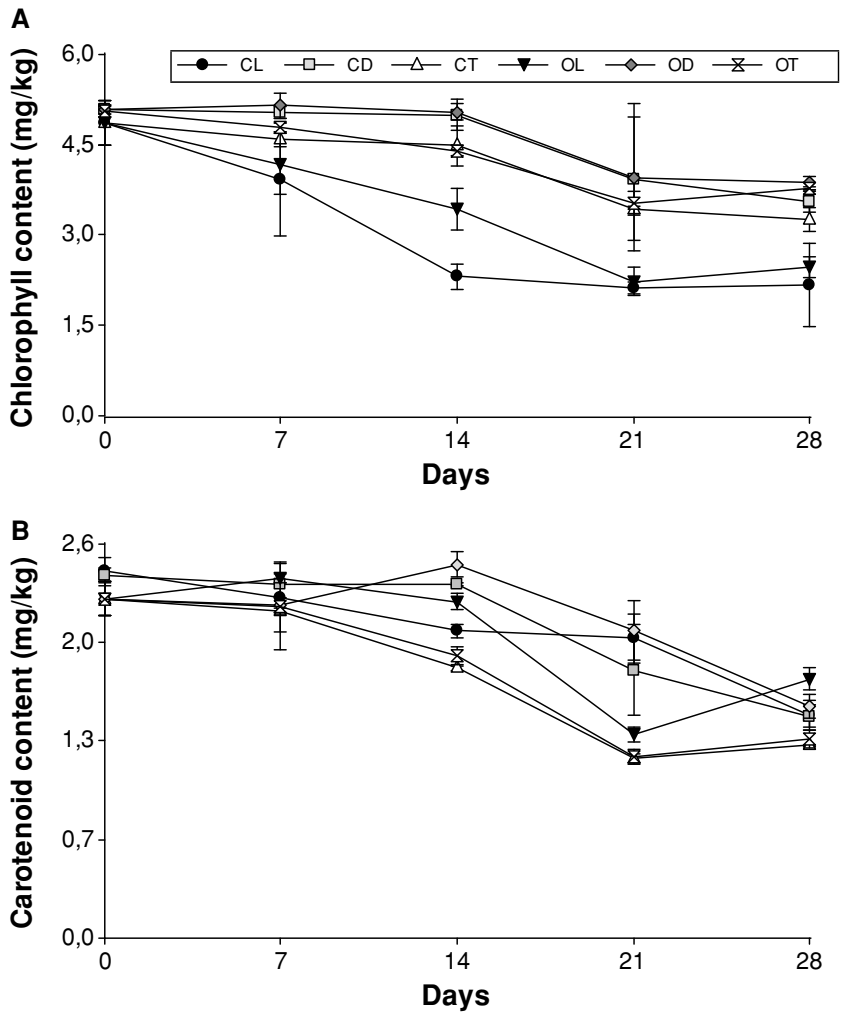

Figure 2–Clorophyll content (mg/kg) (a) and carotenoid content (mg/kg) (b) in samples of olive oil under different treatments: olive oil added with oregano at light (OL), olive oil added with oregano at darkness (OD), olive oil added with oregano at 60 °C (OT), olive oil control sample at light (CL), olive oil control sample at darkness (CD), and olive oil control sample at 60 °C (CT).

treatments (CL and OL) showed significant differences in some storage points. In general, CL and OL had higher AVs than CD and OD, respectively. At day 28 of storage, the lowest value was observed in OD.

Table 2 –Regression equation and adjusted R^2 for the dependent variables: PV, AV, conjugated dienes, FFA, carotenoid, and chlorophyll contents in extra virgin olive oil samples during storage.

Indicators	Treatment	B_0	B_1^b	R^2
Peroxid value	CL[a]	10.91	1.03 d	0.96
Peroxid value	OL	10.73	0.94 d	0.94
Peroxid value	CT	18.71	0.98 c	0.72
Peroxid value	OT	19.23	0.79 d	0.61
Peroxid value	CD	8.1	0.52 b	0.82
Peroxid value	OD	10.63	0.35 a	0.81
P-anisidine	CL	0.48	0.12 c	0.78
P-anisidine	OL	0.67	0.09 bc	0.78
P-anisidine	CT	0.6	0.21 c	0.97
P-anisidine	OT	0.57	0.31 bc	0.96
P-anisidine	CD	0.25	0.11 ab	0.83
P-anisidine	OD	0.33	0.10 a	0.93
K 232	CL	1.31	0.06 a	0.60
K 232	OL	1.36	0.05 a	0.50
K 232	CT	1.04	0.26 b	0.88
K 232	OT	1.9	0.22 b	0.87
K 232	CD	1.13	0.05 a	0.68
K 232	OD	1.32	0.04 a	0.57
Chlorophylls	CL	4.52	−0.1 a	0.71
Chlorophylls	OL	4.78	−0.1 a	0.84
Chlorophylls	CT	5	−0.06 b	0.82
Chlorophylls	OT	5.07	−0.05 b	0.85
Chlorophylls	CD	5.36	−0.06 b	0.60
Chlorophylls	OD	.,35	−0.05 b	0.50
FFA	CL	0.32	7.10 E − 04 a	0.01
FFA	OL	0.26	0.01 a	0.43
FFA	CT	0.23	0.19 a	0.82
FFA	OT	0.26	4.70 E − 03 a	0.39
FFA	CD	0.23	0.01 a	0.59
FFA	OD	0.25	4.80 E − 03 a	0.42
Carotenoids	CL	2.5	−0.03 ab	0.66
Carotenoids	OL	2.43	−0.03 ab	0.54
Carotenoids	CT	2.35	−0.04 a	0.86
Carotenoids	OT	2.37	−0.04 a	0.82
Carotenoids	CD	2.59	−0.04 ab	0.65
Carotenoids	OD	2.45	−0.02 b	0.32

[a]Olive oil added with oregano at light (OL), olive oil added with oregano at darkness (OD), olive oil added with oregano at 60 °C (OT), olive oil control sample at light (CL), olive oil control sample at darkness (CD), and olive oil control sample at 60 °C (CT).
[b]The same letters in the column for every dependent variable mean that the slopes (β_1) of regression equation are not significantly different at $\alpha = 0.05$.

Specific extinction values (K232) is related to the formation of hydroperoxides, conjugated dienes, and carboxylic compounds (Frankel 2005). The maximum value recommended by the EU Regulation 2568/91 for K232 is 2.50 and 2.60 in extra virgin and virgin olive oil, respectively (Ancin and Martinez 1991). Temperature treatments at 60 °C (CT and OT) exhibited the highest values after day 7 of storage. Darkness treatments (CD and OD) and light exposure treatments (CL and OL) showed similar results. In these 2 treatments, K232 values were less than values recommended by the EU Regulation concerning quality characteristics of the extra virgin and virgin olive oils during the 28 d of storage.

Chlorophyll and carotenoid contents during 28 d of storage are shown in Figure 2. Chlorophylls and carotenoid compounds play an important role in the oxidative stability due to their antioxidant nature in the dark and prooxidant activity in the light. In addition, these pigments are responsible for a parameter of quality, color of virgin olive oil, which varies from yellow–green to greenish gold. Chlorophylls give greenish coloration of certain olive oils and carotenoid compounds are responsible for yellowish coloration (Criado and others 2008). Chlorophyll content in all olive oil treatments decreased during storage. Treatments exposed to light (CL and OL) showed the lowest values. Therefore, light treatment was the main factor that degraded chlorophyll causing loss of color in olive oil. OD after 28 d of storage had the highest chlorophyll content (3.87 mg/kg). Significant differences in chlorophyll content were found between olive oil samples with and without oregano essential oil in light exposure (CL and OL), temperature (CT and OT), and darkness (CD and OD) treatments. In all case, olive oil samples added with oregano essential oil had higher chlorophyll content than those samples without essential oil. Oregano essential oil added to olive oil had a protective effect against to chlorophyll degradation. Therefore, this essential oil could be used as a preserving natural additive of the olive oil color quality. Carotenoid content in all olive oil treatments decreased during storage. Treatments exposed to light (CL and OL) showed the lowest values. Significant differences in carotenoid content were found between olive oil exposure to light treatment and the other treatments (darkness and temperature).

Correlation and regression analysis

The regression equations for the dependent variables (PV, AV, FFA, conjugated dienes, and carotenoid and chlorophyll contents) for all treatments are shown in Table 2. All dependent variables

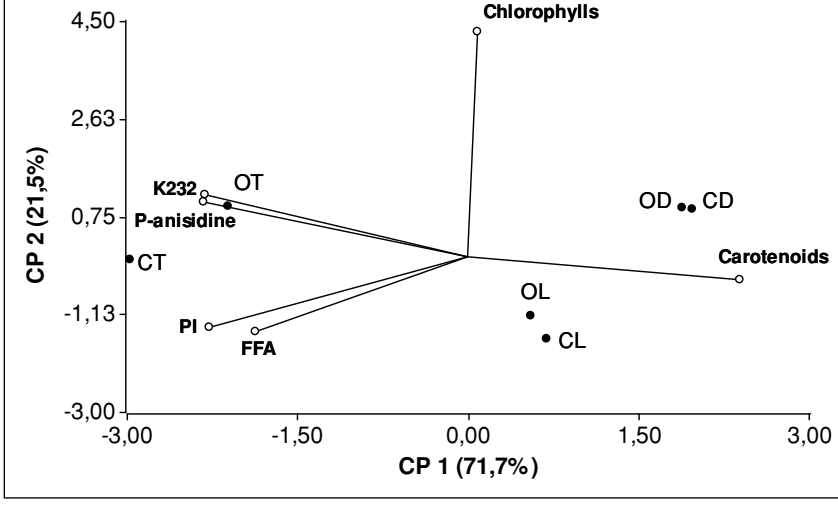

Figure 3–Biplot from the first and second principal components of PCA. Variables: chlorophylls, carotenoids, FFA, PV, conjugated dienes (K232), and p-anisidine. Treatments: olive oil added with oregano at light exposure (OL), olive oil added with oregano at darkness (OD), olive oil added with oregano at 60 °C (OT), olive oil control sample at light exposure (CL), olive oil control sample at darkness (CD), and olive oil control sample at 60 °C (CT).

had R^2 higher than 0.50 except FFA in CL, OL, OT, and OD, and carotenoid content in OD value. The highest R^2 value was 0.97 for AV in CT. Significant differences in the slope (β_1) were found between olive oil samples in PV, AV, K232, and chlorophyll and carotenoid contents. FFA did not show significant differences between treatments. The slope β_1 was lower in olive oil samples added with oregano essential oil than the olive oil samples without essential oil with exception of OT and CT in p-anisidine, OL and CL in chlorophyll content, OL and CL in carotenoid content, and OT and CT also in carotenoid content. These results indicate that the oregano essential oil had a preserving effect of physic and chemical properties in olive oil.

These regression equations could be used to estimate the effect of storage time. According to the European regulation for virgin olive oil, they must present PVs below 20 meq O_2/kg for their classification as extra virgin oils. Using the prediction equation in room temperature treatments, a PV value of 20 meqO_2/kg in this olive oil might be reached in 9, 10, 23, and 27 d for CL, OL, CD, and OD, respectively. This shelf life estimation evidences that oregano essential oil has a protecting effect against lipid oxidation. Olmedo and others (2009) detected antioxidant activity in oregano essential oil when was added in fried-salted peanuts prolonging the shelf-life of this product. Therefore, oregano essential oil as a natural antioxidant could be used for replacement of synthetic antioxidants on food products with high content of lipids.

Principal components analysis

The biplot obtained from the 2 principal components (CP) in the principal component analysis (PCA) is presented in the Figure 3. These 2 principal components explain the 93.2% of the variability in treatments during the 28 storage days. PV is highly associated with FFAs whereas conjugated dienes is with AV. However, carotenoid and chlorophyll content showed no association between them. Temperature treatments at 60 °C in olive oil (CT and OT) exhibited high p-anisidine and K232 values, so they were closed to these lipid oxidation indicators in the PCA. On the other hand, olive oil samples with and without oregano essential oil in darkness were in opposite side to chemical indicators of lipid oxidation (PV, AV, K232, and FFA). Finally, chlorophyll content in olive oil is negatively associated with light exposure treatments (CL and OL).

Conclusions

The results of this study show that olive oil added with oregano essential oil could be stored for longer time. Probably, oregano essential oil retards lipid oxidation process in extra virgin olive oil improving the stability of this product. This essential oil could be used as a natural antioxidant in olive oil and also in other fatty food products to substitute synthetic antioxidants that are questioned for their safety in human health. In addition, this study provides equations to estimate shelf life in olive oil using chemical indicators of lipid oxidation.

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