Sensory Attribute Preservation in Extra Virgin Olive Oil with Addition of Oregano Essential Oil as Natural Antioxidant

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Abstract: Four commercial varieties of oregano are farmed in Argentina: "Compacto," "Cordobes," "Criollo," y "Mendocino." Oregano essential oil is known for antioxidant properties. The objective of this study was to evaluate changes in the intensities of positive and negative attributes in extra virgin olive oil with addition of essential oil obtained from the 4 Argentinean oregano types. Oregano essential oil was added into olive oil at 0.05% w/w. The samples were stored in darkness and light exposure during 126 d at room temperature. The intensity ratings of fruity, pungency, bitterness, oregano flavor, and rancid flavor were evaluated every 21 d by a trained sensory panel. In general, samples with addition of oregano essential oil in olive oil exhibited higher and lower intensity ratings of positive and negative attributes, respectively, during storage compared with the control samples. The first 2 principal components explained 72.3% of the variability in the olive oil samples. In general, positive attributes of olive oil were highly associated with the addition of oregano essential oil in darkness, whereas rancid flavor was negatively associated with them. Olive oil with oregano "Cordobes" essential oil was oppositely associated with light exposure treatments and negative attribute (rancid flavor) suggesting better performance as natural antioxidant of this essential oil in olive oil. The result of this study showed that the presence of oregano essential oil, specially "Cordobes" type, preserve sensory quality of extra virgin olive oil prolonging the shelf life of this product.

Keywords: olive oil, oregano, preserving, sensory, stability

Practical Application: Extra virgin olive oil is highly appreciated for its health benefits, taste, and aroma. These properties are an important aspect in this product quality and need to be preserved. The addition of natural additives instead of synthetic ones covers the present trend in food technology. This research showed that the addition of oregano essential oil preserved the intensity ratings of positive attributes in extra virgin olive oil during storage. The essential oil of the oregano variety called "Cordobes" exhibited better protecting effect on sensory properties of olive oil than the other oregano varieties. The addition of oregano essential oil should be considered for the food industry as a natural source of antioxidant additives for preserving sensory properties in extra virgin olive oil and other similar food products.

Introduction

Oregano is one of the most valued spices. The common name for a general aroma and flavor primarily derived from more than 60 plant species used worldwide as a seasoning (Kintzios 2002). Oregano is also the most important aromatic plant farmed in Argentina, not only because of the surface (80%), but also for the population demand (Di Fabio 2000).

This herb has drawn more attention due to the antimicrobial, antifungal, insecticidal, and antioxidant effects (Bakkali and others 2008; Azizi and others 2009). The abundant essential oils (EOs) located in leaf trichomes are widely used as flavoring in foods and beverages, as fragrances, as fungicides or insecticides in pharmaceutical, and as antioxidant in some food products (Kulisic and others 2004).

Application of antioxidants is one of the technically simplest ways of reducing fat oxidation (O'Brien 2009). Natural antioxidants from edible aromatic plants have many advantages such as (a) to be accepted by consumers, (b) to be considered safe, (c) to come from natural resources, and (d) to have functional and sensory properties (Olmedo and others 2009).

Virgin olive oil is a product widely produced and consumed in the world. It is highly appreciated for its delicious taste and aroma as well as for its nutritional properties (Moldao-Martins and others 2004; Grati-Kamoun 2007). The nutritional benefits are related to the fatty acid composition, due to high content of oleic acid and also to the balanced ratio of saturated and polyunsaturated fatty acids. Monounsaturated fatty acids (MUFA) as oleic acid offers protection against cardiovascular disease by lowering low-density lipoprotein-cholesterol (LDL) ("bad" cholesterol) levels while raising high-density lipoprotein-cholesterol (HDL) (the "good" cholesterol) levels (Ruiz-Gutierrez and others 1999).

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Consumer perception is an important aspect defining food product quality, and attributes resulting from lipid oxidation reactions make food unacceptable to consumers (Boskou and Elmadfa 2011). In vegetable oils, lipid autooxidation can be initiated by light exposure due to free radical generation (O'Brien 2009). In olive oil, it was reported (Asensio and others 2011) higher deterioration in samples under light exposure. The content of chlorophylls and carotenoids decreased as consequence of light exposure effect. Color is a sensory property with a strong influence on food acceptance. Chlorophylls and carotenoids are mainly responsible for this attribute in olive oil. These pigments also play an important role in the oxidative stability due to their antioxidant nature in the dark and prooxidant activity in light (Criado and others 2008). Oxidative rancidity is the most important complex of chemical reactions that limits the shelf life of oils (Kristott 2000). These reactions imply the development of various off-flavors and off-odors, which render foods unacceptable or reduce their shelf life. Oxidative deterioration can result in alterations of sensory properties in the finished products making them unacceptable to the consumers (O'Brien 2009).

Flavored olive oils can be found in markets. They are usually prepared by macerating the aromatic plants in the oil. A flavored olive oil prepared with addition of oregano EO would not only protect olive oil against lipid oxidation but also would preserve its taste, aroma, and health benefit properties.

The main objective of this study was to evaluate changes during storage in the intensities of positive and negative sensory attributes from extra virgin olive oil with the addition of oregano EO coming from 4 different Argentinean varieties.

Materials and Methods

Olive oil

Extra virgin olive oil was pressed by Finca di Fieno (Camino Las Rosas, km 3.3, Cruz del Eje, Cordoba, Argentina).

Oregano

Leaf and flowers of 4 oregano varieties were used in this study: (a) *Origanum x majoricum* "Mendocino," (b) *Origanum vulgare ssp vulgare* "Compacto," (c) *Origanum vulgare ssp hirtum* clone "Cordobés," and (d) *Origanum vulgare ssp hirtum* clone "Criollo." Plants were collected on April 2010 from the Experimental Station of "Facultad Ciencias Agropecuarias (UNC)," Cordoba, Argentina.

EO extraction

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

EO chemical analysis

A Perkin-Elmer® Clarus 600 GC-MS (Shelton, Conn., U.S.A.) coupled with an ion trap mass detector equipped with a capillary column DB-5 (30 m, 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 compounds were identified by comparing their retention time and mass spectra with published data (Adams 1995) and NIST libraries. The main components were further identified by coinjection of authentic standards (SIGMA®, St. Louis, Mo., U.S.A.).

Sample preparation and treatments

Four different olive oils flavored with oregano EO were prepared. EOs (0.05% w/w) of oregano Compacto (Com), Mendocino (Men), Cordobes (Cor), and Criollo (Crio) were added into olive oil. Two groups of treatments were carried out: darkness (D) and artificial light exposure (L). The exposure light treatment was in a 6 m^2 room under 4 Lumilux Plus Eco fluorescent lamps of 58 watts/840 cool white (OSRAM Argentina Compania de Lamparas Electricas SA, Buenos Aires, Argentina) with light intensity of 5200 lumen per lamp. The treatments were Com-L and Com-D, Men-L and Men-D, Cor-L and Cor-D, and Crio-L and Crio-D. A control sample (Control) without EO addition was also prepared and exposed to lightness (Control-L) and darkness (Control-D).

Experimental design

Five olive oil samples and 2 storage conditions were tested using a 5 × 2 × 3 factorial experimental design. Olive oils without EO addition (Control) and flavored with oregano Compacto (Com), Mendocino (Men), Cordobes (Cor), and Criollo (Crio) were used as the olive oil samples. Light exposure and darkness were the storage conditions. A total of 10 treatments were studied. The experiment was replicated 3 times. Replicates were used to assess the reliability of the panel (Meilgaard and others 2006).

Storage and sampling

Each sample of olive oil was packaged in 700 mL glass bottle and stored at 23 ± 1 °C during 126 d. Samples were removed from storage every 21 d to be evaluated by the sensory panel.

Sensory evaluation

Sensory evaluation was made in the "Instituto de Ciencia y Tecnología de los Alimentos, Facultad Ciencias Exactas-Físicas y Naturales, Universidad Nacional de Cordoba" (Cordoba, Argentina) equipped with a sensory lab.

Sensory panel

Panelists were recruited according to the criteria followed by COI 2007. In a first stage, a screening was carried out by the panel leader who interviewed people who work for the National University of Cordoba, and selected: (a) people available for all sessions; (b) people interested in participating; and (c) people capable of verbally communicating the observations regarding the product. The criteria describe by Meilgaard and others (2006) were also included (e) people with age from 25 to 56 y old; (f) people without food allergies; (g) nonsmokers; (h) people who consume nontraditional vegetable oils. At the end of this first stage, 25 people had been recruited. The panelist selection process was performed according to COI (2007). The mean threshold detection of the group for the attributes fusty, winey, rancid, and bitter was determined by paired comparison test. In the 2nd stage of the selection, panelists were evaluated in accordance with the intensity rating method (Rosales and others 1984; COI 2007). Finally, 8 panelists (5 female and 3 male) who showed good levels of perception, olfactory, and gustatory retention and intellectual organization to give the correct order for the 4 stimuli considered (fusty, winey, rancid, and bitter) were selected.

Training

The training was performed to familiarize the panelists with the multiple olfactory-gustatory variants found and to develop the specific sensory methodology of recognizing, identifying, and quantifying the olive oil attributes. The training consisted of 15 sessions of 2 h which, after individually attribute intensities evaluation of the different olive oil samples, panelists commented their results coordinated by the panel leader. The descriptors used by the panel were those mentioned by COI (2010) corresponding to specific vocabulary developed for virgin olive oil. The descriptors were for positive attributes fruity (F), pungency (P), and bitterness (B) and negative attribute rancid (R) (Table 1). Oregano flavor (OF) was also included as a new attribute for this study. The variance and average for each attribute were calculated in every session. A list of definitions and olive oil samples with determined intensity ratings for each attribute (Table 1) was used for the training. Intensity ratings of a warm-up sample were developed during the training sections (Plemmons and Resurreccion 1998). To check panelist performances, reference samples clearly defined were given. Study of the individual variance in the scores obtained by each panelist for these check samples made it possible to determine whether the panelists were keeping up their skills and consistency. When the variance of the panelists evaluating intensities of olive oil attributes was lower than 5%, the panel was considered calibrated. All panelists that participated in this particular study had already had 2 y of experience evaluating olive oils.

Sensory evaluation

All samples were evaluated in partitioned booths under fluorescent light. Room temperature was kept between 20 and 25 °C. The evaluation of the samples was performed following the standard method used for virgin olive oil (COI 2010). The samples were presented in brown glasses covered with a glass watch for preserving the aroma and flavor. Previously to evaluation, the samples were heated at 28 ± 2 °C using hot water bath. All samples were prepared simultaneously 1 h before evaluation so that their head space had enough time to develop. Each glass containing 14 mL of olive oil sample was identified with 3 digit number code. The order of evaluation appeared randomly in each computer. Slices of green apple and cup of distilled water at room temperature were provided to panelists for rinsing their mouth and eliminating residual flavors between samples. The final lists of reference intensity ratings and definitions were posted in the booths for all test sessions. The procedure for evaluation was also posted in each booth.

A data collection automatic system interfaced with a computer in which the screen was performed to register the intensity of each attribute through a 10 cm line scale, where 0 (cero) and 10 (ten) meant the lowest and highest values, respectively. Data were registered by software developed in the University of Cordoba. Every 21 d, panelists evaluated 6 samples per day (3 samples during the morning and 3 samples during the noon) for 5 consecutive days. They were given the references and were instructed to retest them before beginning the evaluation of samples. That was not only to anchor panelist's responses but also to test the calibration and reliability of the panel.

Statistical analysis

The experiment was replicated 3 times. Data were analyzed using the InfoStat software, version 2011p (Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba). The evaluation was arranged into blocks according to the panelist. Block-to-block variability was taken into account so as to increase the sensitivity of the study (Meilgaard and others 2006). Means and standard deviations were calculated for each attribute. Analysis of variance ($\alpha = 0.05$) and the LSD Fisher's Multiple Range test were performed to find significant differences among means (Sokal and Rohlf 1994). The simple regression equations of sensory attributes evaluated during storage were calculated. Principal component analysis (PCA) (Johnson and Wichern 1998; Nepote and others 2009) was performed with the purpose to explore associations between sensory variables and olive oil treatments. Cluster analysis (CA) was carried out to obtain groups of olive oil treatments with similar characteristics. Sample similarities were calculated on the basis of Euclidean distance, and the groups of olive oil treatments with similar characteristics were obtained using the unweighted pair-group method (UPGMA).

Results and Discussion

EO yield and composition

The EO compositions of oregano varieties "Compacto," "Mendocino," "Criollo," and "Cordobes" were studied and the

Table 1–Definitions of attributes, standard references, and warm-up intensity ratings used in descriptive analysis of extra virgin olive oil with addition of oregano essential oils.

Attribute[a]	Definition[b]	Reference	Reference intensity[c]	Warm-up intensity[c,g]
Positive attributes				
Fruity	Olfactory sensations of fruity flavor perceived through the back of the nose, which depend on fresh ripe or unripe and the variety of olives.	Granny Smith apple[e]	8.0	5.5
Pungent	Biting tactile sensation of oils produced from olives that are still unripe perceived throughout the mouth cavity.	Virgin olive oil[d]	7.3	4.8
Bitterness	Characteristic taste obtained from green olives or olives turning color perceived in the "V" region of the tongue.	Virgin olive oil[d]	4.0	2.7
OF	Characteristic taste of oregano essential oil obtained by Hydrodestilation.	Oregano Mendocino essential oil[f]	10.0	4.5[i]
Negative attribute				
Rancid	Flavor of oils that have undergone an intense process of oxidation.	Rancid olive oil[d]	7.5	5.0

[a]Attributes listed in order as perceived by panelists.
[b]The attribute definitions were based on COI/T.20/Doc. No 15/Rev. 3 (COI 2010).
[c]Intensity ratings are based on 10-cm unstructured line scales.
dVirgin olive oil provided by International Olive Oil Council 2009.
[e]Granny Smith Apple.
[f]Essential oil of "Mendocino" oregano crop 2010 Agronomy College Experiment Station (National University of Cordoba).
[g]Warm up: Olive Oil from Empeltre Finca Paso Viejo flavored with 0.05% of Mendocino Essential oil.

main components (concentration greater than 2% relative percentage) are listed in Table 2.

The mayor components in EO-Com were terpinolene (23.09%), carvomenthol (21.65%), and thymol (13.01%). In the related oregano species "Criollo" and "Cordobés," the mayor compounds in the EOs were 4-terpinolene (16.45% and 20.09%, respectively), carvacrol (30.19% and 24.54%, respectively), and o-cymol in "Criollo" (8.72%) and o-cimene in "Cordobés" (14.60%). In EO-Men, the mayor components were terpinen 4-acetato, thymol, and 3-carene (23.90%, 19.98%, and 14.31%, respectively). Dambolena and others (2010) reported trans-sabinene hydrate (32.47%), thymol (20.15%), and γ-terpinene (15.47%) in EO-Com as the mayor components and sabinene hydrate in EO-Men, being this last compound, the typical compound for this oregano species. Samples of oregano were reported to be rich in acyclic compounds and sesquiterpenoids (Essen and others 2007) and samples from Argentina were found rich in sabinyl compounds (Dambolena and others 2010). Phenolic compounds are a class of antioxidant agents that act as free radical terminators. The essential feature of functioning of phenols as an antioxidant (QH) is that QH• species derived from phenoxy radicals produced from monophenolics exist even in aqueous medium as noncharged species. The ortho-substitution with electron donating alkyl or methoxy groups of phenols increases the stability of the free radical, and hence its antioxidant potential. The position and degree of hydroxylation of phenolic compounds are of primary importance in determining their antioxidant activity. The ortho and para positions of hydroxyl groups contribute markedly to the antioxidant activity, while the meta position has little or no effect on the antioxidant property (Dambolena and others 2010). The relationship between the content of particular antioxidants and antioxidant activity is difficult to explain on the basis of only a quantitative analysis because, probably, a synergistic action takes place among the phenolic constituents present in natural extracts (Shan and others 2005). Thymol and carvacrol are a kind of phenolic compound and are, in fact, responsible for the antioxidant activity of many EOs that contain them. Oregano contains these 2 components in its EO (Baratta and others 1998; Quiroga and others 2011). In this study, EO-Com and EO-Men were rich in thymol exhibiting 13.01% and 19.98%, respectively, whereas EO-Cor and EO-Crio were rich in carvacrol showing 24.54% and 30.19%, respectively. These results in the chemical composition of oregano EO indicate that these oregano varieties have potential antioxidant activity. In addition, EO-Com was previously reported as a potential natural antioxidant in extra virgin olive oil measuring chemical lipid oxidation indicators (Asensio and others 2011).

Sensory evaluation

Panelists evaluated the intensities of the positive (Fruity, pungency, bitterness, and OF) and negative (rancid) attributes on day 0 and every 21 d during 126 d of storage. In Table 3, it is shown the mean values for the intensity ratings of attributes that had significant changes during storage, only on storage days 0, 63, and 126. Rancid was the only negative attribute that was perceived by the panelists during storage.

With respect to the positive attributes, fruity experienced changes through storage in all treatments. Control samples (L and D) and all treatments had higher intensity ratings in this attribute at the beginning of storage, then the intensity of this positive attribute decreased during storage. Significant differences were observed between samples. Cor-D had the highest intensity rating for this attribute. Pungency (P) intensity ratings also decreased in control samples and in all treatments. Significant differences were observed among treatments. Samples exposed to light showed higher decrease than those in darkness. Treatments with oregano EO (L and D conditions) except Men-L and Men-D had the highest values of pungency intensity ratings with respect to the control samples in L and D, respectively. Also, significant differences in this attribute were found between control samples (L and D). The intensity ratings of bitterness also decreased during storage. Similar intensity rating behavior as pungency was observed for bitterness (B) during storage. Cor-D was the treatment that better preserved the intensity of this attribute. The intensity of OF decreased significantly in all samples. The highest intensity in OF was developed in Cor-L and Cor-D at the beginning of the storage. Cor-D kept the highest intensity ratings of this attribute in the entire storage with significant differences among treatments.

With respect to negative attribute, all treatments with oregano EO had significant differences with control-L, which presented the highest rancid value. Treatments with oregano EO exposed to light

Table 2–Relative percentages of main terpenoid components in the essential oils of oregano varieties according to their elution order in the GC-MS analysis.

		Relative percentages (%)*							
RI	Compounds	O. Compacto		O. Cordobes		O. Criollo		O. Mendocino	
5.5	β-Phellandrene	2.88	b	0	a	0	a	0	a
5.8	α-Terpinen	0	a	3.08	b	2.99	b	2.57	a
5.93	o-Cimene	8.64	b	14.60	c	0	a	0	a
5.95	o-Cymol	0	a	0	a	8.72	c	1.74	b
6.03	1-Rα-Pinene	0.41	b	4.26	c	0	a	0	a
6.06	β-cis-o cimene	0	a	0	a	4.09	b	0	a
6.43	γ-Terpinen	9.89	b	9.05	b	7.55	c	11.85	a
6.87	Terpinolene	23.09	a	0.92	a	0.85	a	0.69	a
7.15	Terpinen 4-acetato	0	a	0.00	a	0	a	23.90	b
8.29	Borneol	0.7104	b	3.77	d	3.30	c	0.00	a
8.4	4-Terpineol	0	a	20.09	b	16.45	c	8.48	b
8.61	A-Terpineol	2.16	a	5.07	c	4.61	b	2.39	a
9.06	Thymol methyl ether	6.38	c	0.59	a	0.41	a	0.99	b
9.34	3-Carene	0.07	a	0	a	0.00	a	14.31	b
9.53	Carvomenthol	21.65	b	0	a	0.00	a	0.00	a
9.96	Thymol	13.01	b	0	a	0.60	a	19.98	c
9.98	Carvacrol	0.00	a	24.54	c	30.19	d	3.86	b
11.98	Caryophyllene	1.04	a	3.83	b	4.35	c	1.40	a

*The same letter in the row means that there are no significant differences between essential oil from oregano species ($\alpha = 0.05$).

Table 3–Possitive and negative attributes in extra virgin olive oil with addition of oregano essential oil evaluated during storage.

Treatments[a]	Control-L	Com-L[b]	Cor-L[b]	Crio-L[b]	Men-L[b]	Control-D[b]	Com-D[b]	Cor-D[b]	Crio-D[b]	Men-D[b]
Fruity flavor										
Day 0	$5.8 \pm 0.11^{b}_{1}$	$5.5 \pm 0.13^{ab}_{1}$	$5.55 \pm 0.14^{ab}_{1}$	$5.13 \pm 0.26^{a}_{1}$	$5.53 \pm 0.13^{ab}_{1}$	$5.8 \pm 0.1^{b}_{1}$	$5.5 \pm 0.16^{ab}_{1}$	$5.55 \pm 0.14^{ab}_{1}$	$5.35 \pm 0.13^{a}_{1}$	$5.55 \pm 0.13^{ab}_{1}$
Day 63	$4.52 \pm 0.34^{a}_{2}$	$4.57 \pm 0.21^{a}_{2}$	$4.63 \pm 0.24^{a}_{2}$	$4.13 \pm 0.38^{a}_{2}$	$4.3 \pm 0.31^{a}_{2}$	$4.55 \pm 0.14^{a}_{2}$	$4.23 \pm 0.15^{a}_{1}$	$4.84 \pm 0.36^{a}_{2}$	$4.53 \pm 0.42^{a}_{2}$	$4.62 \pm 0.18^{a}_{2}$
Day 126	$3.62 \pm 0.27^{a}_{3}$	$4.03 \pm 0.25^{b}_{3}$	$3.77 \pm 0.2^{b}_{3}$	$3.65 \pm 0.39^{a}_{3}$	$3.48 \pm 0.15^{a}_{3}$	$3.73 \pm 0.42^{a}_{3}$	$4.23 \pm 0.19^{b}_{2}$	$4.52 \pm 0.13^{c}_{3}$	$3.78 \pm 0.19^{b}_{3}$	$4.1 \pm 0.32^{b}_{2}$
Pungency										
Day 0	$2.98 \pm 0.13^{bc}_{1}$	$3.72 \pm 0.2^{bc}_{1}$	$4.3 \pm 0.38^{a}_{1}$	$5.35 \pm 0.15^{c}_{1}$	$5.03 \pm 0.27^{bc}_{1}$	$5 \pm 0.13^{bc}_{1}$	$5 \pm 0.2^{bc}_{1}$	$4.6 \pm 0.27^{ab}_{1}$	$5.36 \pm 0.27^{c}_{1}$	$5.23 \pm 0.21^{bc}_{1}$
Day 63	$2.98 \pm 0.14^{a}_{2}$	$3.72 \pm 0.35^{ab}_{2}$	$3.7 \pm 0.35^{ab}_{2}$	$3.57 \pm 0.38^{ab}_{2}$	$3.73 \pm 0.23^{ab}_{2}$	$3.45 \pm 0.2^{ab}_{1}$	$3.4 \pm 0.35^{ab}_{2}$	$3.72 \pm 0.37^{ab}_{12}$	$4.03 \pm 0.44^{b}_{2}$	$3.63 \pm 0.3^{ab}_{2}$
Day 126	$1.77 \pm 0.27^{a}_{3}$	$2.73 \pm 0.07^{b}_{3}$	$2.4 \pm 0.35^{b}_{3}$	$2.93 \pm 0.45^{b}_{3}$	$2.15 \pm 0.08^{ab}_{3}$	$3 \pm 0.41^{c}_{2}$	$3.13 \pm 0.36^{d}_{3}$	$3.12 \pm 0.49^{d}_{2}$	$3.12 \pm 0.33^{d}_{3}$	$3.04 \pm 0.29^{c}_{3}$
Bitternes										
Day 0	$3.3 \pm 0.16^{a}_{1}$	$3.67 \pm 0.2^{a}_{1}$	$3.85 \pm 0.08^{ab}_{1}$	$3.62 \pm 0.29^{a}_{1}$	$3.28 \pm 0.16^{a}_{1}$	$3.3 \pm 0.16^{a}_{1}$	$3.37 \pm 0.23^{a}_{1}$	$4.35 \pm 0.35^{b}_{1}$	$3.62 \pm 0.36^{a}_{1}$	$3.34 \pm 0.19^{a}_{1}$
Day 63	$2.67 \pm 0.17^{a}_{12}$	$3.05 \pm 0.31^{a}_{12}$	$3.13 \pm 0.72^{a}_{12}$	$2.83 \pm 0.32^{a}_{2}$	$2.63 \pm 0.15^{a}_{1}$	$2.67 \pm 0.17^{a}_{2}$	$3.05 \pm 0.31^{a}_{12}$	$3.2 \pm 0.21^{a}_{1}$	$2.88 \pm 0.31^{a}_{1}$	$2.63 \pm 0.2^{a}_{12}$
Day 126	$2 \pm 0.29^{a}_{2}$	$2.62 \pm 0.2^{c}_{2}$	$2.53 \pm 0.16^{c}_{2}$	$2.62 \pm 0.14^{c}_{3}$	$2.32 \pm 0.17^{b}_{2}$	$2.32 \pm 0.07^{b}_{3}$	$2.63 \pm 0.3^{c}_{2}$	$3.12 \pm 0.46^{d}_{2}$	$2.83 \pm 0.28^{c}_{2}$	2.23 ± 0.33^{b}
OF										
Day 0	$0 \pm 0^{a}_{1}$	$5.63 \pm 0.29^{cd}_{1}$	$6.15 \pm 0.27^{d}_{1}$	$4.88 \pm 0.32^{bc}_{1}$	$4.38 \pm 0.5^{b}_{1}$	$0 \pm 0^{a}_{1}$	$5.63 \pm 0.29^{cd}_{1}$	$6.15 \pm 0.27^{d}_{1}$	$4.9 \pm 0.32^{bc}_{1}$	$4.65 \pm 0.42^{b}_{1}$
Day 63	$0 \pm 0^{a}_{sd}$	$3.63 \pm 0.15^{d}_{2}$	$2.65 \pm 0.07^{b}_{1}$	$2.55 \pm 0.14^{b}_{2}$	$3.54 \pm 0.26^{d}_{1}$	$0 \pm 0^{a}_{2}$	$4.15 \pm 1.85^{c}_{1}$	$4.24 \pm 0.18^{e}_{2}$	$3.23 \pm 0.7^{c}_{2}$	$3.32 \pm 1.23^{c}_{2}$
Day 126	$0 \pm 0^{c}_{sd}$	$2.12 \pm 0.16^{b}_{3}$	$2.12 \pm 0.22^{b}_{2}$	$2.35 \pm 0.4^{b}_{2}$	$1.93 \pm 0.34^{b}_{2}$	$0 \pm 0^{a}_{3}$	$2.35 \pm 0.61^{c}_{2}$	$3.1 \pm 0.62^{d}_{3}$	$2.5 \pm 0.54^{c}_{3}$	$2 \pm 0.26^{b}_{3}$
Rancid										
Day 0	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$	$0 \pm 0^{a}_{1}$
Day 63	$0.35 \pm 0.22^{c}_{2}$	$0.07 \pm 0.07^{b}_{1}$	$0.4 \pm 0.14^{c}_{12}$	$0.45 \pm 0.19^{d}_{12}$	$0.3 \pm 0.14^{c}_{2}$	$0.33 \pm 0.18^{c}_{2}$	$0.02 \pm 0.02^{a}_{12}$	$0 \pm 0^{a}_{1}$	$0.07 \pm 0.07^{b}_{12}$	$0.05 \pm 0.05^{ab}_{1}$
Day 126	$0.88 \pm 0.08^{c}_{3}$	$0.78 \pm 0.32^{b}_{2}$	$0.85 \pm 0.28^{b}_{2}$	$0.66 \pm 0.27^{b}_{2}$	$0.78 \pm 0.1^{b}_{3}$	$0.58 \pm 0.14^{b}_{3}$	$0.25 \pm 0.16^{a}_{2}$	$0.64 \pm 0.29^{ab}_{2}$	$0.35 \pm 0.19^{b}_{2}$	$0.68 \pm 0.24^{b}_{2}$

[a] Olive oil with addition of essential oil of oregano Compacto (Comp), Cordobes (Cor), Criollo (Crio), and Mendocino (Men). Olive oil without addition of essential oil (Control). L = light exposure and D = darkness.
[b] The same letter in the column for every dependent variable means that there are not significant differences between treatments and the same number in the row means that there are not significant differences between evaluated periods ($\alpha = 0.05$).

did not show significant differences among them but treatments with oregano EO in darkness exhibited differences having Com-D the lowest intensity rating at the end of storage.

Positive attributes as bitterness and pungency are related to phenolic compounds present in the extra virgin olive oil. The phenolic components decrease when the olive oils suffer an oxidation process (Inarejos-García and others 2010). Previous study confirmed that the addition of EO-Com to olive oil (Asensio and others 2011) preserved minor components in olive oil as chlorophyll and carotenoids and decrease oxidation process. The preservation of positive attributes especially bitterness and pungency in olive oil samples with addition of oregano EO could be explained for antioxidant activity of this EO that decreased oxidation process on phenolic compounds. In this study, Cor-D could be considered as the treatment that better preserved positive attributes in extra virgin olive oil.

Regression analysis

The regression equations for the dependent variables (fruity, pungency, bitterness, OF, and rancid flavor) for all treatments are shown in Table 4. The slope ($\beta 1$) for fruity, pungency, bitterness, and OF was negative due to these sensory attributes decreased during storage. Significant differences were found between slopes in the same attribute. In general, the highest negative and positive slopes in all dependent variables evaluated were observed in the control sample exposed to light. The lowest slopes were detected in olive oil treatment with addition of oregano EOs in darkness. OF in every treatment had R^2 higher than 0.50. The highest R^2 value was 0.77 for OF in Com-L. However, fruity in Com-L, Com-D, Cor-D, and Crio-D; pungency in Cor-L and Cor-D; and bitterness in all treatments had lower R^2 than 0.5. These low values in R^2t were probably due to that the intensity of these attributes did not decrease enough during storage because of the protective effect of the EO against lipid oxidation process in these olive oil samples. Moreover, the treatments without EO (Control-L and Control-D) and Men-L had R^2 higher than 0.50 in rancid that indicates that these samples had higher deterioration process.

These regression equations could be used to estimate the effect of storage time. If extra virgin olive oil is storage for 150 d exposed to light, the intensity of the positive attributes would be 2.98, 1.35, and 1.6 points in 10-cm line scale for fruity, pungency, and bitterness, respectively. However, if the same extra virgin olive oil is storage for 150 d in darkness with addition of oregano EO (Cor-D), the intensity for the same attributes would be 4.06, 2.66, and 2.57 points, respectively. This prediction evidences the combined protective effect of darkness and the addition of oregano EO acting as a natural antioxidant for preserving sensory quality of olive.

Principal components analysis (PCA)

The biplot obtained from PCA is shown in Figure 1. The first 2 principal components explained 72.3% of the variability in the olive oil samples. In general, positive attributes were highly associated with treatments with addition of oregano EO in darkness, whereas rancid flavor is negatively associated with them. The light exposure is an important factor related to olive oil deterioration. This condition could contribute to the degradation of chlorophylls and carotenoids and activate autooxidation process of lipids (Ayadi and others 2009). It was reported that during light exposure storage, chlorophyll and carotenoid contents decreased and on the contrary, lipid oxidation indicators as peroxide value, conjugated dienes (K232), and anisidine value increased (Asensio and others 2011). Control samples (both darkness and light exposure treatments) were not related with any positive attributes because of the high decrease of the intensity of attributes during storage. There was an association between Com-D and Com-L with bitterness and OF. Crio-D, Men-D, and Cor-D were highly related with fruity and pungency. The dispersion of points in the biplot indicated high variability among samples although some groups could be identified. The variables, fruity, pungency, bitterness, and OF were placed in the left side of the figure. The samples that had high values for these attributes were also place on the left side. All of them had addition of oregano EO and were in darkness except for Com-L that also was placed in this side. Control samples (D and L) and light exposure treatments were placed on the right side of the biplot, and rancid flavor was in this side. Positive attributes and darkness treatments with oregano EOs (Cor-D, Crio-D, Men-D, and Com-D) association indicate antioxidant activity of these EOs that act as preserving agents of sensory quality of olive oil. Cor-D was most oppositely associated with light exposure treatments and negative attribute (rancid flavor) suggesting better performance as natural antioxidant of this EO in olive oil.

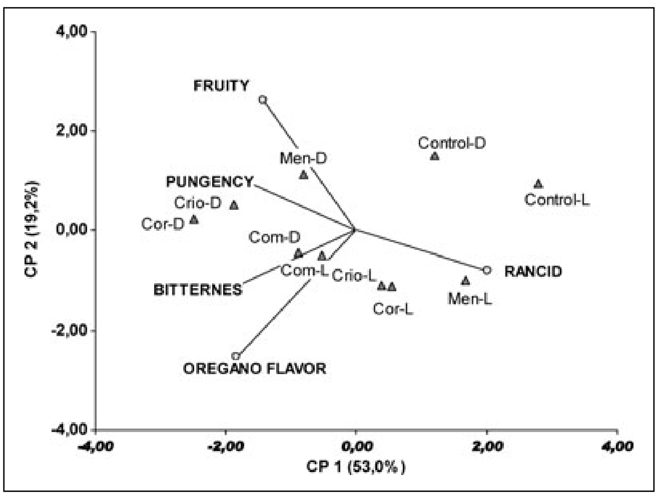

Figure 1–Biplots from the 1st and 2nd principal components of PCA. Sensory variables: fruity, pungency, bitterness, OF, and rancid flavor. Treatments: olive oil exposed to light (L) and darkness (D) with and without oregano essential oils.

CA

The results of the CA from 5 olive oil samples (4 different oregano EO samples + 1 control) in 2 storage conditions (L and D), considering that 4 positive and 1 negative attributes are presented in Figure 2. Four groups in the clusters were identified. Group 1 was formed by olive oil samples with addition of oregano EOs light exposure; group 2 was formed by samples of 3 olive oils with EOs (Com, Crio, and Men) in darkness; group 4 was only formed by Cor-D; and group 3 was formed by control treatments (L-D). These CA results indicate that control samples (L and D) experienced similar changes through storage. Similar results were obtained in light exposure treatments (Com-L, Cor-L, Crio-L, and Men-L). Cor-D was alone in one group (group 4) due to its different performance during storage. This result matches the PCA analysis in which Cor-D was the sample most separated of the rest and with the most opposite place with respect to rancid flavor.

Mean values for sensory variables are presented in Table 5 according to the groups formed from the CA. Significant differences were found between groups in all variables. Fruity intensity rating in groups 1 and 3 had the lowest ratings. Groups 4 and 1 exhibited higher and lower intensity ratings of positive and negative attributes, respectively. In bitterness and pungency, the lowest intensity ratings were observed in light exposure treatments (group 1) and control samples (group 3). Moreover, the samples of groups 1 and 3 had the highest rancid ratings. Cor-D (group 4) preserved the strongest intensity rating of OF during storage followed by group 2. Group 4 had the highest intensity ratings in positive attributes and the lowest intensity in rancid flavor. Once again, this result suggests much better antioxidant activity in the EO of "Cordobes" oregano variety than the other studied oregano EOs.

Table 4–Regression coefficients and adjusted R^2 for the dependent variables: fruity, pungency, bitterness, OF, and rancid flavor in extra virgin olive oil samples without and with essential oils during storage.

Treatment[a]	Control-L[b]	Com-L[b]	Cor-L[b]	Crio-L[b]	Men-L[b]	Control-D[b]	Com-D[b]	Cor-D[b]	Crio-D[b]	Men-D[b]
Fruity flavor										
$\beta 0$	5.681	5.628	5.269	4.964	5.427	5.566	5.076	5.515	5.369	5.695
$\beta 1$[b]	−0.018a	−0.016ab	−0.011c	−0.008d	−0.014b	−0.010c	−0.012bc	−0.014b	−0.017a	−0.015b
R2	0.68	0.56	0.39	0.15	0.59	0.4	0.33	0.59	0.66	0.55
Pungency										
$\beta 0$	4.651	4.931	5.003	4.792	4.266	4.618	5.024	5.485	5.018	5.089
$\beta 1$[b]	−0.022a	−0.017b	−0.019b	−0.016b	−0.015b	−0.013b	−0.017b	−0.020ab	−0.023a	−0.018b
R2	0.72	0.58	0.642	0.44	0.36	0.33	0.46	0.58	0.68	0.58
Bitterness										
$\beta 0$	3.402	3.244	3.627	3.408	3.701	4.027	3.587	3.459	3.156	3.376
$\beta 1$[b]	−0.012a	−0.007c	−0.008bc	−0.007c	−0.009b	−0.010b	−0.009b	−0.006d	−0.007c	−0.009b
R2	0.38	0.38	0.21	0.25	0.27	0.28	0.34	0.21	0.29	0.3
OF										
$\beta 0$	0	0	5.264	5.475	5.634	5.872	4.74	5.085	4.744	4.795
$\beta 1$[b]	0d	0d	−0.026b	−0.026b	−0.032a	−0.026b	−0.021c	−0.023b	−0.025b	−0.025b
R2	0	0	0.77	0.49	0.78	0.67	0.64	0.59	0.54	0.66
Rancid										
$\beta 0$	−0.124	−0.039	−0.141	−0.048	−0.114	−0.128	−0.008	−0.056	−0.095	−0.119
$\beta 1$[b]	0.0081d	0.0061bc	0.0062bc	0.0017a	0.0071c	0.0045b	0.0057b	0.003ab	0.0072c	0.0071c
R2	0.53	0.37	0.39	0.21	0.4	0.25	0.26	0.24	0.49	0.38

[a]Olive oil with addition of essential oil of oregano Compacto (Comp), Cordobes (Cor), Criollo (Crio), and Mendocino (Men). Olive oil without addition of essential oil (Control). L = light exposure and D = darkness.
[b]The same letters in the column for every dependent variable mean that the slopes ($\beta 1$) of regression equation are not significantly different at $\alpha = 0.05$.

Table 5–Means of attribute intensity ratings in each olive oil group form CA.

Groups of olive oils treatments	Fruity		Pungency		Bitterness		OF		Rancid	
Group 1	4.44	a	3.67	a	2.99	b	3.49	b	0.33	b
Group 2	4.66	b	4.0	b	2.95	b	3.64	b	0.14	a
Group 3	4.6	a	3.57	a	2.72	a	0.00	a	0.33	b
Group 4	4.94	c	3.79	ab	3.44	c	4.22	c	0.16	a

The same letters in the column mean that the intensity ratings for each attribute are not significantly different at $\alpha = 0.05$.

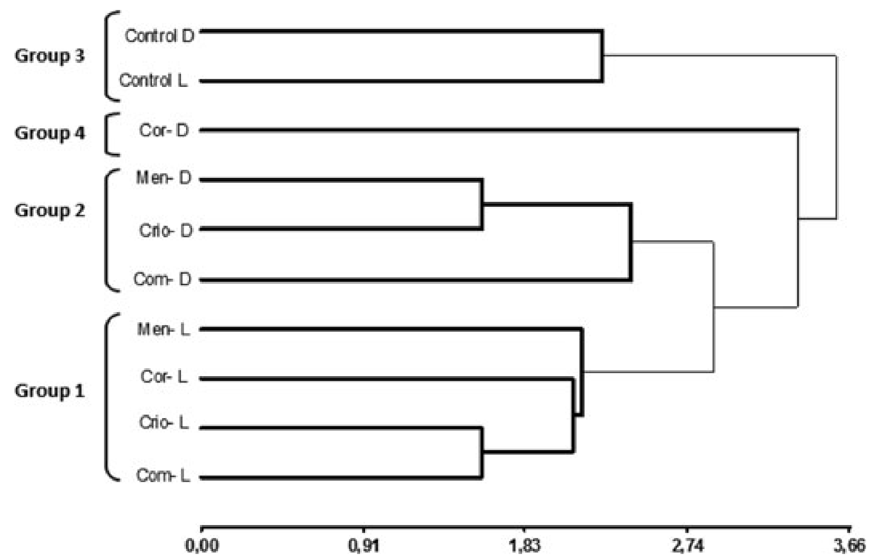

Figure 2–Dendogram from CA of olive oil with addition of oregano essential oils in darkness and light exposure considering sensory variables.

Conclusions

The results from the PCA suggest that treatments in darkness and with addition of oregano EOs are related to positive attributes of extra virgin olive oil. Moreover, the result of this study showed that the presence of oregano EO, specially the "Cordobes" type, preserves sensory quality of extra virgin olive oil prolonging the shelf life of this product. Based on this study and others, oregano EO presents antioxidant activity decreasing lipid oxidation process. The addition of this kind of substance for preserving food with high lipid content renews the interest in using natural products as antioxidants in food industry. Finally, this study also provides regression equations to estimate shelf life of extra virgin olive oil using intensity ratings of sensory attributes from descriptive analysis.

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