International Journal of
Food Science & Technology

International Journal of Food Science and Technology 2011, **46**, 2648–2655

Original article

Antioxidant activity of essential oil of oregano species from Argentina in relation to their chemical composition

Patricia R. Quiroga,[1] Cecilia G. Riveros,[1] Julio A. Zygadlo,[2] Nelson R. Grosso[1] & Valeria Nepote[2]*

1 Facultad de Ciencias Agropecuarias (UNC), Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Av. Valparaiso s/n, CU, CC 509, X5016GCA, Córdoba, Argentina

2 Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET), Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Facultad de Ciencias Exactas, Físicas y Naturales (UNC), Av. Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina

(Received 31 March 2011; Accepted in revised form 13 August 2011)

Summary The purpose of this work was to determine chemical composition and antioxidant activity of essential oil of different oregano species from Argentina: 'Cordobes', 'Criollo', 'Mendocino' and 'Compacto'. The essential oil composition was determined by gas–liquid chromatography and mass spectrometry. Scavenging activity was analysed by DPPH test. The antioxidant activity of the essential oils was determined by an accelerated oxidation test in canola oil. Thirty-nine compounds were identified in the oregano essential oils. The oregano species showed differences in their chemical composition, radical scavenging activity and antioxidant activity. The main compounds in the studied oregano species were thymol and *trans*-sabinene hydrate followed by γ-terpinene, terpinen-4-ol and α-terpinene. The oregano, 'Criollo', was rich in γ-terpinene and had lower thymol and *trans*-sabinene hydrate and higher α-terpinene and carvacrol contents than the other oregano species. 'Mendocino' had higher *trans*-sabinene hydrate and limonene than the other oregano species. 'Cordobes' and 'Compacto' had higher thymol content, radical scavenging activity and antioxidant activity in canola oil.

Keywords Antioxidant, canola, essential oil, oregano.

Introduction

Natural antioxidants as food ingredients are preferred by consumers because these kinds of compounds have positive effects on human health. Tocopherols are natural antioxidants present in most vegetable oils, but they are unstable during heating process (Nepote *et al*., 2006; Ryan *et al*., 2008; Silva *et al*., 2010). Many researchers are interested in new natural antioxidant sources.

Since ancient times, herbs and species have been added to different types of food to improve the flavour and organoleptic properties (Neffati *et al*., 2009). Essential oils from aromatic species are known to possess potential as natural agents for food preservation; in fact, their effectiveness against a wide range of microorganisms has been repeatedly demonstrated (Baratta *et al*., 1998a; Deans, 1991; Helander *et al*., 1998). In addition, many essential oils have shown antioxidant activity (Aeschbach *et al*., 1994; Baratta *et al*., 1998a; Yanishlieva *et al*., 1999; Olmedo *et al*., 2008, 2009).

Oregano is an important aromatic plant widely used in many countries for flavouring foods. The oregano leaves and essential oil were used for centuries because of its medicinal properties. The positive effect of oregano on human health has been attributed to the antioxidant activity of the essential oil and soluble phenolic fractions (Eguchi *et al*., 1996; Englberger *et al*., 1988; Peake *et al*., 1991). The antioxidant activity of the essential oil may be attributed primarily to the high content of phenolic components (Ruberto *et al*., 2002).

Argentina is an important producer and exporter country of oregano. The cultivation areas of oregano are in central and south-west regions in Argentina (Barreyro *et al*., 2005). Commercial oregano comes from *Origanum vulgare* L. spp. *vulgare* (Compacto), *vulgare* spp. *virens* (Cordobes), *Origanum* × *applii* (Criollo) and *Origanum* × *majoricum* (Mendocino) (Barreyro *et al*., 2005).

The objective of this work was to determine chemical composition and antioxidant activity of essential oil of different oregano species from Argentina.

Materials and methods

Plant material

The studied species were the following:

1 *O. vulgare* spp. *virens* (Hoffm. et Link) letswaart

*Correspondent: Fax: +54 0351 4334439;
e-mail: vnepote@efn.uncor.edu

doi:10.1111/j.1365-2621.2011.02796.x

2 *Origanum* × *applii* (Domin) Boros,
3 *Origanum* × *majoricum* Cambess,
4 *O. vulgare* L. spp. *vulgare*.

These species are known in Argentina as oregano-type 'Cordobes' (Cor), 'Criollo' (Cri), 'Mendocino' (M) and 'Compacto' (Com), respectively. Plants were collected from the 'Instituto Nacional de Tecnología Agropecuaria' research station in Santa Lucia, San Juan, Argentina and 'Universidad Nacional de La Pampa', Santa Rosa, La Pampa, Argentina, crop 2008. For harvesting, the plant material was cut 5 cm above the soil surface. Plant samples were air-dried at room temperature. Air-drying at ambient temperature seems to be an appropriate method in preserving most of the phenolic compounds present in plant materials (Papageorgiou *et al*., 2008).

Canola oil was provided by Compañía Oleaginosa del Sur S.A., Lobería, Buenos Aires, Argentina.

Essential oil extraction

Essential oils were extracted by hydrodistillation for 2 h in a Clevenger-type apparatus. The resulting essential oils were dried over anhydrous sodium sulphate and analysed by gas–liquid chromatography and mass spectrometry (GC-MS).

Essential oil composition

The essential oils were analysed by GC-MS in a Perkin Elmer Q-700 equipment coupled with an ion trap mass detector (Perkin Elmer, Shelton, CT, USA). A column DB-5 (30 m × 0.25 mm i.d. and 0.25 m coating thickness) was used. The temperature programme was 3 min at 60 °C and after a rate of 4 °C min^{-1}–240 °C. The injector was held at 250 °C. Helium was used as the carrier gas with a flow rate of 0.9 mL min^{-1}. Ionisation was realised by electron impact at 70 eV. The compounds from the different oregano essential oils were identified by comparing their retention index and mass spectra with published data (Dambolena *et al*., 2010), libraries NIST and Adams (1995). Co-injection of Authentic standards (Sigma, St. Louis, MO, USA) was also used for identification of the main components. Internal standard of fenchone was used at a concentration of 0.1 mg mL^{-1} dichloromethane. The quantitative composition was obtained by peak area normalisation, and the response factor for each component was considered to equal 1.

Free radical scavenging activity on DPPH (FRSA)

This method was adapted from Chen & Ho (1997). Tested essential oils were added in 3.9 mL of 0.05 mM DPPH methanolic solution, and their final concentrations were 5.77, 2.77, 1.39 and 0.69 μg mL^{-1}. The absorbance of the samples was measured after 30 min on a spectrophotometer (Perkin Elmer, Lambda 1A, UV/VIS spectrophotometer) at 517 nm. Per cent inhibition of the DPPH radical by the samples was calculated according to the following formula:

$$\%\ \text{DPPH inhibition} = \left(1 - \frac{A - Ab}{Ao}\right) x100$$

where A is the absorbance of DPPH solution with the essential oils, Ab is the absorbance of 60% methanol with the essential oil, and Ao is the absorbance of DPPH solution.

The inhibitory concentration 50% (IC_{50}) was calculated from the curve obtained by plotting the percentage of inhibition vs. the final essential oil concentrations (Loizzo *et al*., 2009).

Antioxidant activity of oregano essential oils in canola oil

The antioxidant activity of oregano essential oils in canola oil was determined by an oxidation accelerated test according to Nepote *et al*. (2002). Oil treatments were prepared by mixing 0.02% (w/w) essential oil of oregano 'Cordobes' (EO-Cor), 'Criollo' (EO-Cri), 'Mendocino' (EO-M) and 'Compacto' (EO-Com) in canola oil. BHT (0.02%, w/w) in canola oil was used as reference, and canola oil without additives was used as control sample. Argentinean Food Code (CAA, 1996) allows a maximum of 0.02% of BHT and other synthetic antioxidants in vegetable oils. Because of that, 0.02% concentration of essential oil and BHT were used. The oxidation accelerated test was carried out in a laboratory oven (Reliance Enterprise, Kolkata, India) at 60 ± 1 °C during 12 days. Samples were investigated at 0, 5, 7, 9 and 12 days. Lipid oxidation of samples was evaluated by determining peroxide (PV) and *p*-anisidine (*p*-AV) values.

Peroxide value was measured following AOAC (1995) method. PV was expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2 kg^{-1}).

p-Anisidine value was evaluated following IUPAC (1987) method using a spectrophotometer (UV-V Diode Array Spectrophotometer Hewlett Packard HP 8452 A; Palo Alto, CA, USA).

Statistical analysis

The experiment was replicated three times. The data were analysed using the InfoStat software, version 2010p (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba). Means and standard deviations were calculated. Analysis of variance (ANOVA, $\alpha = 0.05$) and the LSD Fisher's multiple range test were performed to find significant differences among means. Principal component analysis (PCA) (Johnson & Wich-

ern, 1998) was performed on the correlation matrix of the standardised data from the oregano essential oil composition and the regression coefficient β_1 of the chemical variables from the storage study of canola oil (PV and AV). The purpose of the PCA was to explore associations between the main components of the essential oils of the different oregano species, the IC_{50} on DPPH, and the lipid deterioration indicators of canola oil. Cluster analysis (CA) was performed to find out groups of oregano species with similar characteristics. Sample similarities were calculated on the basis of the Euclidean distance, and the groups of oregano species with similar characteristics were obtained using the average linkage or the unweighted pair-group method using an arithmetic average (UPGMA).

Results and discussion

Essential oil composition

The essential oil composition from different oregano specie is listed in Table S1. Only the components with concentrations $>0.05\%$ were reported. The main compounds found in all oregano samples were the monoterpenes *trans*-sabinene hydrate, thymol and γ-terpinene. The studied oregano species showed difference in their essential oil composition. EO-Com, EO-Cor and EO-M had high content of *trans*-sabinene hydrate that varied between 27% and 38.2%. EO-Cri had the lowest amount of this compound (6.3%) and the highest amount of γ-terpinene (18.2%). EO-Com and EO-Cor showed higher amount of thymol (26.6–29.7%, respectively) than EO-M (17.1%) and EO-Cri (14.9%).

EO-Cordobes was characterised by the presence of twenty-five components, representing 95.8% of its essential oil. Other authors reported similar components in the essential oil of this oregano species (Esen *et al*., 2007; Dambolena *et al*., 2010). Twelve compounds characterised the EO-Com representing 99.6% of its essential oil. Other researchers have found that EO-Com and EO-Cor were rich in acyclic compounds and sesquiterpenoids (Sezik *et al*., 1993; Kula *et al*., 2007). Oregano species from Argentina were characterised for being rich in sabinyl compounds (Dambolena *et al*., 2010). Twenty-one compounds were found in the EO-M that represents 99.4% of its essential oil. Other authors found similar composition in this last oregano specie (Figueredo *et al*., 2005; Dambolena *et al*., 2010). Thirty-three components were found in EO-Cri representing 95.9% of its essential oil. Other authors reported high amounts of thymol and *trans*-sabinene hydrate in EO-Cri (Tabanca *et al*., 2004; Dambolena *et al*., 2010). The differences in the composition of essential oils could be attributed to different factors such as genotype of subspecies and/or environmental factors like climate conditions, temperature, humidity and rainfall among others (Dambolena *et al*., 2010).

Free radical scavenging activity on DPPH

DPPH inhibition percentages at different concentrations (5.77, 2.77, 1.39 and 0.69 μg mL^{-1}) and IC_{50} of the four oregano essential oils (Compacto, Cordobes, Criollo and Mendocino) are shown in Table S2. Oregano, Cordobes and Compacto had higher scavenging activity than the other oregano species. Mendocino had the lowest DPPH inhibition percentages. Loizzo *et al*. (2009) informed IC_{50} (μg mL^{-1}) between 1.0 and 1.7 in *Origanum* essential oils. Dambolena *et al*. (2010) reported FRSA between 17.5 and 75.3 in water extracts from dry oregano plant material studied in different locations measured at concentration of 40 mg plant mL^{-1}. These authors found that Compacto type had the highest FRSA.

Antioxidant activity of oregano essential oils in canola oil

Peroxide value is a chemical indicator used to determine primary reaction products of lipid oxidation (Frankel, 2005). PV of the different canola oil samples (canola oil control and canola oil added with EO-Com, EO-Cor, EO-Cri and EO-M, and with BHT) during storage at 60 °C are shown in Fig. 1. In general, PV of all treatments increased significantly ($\alpha = 0.05$) with storage time. Initially, all treatments had similar PVs (between 12.99 and 13.59 meq O_2 kg^{-1}). At day 5, canola oil with BHT showed the lowest value. From seventh day onwards, all treatments had significantly lower PV compared with the control having BHT treatments the lowest values. On 12th day of storage, the canola oil enriched with oregano essential oils showed lower PV (between 37.00 and 40.96 meq O_2 kg^{-1}) than canola oil control sample (43.12 meq O_2 kg^{-1}) and higher PV than BHT treatment (34.56 meq O_2 kg^{-1}). At this storage day, canola oils added with EO-Cor and EO-M had lower PV in comparison with the other treatments with oregano essential oils (Criollo and Compacto). These results indicate that essential oil of the studied oregano species showed antioxidant activity in canola oil.

In advanced stages of lipid oxidation, organic peroxides decompose into secondary products, including alcohols, carboxylic acids, aldehydes and ketones, which can be measured by the *p*-AV method (Frankel, 2005). *p*-AV of the different canola oil samples (canola oil control and canola oil added with EO-Com, EO-Cor, EO-Cri and EO-M, and with BHT) during storage at 60 °C are shown in Fig. 2. In general, *p*-AV of all treatments increased significantly with storage time. Initially, all treatments showed similar *p*-AV. Since 5–12 days of storage, canola oil samples had significant differences in *p*-AV and all

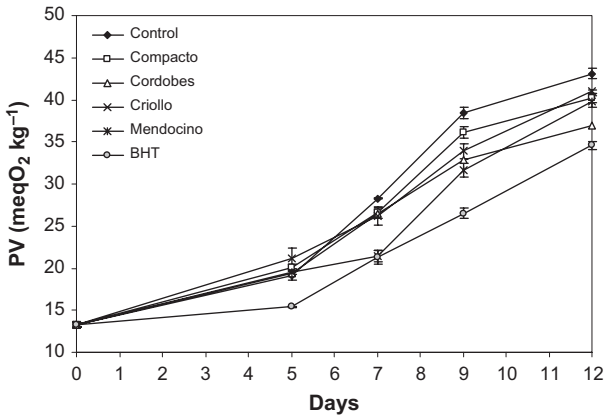

Figure 1 Peroxide values (meq O_2 kg^{-1}) from different canola oil samples: canola oil control and canola oil added with oregano essential oils (Compacto, Cordobés, Criollo, and Mendocino) and with BHT stored at 60 °C.

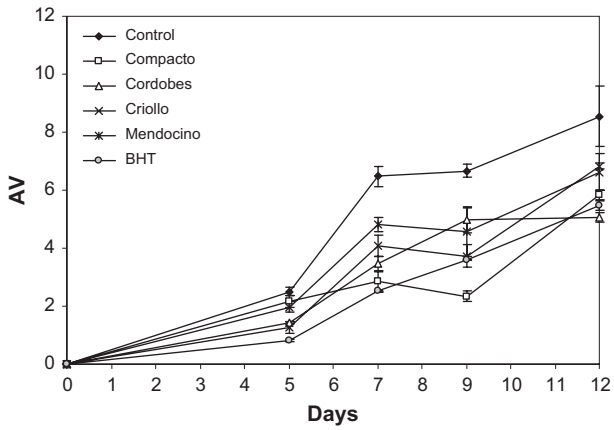

Figure 2 *p*-Anisidine values from different canola oil samples: canola oil control and canola oil added with oregano essential oils (Compacto, Cordobés, Criollo and Mendocino) and with BHT stored at 60 °C.

samples added with oregano essential oils and BHT had lower AV than canola oil control sample. At 12 days of storage, the samples with EO-Com and EO-Cor, and BHT had lower *p*-AV in comparison with those samples added with EO-Cri and EO-M. The *p*-AV at 12 storage days were 8.55, 5.47, 5.05, 5.84, 6.63 and 6.83 in canola oil control sample, and in canola oil added with BHT, EO-Cor, EO-Com, EO-M and EO-Cri, respectively. According to *p*-AV results, EO-Com and EO-Cor had antioxidant activity similar to BHT in canola oil.

Other authors (Dambolena *et al.*, 2010) reported total phenol content and FRSA of extracts from these oregano species (Compacto, Criollo, Cordobes and Mendocino) from different Argentinean locations (Santa Lucia, La Pampa, La Consulta). They found differences in the phenol content and FRSA among oregano species and locations. They reported that oregano extracts from Santa Lucia showed higher phenol content and FRSA. In that study, the authors also reported that Mendocino had the highest amount of phenolics (19.36 mg g^{-1}) followed by Compacto (18.88 mg g^{-1}), Cordobes (18.21 mg g^{-1}) and Criollo (18.09 mg g^{-1}), and Compacto had the highest FRSA (75.3%) followed by Criollo (67.3%), Mendocino (54.6%) and Cordobes (17.5%). Those authors concluded that O. Compacto from Santa Lucia could have higher antioxidant activity than the other oregano varieties.

Regression coefficients and R^2 for the dependent variables, PV and *p*-AV, in canola oil samples (canola oil control, canola oils enriched with EO-Com, EO-Cor, EO-Cri and EO-M, and with BHT) from the storage study at 60 °C are shown in Table 1. In general, the

Table 1 Regression equations and R^2 for dependant variables: peroxide (PV) and *p*-anisidine (AV) values in canola oil (control sample) and canola oil added with oregano essential oils (Compacto, Cordobes, Criollo, and Mendocino), and with BHT

		Regression*		
Dependent variable	**Canola oil sample**	β_0	β_1†	R^2
PV	Control	10.718645	2.686872 b	0.925670
	Compacto	11.434828	2.398966 ab	0.942724
	Cordobes	11.927463	2.099778 ab	0.955474
	Criollo	9.862291	2.308744 ab	0.764765
	Mendocino	10.462217	2.223300 ab	0.903530
	BHT	10.299631	1.802783 a	0.883125
AV	Control	−0.190107	0.797693 b	0.936115
	Compacto	−0.137898	0.439278 a	0.854419
	Cordobes	−0.123391	0.487989 a	0.895857
	Criollo	−0.444163	0.570025 a	0.909248
	Mendocino	−0.130123	0.604261 a	0.944129
	BHT	−0.617923	0.474130 a	0.922385

*Regression equations: $Y = \beta_0 + \beta_1 X$, where Y = dependent variable (PV and AV) and X = independent variable (days).

†ANOVA and LSD Fisher's multiple range test: The slope (β_1) of each variable followed with the same letters in the column are not significantly different at $\alpha = 0.05$.

regression models for these variables showed R^2 higher than 0.76. Therefore, these regression equations represented quite well the effect of the time on chemical variables (PV and AV) analysed in canola oil during storage at 60 °C. In addition, the stability differences between samples could be analysed through their oxidation tendencies. In Table 1, significant differences between the slopes (β_1) from regression analysis were detected for dependent variables PV and AV. Higher slopes (β_1) indicate higher tendency to oxidation. PV slope in canola oil added with BHT was lower than in the control. The canola oil with addition of oregano essential oils did not showed significant differences in their PV slopes in comparison with control and BHT treatments. β_1 values of AV in canola oils enriched with oregano essential oils and BHT were lower than in canola oil control. These results evidenced the antioxidant activity of oregano essential oils in canola oil.

Principal component analysis

Principal component analysis is an unsupervised technique that reduces the dimensionality in a data set. By projecting the objects of the data set into the space of the first few components, it is possible to demonstrate differences among the various objects, determining at the same time which variables are principally involved. Multivariate analysis methods are becoming increasingly important for food characterisation, classification, authenticity determination and quality control (Arvanitoyannis *et al*., 1999; Tzouros & Arvanitoyannis, 2001). In other works, multivariate analysis was used to explore authenticity of different food such as red wine (Arvanitoyannis *et al*.,1999; Kallithraka *et al*., 2001a,b), meat and meat products (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2003), olive oil (Arvanitoyannis & Vlachos, 2007), potato (Arvanitoyannis *et al*., 2008), rice (Arvanitoyannis & Vlachos, 2008), maize (Arvanitoyannis & Vlachos, 2009), and for classification of foods according to their variety or geographical origin (Kallithraka *et al*., 2001a,b).

Figure 3 is the biplot obtained from the first two principal components (PC) of PCA. The first two PC of the PCA explain 87.8% of total variability in the oregano species. This percentage was considered acceptable to draw correlation between the variables. The dispersion of the points indicated high variability among samples. Oxidation indicators (PV and AV) from canola oil storage study were positively associated between them and were negatively associated with thymol and carvacrol methyl ether contents. IC_{50} was positively correlated with limonene content and with the oxidation indicators (PV and AV). EO-Com and EO-Cor had higher contents of thymol, lower IC_{50} on DPPH, and relatively lower PV and AV. These oregano species showed antioxidant activity in canola oil. Thymol is a phenolic compound with demonstrated antioxidant activity (Baratta *et al*., 1998b; Lagouri *et al*., 1993; Aeschbach *et al*., 1994; Neffati *et al*., 2009). Ruberto & Baratta (2000), and this component together with carvacrol is the most active components of oxygenated monoterpenes. It is known that phenols are efficient antioxidants (Deighton *et al*., 1993; Ruberto & Baratta, 2000; Yanishlieva *et al*., 1999). Thymol and carvacrol are, in fact, responsible for the antioxidant activity of many essential oils that contain them (Baratta *et al*.,

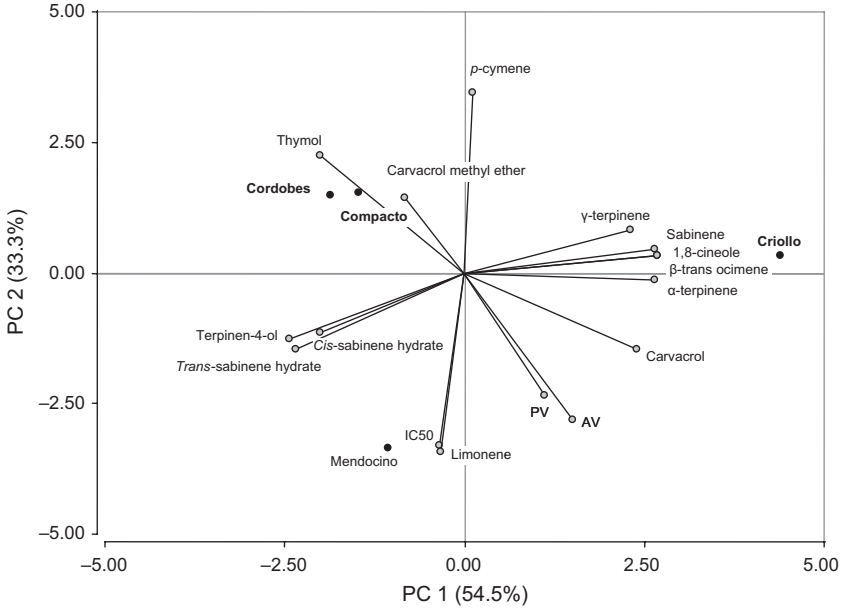

Figure 3 Biplot from the first and second principal components of principal component analysis. Variables: inhibitory concentration 50% on DPPH, chemical indicators (peroxide values and anisidine values) from canola oil stability study, and the main components of the essential oil from the studied oregano species (Compacto, Cordobes, Criollo and Mendocino).

1998b; Lagouri *et al*., 1993; Aeschbach *et al*., 1994; Neffati *et al*., 2009). The oregano species studied in this work had low amounts of carvacrol but their antioxidant activity may be due to high thymol content. Thymol and carvacrol are compounds used to characterise oregano chemotype. Thymol with sabinene hydrate characterised essential oils of *O. vulgare* subspecies *vulgare* and *virens*. While Criollo and Mendocino are hybrid oreganos, the chemistry of their essential oils would depend on their parents (Skoula *et al*., 1999; Skoula & Harborne, 2002). High carvacrol content in essential oil is the key to the concept of the oregano spice and is a prerequisite determining a plant's suitability for the preparation of this condiment. *Origanum heracleoticum*, the main source of Greek oregano, is a chemically non-uniform species. Within its wild population, there are at least three chemovarieties that, although similar in their external appearance, differ in their odours. On the basis of their flavours and essential oil compositions, these varieties could be defined as marjoram, oregano types. Oregano, which contains mainly carvacrol, is traditionally collected for oregano spice preparation on the basis of its odour (Fleisher & Sneer, 1982).

On the other hand, *cis* and *trans*-sabinene hydrate and terpinen-4-ol contents were positively related among them and negatively associated with α-terpinene, 1,8-cineole, β-*trans*-ocimene, sabinene and γ-terpinene contents in the essential oils of the studied oregano species. EO-Cri had higher amounts of these lasts compounds (α-terpinene, 1,8-cineole, β-*trans*-ocimene, sabinene and γ-terpinene) and lower amounts of *cis* and *trans*-sabinene hydrate and 4-terpineol than the essential oils from the other species. Limonene and IC_{50} were negatively associated mainly with *p*-cymene. Oregano Mendocino essential oil had higher contents of limonene, *cis* and *trans*-sabinene hydrate, IC_{50}, and lower *p*-cimene amount than the essential oils from the other oregano species.

The biplot suggests a poor association between the oxidation indicators in canola oil (PV and AV), the IC_{50}, and the following compounds in these oregano species: *cis* and *trans*-sabinene hydrate, terpinen-4-ol, α-terpinene, 1,8-cineole, β-*trans*-ocimene, sabinene and γ-terpinene.

Previous researches (Dambolena *et al*., 2010) reported variability in the composition and found associations between oregano essential oil composition, phenolic content and FRSA in water oregano extracts owing to different environmental factors.

Cluster analysis

Cluster analysis is an unsupervised classification procedure that involves a measurement of either the distance or the similarity between objects to be clustered. Objects are grouped in clusters in terms of their nearness or similarity (Arvanitoyannis *et al*., 1999, 2008). The results achieved by the CA of the oregano species considering the chemical indicators from storage study in canola oil (PV, AV and IC_{50}) and the main compounds in the oregano essential oils are presented as a dendrogram in Fig. 4. Three clusters of groups were observed: group 1 was formed by Compacto and Cordobes; group 2 and 3 were formed by Mendocino and Criollo, respectively. These results disclosed that there were differences between groups of oregano species. These variables may contain adequate information to attain oregano species differentiation according to the established classes. Figure 3 shows that *O. vulgare* ssp *virens* (Cordobes) and *O. vulgare* ssp *vulgare* (Compacto) had higher concentration of thymol and *trans*-sabinene hydrate. This composition made grouped together both subspecies. These results are consistent with the chemotaxonomic classification reported by Skoula *et al*. (1999). On the other hand, essential oil composition of *Origanum* × *majoricum* (Mendocino) was similar to that of their ancestors (Dambolena *et al*., 2010). Finally, *Origanum* × *appli* (Criollo) had a composition enriched in the biosynthetic precursors of oxygenated compounds as γ-terpinene, α-terpinene and sabinene. All these results affected the grouping of the four studied oregano (Fig. 4) determining higher similarity between subspecies of *O. vulgare* (Cordobes and Compacto) than the hybrid oreganos (Criollo and Mendocino). These last two oreganos were also not grouped.

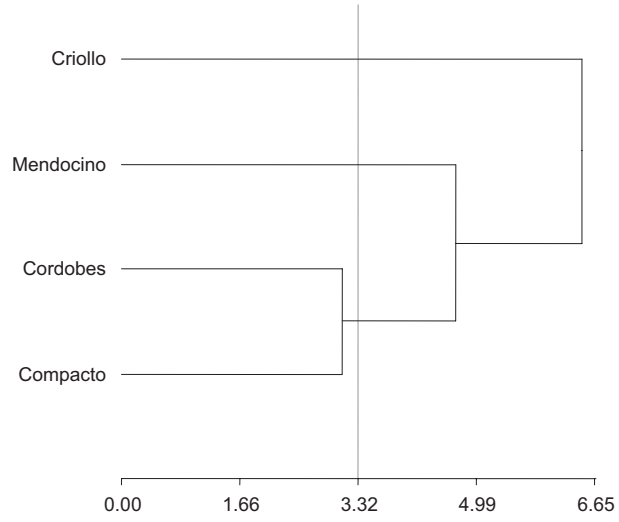

Figure 4 Dendrogram from cluster analysis of the different oregano species (Compacto, Cordobes, Criollo and Mendocino) considering the variables: main components in the oregano essential oils, inhibitory concentration 50% on DPPH, and the chemical indicators (peroxide values and anisidine values) from canola oil stability study.

Conclusions

The different oregano essential oils from Argentina showed variability in the relative percentages of the two main components: thymol and *trans*-sabinene hydrate. The different oregano species showed differences in radical scavenging activity and antioxidant activity in canola oil. In general, EO-Cor and EO-Com had higher scavenging activity and antioxidant activity than EO-Cri and EO-M. This antioxidant activity could be mainly associated with the high amount of thymol on these essential oils. The addition of these oregano essential oils in canola oil improves the stability of this oil preventing lipid oxidation. The present research renews interest in the use of naturally occurring antioxidants in food industry. For that reason, oregano essential oil as a natural antioxidant could be used for replacement of synthetic antioxidants on food products with high content of lipids. This study also provides associations between chemical composition of the essential oils, radical scavenging activity and oxidation indicators from the canola oil storage study.

Acknowledgments

This work was financed with grants from SeCyT-UNC and CONICET.

References

Adams, R.P. (1995). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Carol Stream, IL, USA: Allured Publishing.

Aeschbach, R., Loliger, J., Scott, B.C. *et al.* (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology*, **32**, 31–36.

AOAC (1995). *Official Methods of Analysis of the AOAC*. Washington, DC, USA: Association of Official Analytical Chemists.

Arvanitoyannis, I.S. & Van Houwelingen-Koukaliaroglou, M. (2003). Implementation of chemometrics for quality control and authentication of meat and meat products. *Critical Reviews in Food Science and Nutrition*, **43**, 173–218.

Arvanitoyannis, I.S. & Vlachos, A. (2007). Implementation of physicochemical and sensory analysis in conjunction with multivariate analysis towards assessing olive oil authentication/adulteration. *Critical Reviews in Food Science and Nutrition*, **47**, 441–498.

Arvanitoyannis, I.S. & Vlachos, A. (2008). A review of rice authentication/adulteration methods and results. *Critical Reviews in Food Science and Nutrition*, **48**, 553–598.

Arvanitoyannis, I.S. & Vlachos, A. (2009). Maize authentication: quality control methods and multivariate analysis (chemometrics). *Critical Reviews in Food Science and Nutrition*, **49**, 501–537.

Arvanitoyannis, I.S., Katsota, M.N., Psara, E.P., Soufleros, E.H. & Kallithraka, S. (1999). Application of quality control methods for assessing the wine authenticity: use of statistical (multivariate) analysis. *Trends in Food Science and Technology*, **10**, 321–336.

Arvanitoyannis, I.S., Vaitsi, O. & Mavromatis, A. (2008). Physicochemical and sensory attributes in conjunction with multivariate analysis of two potato (*Solanum tuberosum* L.) cultivars after 90 days of storage: an exploratory authentication study. *International Journal of Food Science and Technology*, **43**, 1960–1970.

Baratta, M.T., Dorman, H.J.D., Deans, S.G., Biondi, D.M. & Ruberto, G. (1998a). Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. *Journal of Essential Oil Research*, **10**, 618–627.

Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A.C., Barroso, J.G. & Ruberto, G. (1998b). Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal*, **13**, 235–244.

Barreyro, R., Ringuelet, J. & Agricola, S. (2005). Fertilización nitrogenada y rendimiento en orégano (*Origanum* x *applii*). *Ciencia e Investigación Agraria*, **32**, 39–43.

CAA (1996). Capítulo VII, Alimentos Grasos, Artículo nº 531 (Res. 2012, 19.10.84). In: *Código Alimentario Argentino, Ley 18.248 (18.7.1969)* (edited by S.R.L. De la Canal y Asociados). Pp. 164–165. Buenos Aires, Argentina: De la Canal y Asociados SRL.

Chen, J.H. & Ho, C.-T. (1997). Antioxidant activities of caffeic acid and iIts related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry*, **45**, 2374–2378.

Dambolena, J.S., Zunino, M.P., Lucini, E.I. *et al.* (2010). Total phenolic content, radical scavenging properties, and essential oil composition of *Origanum* species from different populations. *Journal of Agricultural and Food Chemistry*, **58**, 1115–1120.

Deans, S.G. (1991). Evaluation of antimicrobial activity of essential (volatile) oils. In: *Modern Methods of Plant Analysis, New Series, Vol. 12, Essential Oils and Waxes* (edited by H.F. Linskens & J.F. Jackson). Pp. 310–320. Berlin, Germany: Springer-Verlag.

Deighton, N., Glidewell, S.M., Deans, S.G. & Goodman, B.A. (1993). Identification by EPR spectroscopy of carvacrol and thymol as the major sources of free radicals in the oxidation of plant essential oils. *Journal of the Science of Food and Agriculture*, **63**, 221–225.

Eguchi, Y., Curtis, O.F. & Shetty, K. (1996). Interaction of hyperhydricity-preventing Pseudomonas sp. with oregano (*Origanum vulgare*) and selection of high phenolics and rosmarinic acid-producing clonal lines. *Food Biotechnology*, **10**, 191–202.

Englberger, W., Hadding, U., Etschenberg, E. *et al.* (1988). Rosmarinic acid: a new inhibitor of complement C3-convertase with anti-inflammatory activity international. *Journal of Immunopharmacology*, **10**, 729–737.

Esen, G., Azaz, A.D., Kurkcuoglu, M., Baser, K.H.C. & Tinmaz, A. (2007). Essential oil and antimicrobial activity of wild and cultivated *Origanum vulgare* L. subsp. hirtum (Link) letswaart from the Marmara region, Turkey. *Flavour and Fragrance Journal*, **22**, 371–376.

Figueredo, G., Chalchat, J.-C. & Pasquier, B. (2005). Studies of mediterranean oregano population IV – chemical composition of essential oils of hybrids *Origanum* × *majoricum* cambassedes from France, *Origanum* × intercedons Rechinger and *Origanum* × *minoanum* davis from Turkey and Crete. *Journal of Essential Oil Research*, **17**, 296–300.

Fleisher, A. & Sneer, N. (1982). Oregano spices and *Origanum* chemotypes. *Journal of the Science of Food and Agriculture*, **33**, 441–446.

Frankel, E.N. (2005). *Lipid Oxidation*. Pp. 1–470. Bridgewater, UK: The Oily Press.

Helander, I.M., Alakomi, H.-L., Latva-Kala, K. *et al.* (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. *Journal of Agricultural and Food Chemistry*, **46**, 3590–3595.

IUPAC (1987). Method number 2.504. Determination of the p-anisidine value. In: *Standard Methods for the Analysis of Oils, Fats and Derivatives* (edited by C. Paquot & A. Hautfenne). Pp. 210–212. Oxford, UK: Blackwell.

Johnson, R.A. & Wichern, D.W. (1998). *Applied Multivariate Statistical Analysis*. Pp. 816. Englewood Cliffs, N.J.: Prentice Hall.

Kallithraka, S., Arvanitoyannis, I.S., El-Zajouli, A. & Kefalas, P. (2001a). A new method for transresveratrol determination in red wines; Implementation of chemometrics for Greek red wines

classification according to geographical origin. *Food Chemistry*, **75**, 355–363.

Kallithraka, S., Arvanitoyannis, I.S., Kefalas, P., El-Zajouli, A., Soufleros, E. & Psarra, E. (2001b). Instrumental and sensory evaluation of Greek red and white wines; implementation of principal component analysis for classification according to geographic origin. *Food Chemistry*, **73**, 501–514.

Kula, J., Majda, T., Stoyanova, A. & Georgiev, E. (2007). Chemical composition of *Origanum vulgare* L. essential oil from Bulgaria. *Journal of Essential Oil – Bearing Plants*, **10**, 215–220.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S. & Boskou, D. (1993). Composition and antioxidant activity of essential oils from Oregano plants grown wild in Greece. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, **197**, 20–23.

Loizzo, M.R., Menichini, F., Conforti, F. *et al*. (2009). Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. *Food Chemistry*, **117**, 174–180.

Neffati, A., Bouhlel, I., Ben Sghaier, M. *et al*. (2009). Antigenotoxic and antioxidant activities of Pituranthos chloranthus essential oils. *Environmental Toxicology and Pharmacology*, **27**, 187–194.

Nepote, V., Grosso, N.R. & Guzman, C.A. (2002). Extraction of antioxidant components from peanut skins. *Grasas y Aceites*, **53**, 391–395.

Nepote, V., Mestrallet, M.G. & Grosso, N.R. (2006). Oxidative stability in fried-salted peanuts elaborated with high-oleic and regular peanuts from Argentina. *International Journal of Food Science and Technology*, **41**, 900–909.

Olmedo, R.H., Nepote, V., Mestrallet, M.G. & Grosso, N.R. (2008). Effect of the essential oil addition on the oxidative stability of fried-salted peanuts. *International Journal of Food Science and Technology*, **43**, 1935–1944.

Olmedo, R.H., Asensio, C., Nepote, V., Mestrallet, M.G. & Grosso, N.R. (2009). Chemical and sensory stability of fried-salted peanuts flavored with oregano essential oil and olive oil. *Journal of the Science of Food and Agriculture*, **89**, 2128–2136.

Papageorgiou, V., Mallouchos, A. & Komaitis, M. (2008). Investigation of the antioxidant behavior of air- and freeze-dried aromatic plant materials in relation to their phenolic content and vegetative cycle. *Journal of Agricultural and Food Chemistry*, **56**, 5743–5752.

Peake, P.W., Pussell, B.A., Martyn, P., Timmermans, V. & Charlesworth, J.A. (1991). The inhibitory effect of rosmarinic acid on complement involves the C5 convertase. *International Journal of Immunopharmacology*, **13**, 853–857.

Ruberto, G. & Baratta, M.T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*, **69**, 167–174.

Ruberto, G., Baratta, M.T., Sari, M. & Kaabeche, M. (2002). Chemical composition and antioxidant activity of essential oils from Algerian *Origanum* glandulosum Desf. *Flavour and Fragrance Journal*, **17**, 251–254.

Ryan, L.C., Mestrallet, M.G., Nepote, V., Conci, S. & Grosso, N.R. (2008). Composition, stability and acceptability of different vegetable oils used for frying peanuts. *International Journal of Food Science and Technology*, **43**, 193–199.

Sezik, E., Tumen, G., Kirimer, N., Ozek, T. & Baser, K.H.C. (1993). Essential oil composition of four *Origanum vulgare* subspecies of Anatolian origin. *Journal of Essential Oil Research*, **5**, 425–431.

Silva, M.P., Martinez, M.J., Casini, C. & Grosso, N.R. (2010). Tocopherol content, peroxide value and sensory attributes in roasted peanuts during storage. *International Journal of Food Science and Technology*, **45**, 1499–1504.

Skoula, M. & Harborne, J.B. (2002). The taxonomy and chemistry of *Origanum*. In: *The Genera Origanum* and Lippia (edited by S.E. Kintzios). Pp. 67–108. New York: Taylor and Francis.

Skoula, M., Gotsiou, P., Naxakis, G. & Johnson, C.B. (1999). A chemosystematic investigation on the mono- and sesquiterpenoids in the genus *Origanum* (Labiatae). *Phytochemistry*, **52**, 649–657.

Tabanca, N., Ozek, T., Baser, K.H.C. & Turnen, G. (2004). Comparison of the essential oils of *Origanum majorana* L. and *Origanum* × *majoricum* cambess. *Journal of Essential Oil Research*, **16**, 248–252.

Tzouros, N.E. & Arvanitoyannis, I.S. (2001). Agricultural produces: synopsis of employed quality control methods for the authentication of foods and for the classification of foods according to their variety or geographical origin. *Critical Reviews in Food Science and Nutrition*, **41**, 287–319.

Yanishlieva, N.V., Marinova, E.M., Gordon, M.H. & Raneva, V.G. (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chemistry*, **64**, 59–66.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Terpenoid concentrations (relative percentage) from the four oregano species presented according to their elution order in the gas–liquid chromatography and mass spectrometry analysis.

Table S2. Means of DPPH inhibition percentages of the four oregano essential oils (Compacto, Cordobes, Criollo and Mendocino) at different concentrations (5.77, 2.77, 1.39, and 0.69 μg mL^{-1}), and the inhibitory concentration 50% (IC_{50}).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.