International Journal of
Food Science & Technology
www.ifst.org

Original article

Chemical composition, antioxidant activity and anti-lipase activity of *Origanum vulgare* and *Lippia turbinata* essential oils

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(Received 12 April 2012; Accepted in revised form 31 August 2012)

Summary This study reported the chemical composition, phenolic content, antioxidant and anti-lipase activity of oregano and *Lippia* essential oils. The major compounds found in oregano essential oil were γ-terpinene (32.10%), α-terpinene (15.10%), *p*-cymene (8.00%) and thymol (8.00%). In *Lippia* essential oil, α-limonene (76.80%) and 1,8-cineole (4.95%) represented the major compounds. Oregano essential oil had higher phenolic content (12.47 mg gallic acid mL^{-1}) and DPPH scavenging activity (IC_{50} 0.357 μg mL^{-1}) than *Lippia* essential oil (7.94 mg gallic acid mL^{-1} and IC_{50} 0.400 μg mL^{-1}, respectively). Both essential oils had similar antioxidant indexes (about 1.2) determined by Rancimat. Moreover, oregano essential oil had also higher anti-lipase activity (IC_{50} 5.09 and 7.26 μg mL^{-1}). Higher phenolic content in the essential oils was related with higher scavenging and anti-lipase activities. Oregano and *Lippia* essential oils could be used as natural antioxidants on food products.

Keywords Anti-lipase, antioxidant, essential oils, *Lippia*, oregano.

Introduction

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). Moreover, essential oils from aromatic species are known to possess potential as natural agents for food preservation, and their effectiveness against a wide range of micro-organisms has been repeatedly demonstrated (Baratta *et al*., 1998; Helander *et al*., 1998). In addition, many essential oils have shown antioxidant activity (Olmedo *et al*., 2008; Quiroga *et al*., 2011).

Growing interest in the replacement of synthetic antioxidants by natural ones has led to multiple investigations in the field of new antioxidants (Lante *et al*., 2011). Essential oils have shown to be effective in retarding the process of lipid oxidation. For that reason, essential oils are gaining more attention because of their relatively safe status and their wide acceptance by consumers. Essential oils are a rich source of polyphenols recognised as antioxidants stronger than tocopherols. These last ones are present in most vegetable oils, but they are unstable during heating process (Nepote *et al*., 2006; Ryan *et al*., 2008; Silva *et al*., 2010).

Oregano is an important aromatic plant widely used in many countries for flavouring foods. Mexico, Greece, Israel, Albania, Morocco and Turkey are the main countries involved in production and export of oregano (Koksal *et al*., 2010). Species of the genus *Origanum* are the most commonly used in the oregano commercial production. *Origanum* is a genus with several species where all of them are known as oregano in most countries (Dambolena *et al*., 2010). *Origanum vulgare* L. is the most important aromatic herb cultivated in Argentina, not only for the surface (80%) but also for the population demand (Di Fabio, 2008; Dambolena *et al*., 2010). The main areas of farming are located in Mendoza Province (250 ha) followed by Córdoba (113 ha) and San Juan (56 ha) provinces. The oregano leaves and essential oil were used for centuries because of its medicinal properties, in particular for its positive effect on human health that has been attributed to the antioxidant activity of essential oil and soluble phenolic fractions (Peake *et al*., 1991; Eguchi *et al*., 1996; Ruberto *et al*., 2002).

Lippia turbinata Griseb. or 'poleo' is an aromatic plant belonging to the *Verbenaceae* family. It is a

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doi:10.1111/ijfs.12011

native shrub from South America, commonly found in the north-eastern Argentina, which is widely used in folk medicine and food industry for its flavour. *Lippia* essential oil from the Argentinean north-eastern region has phyletic markers such as lippione and dihydrolippione (Ricciardi *et al*., 2005).

Oregano and *Lippia* essential oils are compounds with preserving properties because of their antioxidant and anti-lipase properties. The introduction of an antioxidant into the lipid system leads to a change in mechanism of the oxidising process. The effect of the antioxidant depends on the participation of its molecules and the radicals formed from the latter in the chain reactions. Yanishlieva *et al*. (1999) reported two kinetic characteristics to explain the inhibitory action: (i) the possibility of blocking the radical chain process by interaction with peroxide radicals, affecting the induction period duration and (ii) the possibility for the inhibitor moieties to participate in other reactions, changing the oxidation rate during the induction periods. Thymol and carvacrol are isomeric molecules characteristic of the Lamiaceae essential oils like oregano is (Tsimidou & Boskou, 1994). Yanishlieva *et al*. (1999) observed that these molecules took part in chain reaction initiation during the oxidation of lard and sunflower oil triglycerides. In addition, it was also observed that the radicals of carvacrol participated in one reaction of chain propagation but thymol does not. Thymol was more active and effective antioxidant than carvacrol. Both molecules differed in the mechanism of their inhibiting action, depending on the lipid medium.

Lipases (triacylglycerol hydrolases) are an important group of enzymes having biotechnological significance, and they have numerous applications in food, dairy, detergent and pharmaceutical industries (Gupta *et al*., 2004). Lipases are widely distributed in animal, plant, bacteria, yeast and fungal species (Saxena *et al*., 1999). Microbial lipases have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds and decaying food (Sztajer *et al*., 1988). These enzymes catalyse the hydrolysis of the ester bonds of triacylglycerols enhancing free fatty acid content; therefore, lipase inhibitors may be considered to control oil acidity and lipid stability. Considerable efforts in the search of new lipase inhibitors derived from natural sources give access to novel potential food additives. Natural compounds such as terpenoids (Jang *et al*., 2008; Kim *et al*., 2009), triterpene glycosides (Morikawa *et al*., 2009; Sugimoto *et al*., 2009), flavonoids (Won *et al*., 2007), catechins (Yoshikawa *et al*., 2002; Nakai *et al*., 2005), stilbenes (Matsuda *et al*., 2009), chitosan (Ostanina *et al*., 2007) and phenyl compounds (Raghavendra & Prakash, 2002; Han *et al*., 2007) showed lipase inhibition activity.

The biochemical activity of enzymes, such as lipases, is often associated with structural changes in the enzyme, resulting in selective and stereospecific reactions between the lipase binding site and the substrate (Peters *et al*., 1997). The mechanism of action of these enzymes involves the nucleophilic cleavage of an ester bond by an activated serine that belongs to the catalytic triad Ser-His-Asp/Glu. Inhibitors react with the nucleophilic serine of lipase active site, thus leading to the formation of covalent and equimolar lipid–protein complexes that are stable in aqueous and organic solutions. This binding between inhibitors and lipase active site is reversible (Oskolkova *et al*., 2003). Plant secondary metabolites present in herbal food have shown to be lipase inhibitors. Among these metabolites, saponins, flavonoids and alkaloids, present in high concentrations in plant extract, are a promising source of lipase inhibitors because they are capable of inhibiting lipase activity (Ruiz *et al*., 2006). Probably, determined compounds from the essential oils could be inhibiting the lipase activity interacting in the active site of the enzyme.

The objective of the present study was to determine antioxidant and anti-lipase activity of *O. vulgare* L. and *L. turbinata* Griseb. essential oils in relation to their chemical composition and phenolic content.

Materials and methods

Plant material

The studied species were *Origanum vulgare* L. from San Carlos farm (crop 2010), Mendoza, Argentina, and *Lippia turbinata* Griseb. from Altas Cumbres (crop 2010), Córdoba, Argentina. Three different lots of each plant species were collected separately, and each lot was considered a repetition. Plant materials of both species were harvested at the full flowering stage in November 2010. Two-year-old plants (leaves, flowers and stems) were cut 5 cm above the soil surface. Voucher specimens were deposited at the herbarium of Botanical Museum (ACORD), Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba, Argentina. The plant identification was according to the voucher specimen numbers ACORD – AMP 2544 for *Origanum vulgare* L. and LMC 654 for *Lippia turbinata* Griseb.

Experimental design

An experimental design of two treatments ×3 repetitions was used. Two treatments were studied: *O. vulgare* L. and *L. turbinata* Griseb. essential oils. The experiment was replicated three times. The analysed variables in this experiment were essential oil composition, total

phenol content, free radical scavenging activity (FRSA), antioxidant activity index (AAI) by Rancimat and anti-lipase activities in *Candida antarctica* and *Pseudomonas fluorescens* lipases.

Essential oil extraction

Essential oils were extracted by hydro-distillation 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} to 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.

Total phenolic compounds

Total phenolic compounds were determined by Folin–Ciocalteu method (Singleton & Rossi, 1965). Folin–Ciocalteu reagent (0.5 mL; Anedra, Sanfernando Buenos Aires, Argentina) was used, and the total phenols were determined on a spectrophotometer (Perkin Elmer, Lambda 1A, UV/VIS spectrophotometer, Perkin Elmer Inc., Waltham, MA, USA) at 760 nm. Total phenol content was expressed in mg gallic acid equivalent mL^{-1} of essential oil.

Antioxidant activity

Free radical scavenging activity on DPPH

Tested essential oils were added in 0.05 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) methanolic solution (Chen & Ho, 1997), and their final concentrations were 0.062, 0.123, 0.247, 0.494, 0.988 and 1.975 μg mL^{-1}. The absorbance of the samples was measured on a spectrophotometer (Perkin Elmer, Lambda 1A, UV/VIS spectrophotometer, PerkinElmer Inc., Waltham, MA, USA) at 517 nm. Per cent inhibition of the DPPH radical by the samples was calculated according to the following formula:

$$\% \quad \text{DPPH inhibition} = \left(1 - \frac{A - Ab}{Ao}\right) \times 100$$

where A is the absorbance of DPPH solution with 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; Quiroga *et al.*, 2011).

Antioxidant activity index by RANCIMAT

A Rancimat 743 (Metrohm Ltd., Herisau, Switzerland) was used to determine the antioxidant activity of oregano and *Lippia* essential oils in butter (Auchan, Padova, Italy). Three treatments were prepared: pure butter without essential oils, butter with oregano and *Lippia* essential oils (16.458 mg g^{-1} final concentration). Air flow rate was set at 20 L h^{-1}, and temperature of the heating block was maintained at 100 °C (Politeo *et al.*, 2006). The AAI was calculated from the measured induction times (break points in the plotted curves), according to the following formula (Viuda-Martos *et al.*, 2010):

$$\text{AAI} = (\text{Induction time of butter with essential oil})/(\text{Induction time of pure butter})$$

Anti-lipase activity

Anti-lipase activity of essential oils was determined following Lomolino *et al.*'s (2003) method with modifications. For the anti-lipase activity determination, β-naphthyl acetate (Sigma) was used as substrate. Commercial lipases from *C. antarctica* (L-C) and from *P. fluorescens* (L-P) were used (Sigma). β-Naphthyl acetate in Triton 100× solution (Sigma) with essential oil at different concentrations (0.617, 1.234, 2.469, 4.938 and 9.875 μg mL^{-1}) was analysed. Samples were incubated at 40 °C for 90 min. Fast Blue BB Salt (Fluka, Buchs, Switzerland) was added to the reaction, and the absorbance of the samples was measured on a spectrophotometer (Perkin Elmer, Lambda 1A, UV/VIS spectrophotometer) at 500 nm. Per cent lipase inhibition was calculated as following:

$$\% \quad \text{Lipase inhibition} = \left(1 - \frac{A - Ab}{Ao}\right) \times 100$$

where A is the absorbance of sample with essential oil, Ab is the absorbance of sample without lipase (blank), and Ao is the absorbance of control without essential oil.

The inhibitory concentration 50% (IC_{50}) was calculated from the curve obtained by plotting the percentage of inhibition vs. the final essential oil concentrations (Quiroga *et al.*, 2011).

Statistical analysis

The data were analysed using the InfoStat software, version 2010p (Facultad de Ciencias Agropecuarias, Univ. Nacional de Cordoba). 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. Correlation analyses were performed employing Pearson's test.

Results and discussion

Essential oil composition

Volatile composition in oregano and *Lippia* essential oils is listed in Table 1. Only components with concentrations >0.1% are reported.

Oregano was characterised by the presence of thirty-seven components. The major compounds found in oregano sample were the monoterpenes: γ-terpinene (32.10%), α-terpinene (15.10%), *p*-cymene (8.00%), thymol (8.00%) and β-phellandrene (7.10%). Previous studies reported that the major compounds found in four different Oregano species were the monoterpenes *trans*-sabinene hydrate, thymol and γ-terpinene (Rodríguez Vaquero *et al.*, 2010; Quiroga *et al.*, 2011).

Other authors reported thymol, α-terpineol, linalyl acetate and linalool (Pérez *et al.*, 2007; De Martino *et al.*, 2009) and linalool, thymol and α-terpineol (Khosravi *et al.*, 2011) as major components in oregano essential oil. Oregano species growing in Argentina does not have a significant concentration of carvacrol (Dambolena *et al.*, 2010; Asensio *et al.*, 2011; Quiroga *et al.*, 2011). This may be related to genetic causes associated with the original material introduced to Argentina which was renewed by clones and environmental factors that affect the composition of essential oils like climate conditions, temperature, humidity and rainfall among others (Figueiredo *et al.*, 2008; Dambolena *et al.*, 2010).

Forty-five compounds were found in the *Lippia* essential oil. α-Limonene (76.80%), 1,8-cineole (4.95%), caryophyllene oxide (1.55%), β-pinene (1.45%) and sabinene (1.40%) were the major compounds found in this essential oil. Other authors have

Table 1 Terpenoid concentrations (relative percentage) from the oregano and *Lippia* essential oils presented according to their elution order in the GC–MS analysis

			Relative percentages%	
RI	**MOI**	**Compounds**	**Oregano**	***Lippia***
924	MS	α-Thujene	0.70 ± 0.06	n.d.
930	MS	α-Pinene	1.40 ± 0.10	0.90 ± 0.08
944	MS	Camphene	0.50 ± 0.04[a]	1.15 ± 0.10[b]
970	MS	Sabinene	3.10 ± 0.26[b]	1.40 ± 0.08[a]
974	Co-MS	β-Pinene	n.d.	1.45 ± 0.13
989	MS	β-Myrcene	0.50 ± 0.04	0.25 ± 0.02
1002	Co-MS	α-Phellandrene	2.9 ± 0.25	n.d.
1014	Co-MS	α-Terpinene	15.10 ± 1.20	n.d.
1020	MS	*p*-Cymene	8.00 ± 0.60[b]	0.15 ± 0.01[a]
1024	MS	Limonene	0.6 ± 0.05[a]	76.80 ± 6.90[b]
1026	Co-MS	β-Phellandrene	7.10 ± 0.62	n.d.
1027	MS	1,8-Cineole	n.d.	4.95 ± 0.35
1032	MS	Z β-ocimene	0.60 ± 0.05[b]	0.15 ± 0.01[a]
1054	Co-MS	g-Terpinene	32.10 ± 2.60	n.d.
1086	MS	Terpinolene	4.10 ± 0.33	n.d.
1089	MS	*p*-Cymenene	0.20 ± 0.02	n.d.
1095	MS	Linalool	2.10 ± 0.16	n.d.
1124	MS	Chrysanthenone	n.d.	0.10 ± 0.01
1140	Co-MS	Z β-terpineol	0.10 ± 0.01	n.d.
1165	MS	Borneol	n.d.	0.15 ± 0.01
1172	Co-MS	Terpinen 4 ol	0.30 ± 0.02	0.15 ± 0.01
1183	Co-MS	α-Terpineol	0.20 ± 0.01	0.35 ± 0.032
1232	MS	Thymil methyl ether	0.30 ± 0.02	n.d.
1239	MS	Carvone	n.d.	1.10 ± 0.09
1241	MS	Carvacrol methyl ether	0.50 ± 0.04	n.d.
1249	MS	Piperitone	n.d.	0.15 ± 0.01
1253	MS	Piperitone oxide	n.d.	0.25 ± 0.02
1285	MS	Bornyl acetate	n.d.	0.15 ± 0.011
1289	Co-MS	Thymol	8.00 ± 0.71	n.d.
1298	MS	Carvacrol	4.00 ± 0.33	n.d.
1340	MS	Carvyl acetate	n.d.	0.30 ± 0.02
1356	MS	Eugenol	n.d.	0.45 ± 0.04
1366	MS	Piperitenone oxide	n.d.	0.90 ± 0.08
1374	MS	α-Copaene	n.d.	0.15 ± 0.01
1385	MS	β-Bourbonene	0.10 ± 0.01[a]	0.85 ± 0.07[b]
1389	MS	β-Elemene	n.d.	0.25 ± 0.02
1409	MS	α-Gurjunene	n.d.	0.15 ± 0.01
1417	MS	β-Caryophyllene	2.00 ± 0.13	1.25 ± 0.11
1434	MS	g-Elemene	n.d.	0.10 ± 0.01
1439	Co-MS	Aromadendrene	0.40 ± 0.03	0.15 ± 0.01
1450	MS	α-Humulene	0.30 ± 0.02	0.30 ± 0.02
1458	Co-MS	Allo-aromadendrene	n.d.	0.2 ± 0.01
1478	MS	g-Muurolene	0.10 ± 0.01	n.d.
1479	MS	α-Curcumene	n.d.	0.25 ± 0.02
1484	MS	Germacrene D	n.d.	0.40 ± 0.03
1505	MS	β-Bisabolene	0.40 ± 0.03	0.10 ± 0.01
1513	MS	γ-Cadinene	0.30 ± 0.02	0.15 ± 0.01
1522	MS	δ-Cadinene	1.00 ± 0.09[b]	0.25 ± 0.02[a]
1528	MS	cis-Calamenene	n.d.	0.15 ± 0.01
1537	MS	α-Cadinene	0.10 ± 0.01	n.d.
1544	MS	α-Calacorene	n.d.	0.20 ± 0.01
1559	MS	Germacrene B	0.10 ± 0.01	n.d.
1575	MS	Spathulenol	0.90 ± 0.08	0.40 ± 0.04

Table 1 (*Continued*)

RI	MOI	Compounds	Relative percentages%	
			Oregano	*Lippia*
1590	MS	Caryophyllene oxide	0.80 ± 0.07	1.55 ± 0.13
1610	MS	Cedrol	n.d.	0.30 ± 0.03
1631	MS	T-muurolol	n.d.	0.15 ± 0.01
1640	MS	T-cadinol	n.d.	0.40 ± 0.03
1655	MS	α-Cadinol	n.d.	0.35 ± 0.03
1988	MS	M + 222	n.d.	0.25 ± 0.02
1993	MS	M + 234	n.d.	0.20 ± 0.02
2003	MS	M + 236	n.d.	0.25 ± 0.02
2508	MS	M + 290	0.50 ± 0.04	n.d.
2526	MS	M + 308	0.40 ± 0.03	n.d.
2717	MS	M + 310	0.20 ± 0.02	n.d.

n.d., not detected; RI, retention index; MOI, methods of identification by Co (co-injection with standard) and MS (From libraries data – Nist and Adams, 1995); GC–MS, gas–liquid chromatography and mass spectrometry.
Values with different letters are significantly different from each other according to LSD Fisher's multiple range test at $\alpha = 0.05$ ($n = 3$).

found limonene, *cis*-α-bisabolene, germacrene D and β-caryophyllene as major components in the essential oil (Moriconi *et al*., 2009; Passone *et al*., 2012).

Total phenolic compounds and antioxidant activity

Total phenolic compounds, DPPH inhibitory concentration 50% (DPPH IC_{50}), AAI and the lipase inhibitory concentration 50% (L-C-IC_{50} and L-P-IC_{50}) of oregano and *Lippia* essential oils are showed in Table 2. Phenolic compounds have proved radical scavenging and antioxidant activity (Nepote *et al*., 2004, 2005). Oregano essential oil had almost double phenolic content than *Lippia* essential oil. Other authors reported total phenol content and FRSA of aqueous extracts from four oregano species (Compacto, Criollo, Cordobes and Mendocino) from different Argentinean locations. They found differences in the phenol content and FRSA among oregano species and locations and also found total phenolic compounds between 8.88 and 19.36 mg gallic acid equivalent g^{-1} in oregano water extract (Dambolena *et al*., 2010). *Lippia turbinata* infusions showed a content of about 0.123 mg gallic acid equivalent g^{-1} (Rodríguez Vaquero *et al*., 2010). Other authors found 376.7 mg gallic acid equivalent g^{-1} in methanolic extracts from *Lippia schomburgkiana* (Da Silva *et al*., 2009).

Antioxidant activity of essential oils from aromatic plants is mainly attributed to phenolic compounds present in them (Olmedo *et al*., 2008; Dambolena *et al*., 2010; Asensio *et al*., 2011; Quiroga *et al*., 2011). DPPH inhibition percentages of oregano and *Lippia* essential oils at different concentrations were used to determine DPPH-IC_{50} of each essential oil (Table 2).

Table 2 Total phenolic compounds, DPPH inhibitory concentration 50% (DPPH-IC_{50}), antioxidant activity index by Rancimat test (AAI) and the lipase inhibitory concentration 50% (L-C-IC_{50}: activity on lipase from *Candida antarctica*, and L-P-IC_{50}: activity on lipase from *Pseudomonas fluorescens*) measured in oregano and *Lippia* essential oils

	Oregano*	*Lippia**
Total phenolic compounds (mg gallic acid mL^{-1})	12.47 ± 0.22[b]	7.94 ± 0.30[a]
DPPH-IC_{50} (μg mL^{-1})	0.357 ± 0.001[a]	0.400 ± 0.022[b]
AAI	1.20 ± 0.09[a]	1.24 ± 0.05[a]
L-C-IC_{50} (μg mL^{-1})	5.09 ± 0.06[a]	7.49 ± 0.13[b]
L-P-IC_{50} (μg mL^{-1})	7.26 ± 0.11[a]	11.13 ± 0.47[b]

*Values with different letters within each raw are significantly different (LSD Fisher's test, $\alpha = 0.05$, $n = 3$).

In general, oregano and *Lippia* essential oils showed similar radical scavenging activity at different essential oil concentrations. However, oregano showed higher radical scavenging activity than *Lippia* at 0.247 and 0.494 μg mL^{-1}. According to the results showed in Table 2, oregano and *Lippia* essential oils showed similar DPPH-IC_{50} values; however, significant differences were found between the samples. Oregano essential oil had lower DPPH-IC_{50} (0.357 ± 0.001 μg mL^{-1}), and therefore, it also had stronger radical scavenging activity than *Lippia* essential oil (0.400 ± 0.022 μg mL^{-1}). Lower DPPH-IC_{50} is related to stronger radical scavenging activity. In the previous studies, essential oils from different oregano species showed DPPH-IC_{50} between 0.90 and 2.38 μg mL^{-1} (Quiroga *et al*., 2011). Other authors reported DPPH-IC_{50} (μg mL^{-1}) between 1.0 and 1.7 in oregano essential oils (Loizzo *et al*., 2009) and 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} (Dambolena *et al*., 2010).

Rancimat method involves lipidic sample exposure to temperatures between 50 and 220 °C and air flow. The volatile oxidation products (mainly formic acid) are transferred with the air flow to the conductivity measurement equipment. The induction time corresponded to the break points in the plotted curves of conductivity. A low induction time indicates fast lipid oxidation. The AAI is the ratio between the induction times of the sample with antioxidant and the sample without antioxidant. The oregano and *Lippia* essential oils did not show significant differences in their AAI (Table 2), which indicates that these essential oils had similar antioxidant activity in the studied conditions (1.20–1.24). Other authors reported that oregano, thyme, clove, sage and rosemary essential oils had AAI between 1.05 and 1.67 being these values lower than butylated hydroxytoluene (BHT) (AAI = 2.42, Viuda-Martos *et al*., 2010). Considering the test

condition of Rancimat method and that the essential oils are volatile compounds, part of the essential oils could have been lost partially during the test. These causes could explain low antioxidant activity showed by the essential oils in Rancimat method. The results obtained by the Rancimat method are sometimes ambiguous and may guide to incorrect conclusions (Politeo *et al*., 2006).

Essential oils with phenolic components such as eugenol, thymol or carvacrol have shown remarkable antioxidant activity because these compounds have a phenolic base. Phenols with ortho-substitution with electron-donating alkyl or methoxy groups increase the stability of the free radical. The position and degree of hydroxylation of phenolic compounds determines their antioxidant activity (Dambolena *et al*., 2010; Asensio *et al*., 2011). Oregano essential oil reported in the present study, rich in terpenes like g-terpinene, β-phellandrene and terpinolene and phenols like thymol and carvacrol, exhibited higher antioxidant activity than *Lippia* essential oil. Probably, this higher activity was influenced by their phenol constituents (thymol and carvacrol). *Lippia turbinata* essential oil exhibited high concentration of terpenes like limonene and 1,8-cineole. Despite the fact that *Lippia* essential oil showed low concentration of phenolic compounds, this essential oil also exhibited antioxidant activity. Many essential oils without phenols in their composition have also shown antioxidant activity. Essential oils from *Citrus sinensis* rich in limonene, and from *Eucalyptus camaldulensis* rich in *p*-cymene, 1,8-cineole, β-phellandrene and spathulenol, and from *Myrtus communis* L. rich in 1.8-cineole and methyl eugenol were reported with moderate antioxidant activity (Graça, 2010). In addition, some alkenes such as terpinenes and caryophyllene were also reported to retard the linoleic acid peroxidation. Alpha and gamma terpinenes can be oxidised to form *p*-cymene preventing oxidation of linoleic acid (Li & Liu, 2009).

Anti-lipase activity

Lipase inhibition percentages of the oregano and *Lippia* essential oils at different concentrations on different lipases from *C. antarctica* and *P. fluorescens* were used to obtain the IC_{50} presented in Table 2. Both essential oils showed inhibition of the two lipases. In addition, all concentrations of oregano essential oil showed higher inhibition percentages than those of *Lippia* essential oil. Oregano essential oil had lower lipase inhibitory concentration (IC_{50}, 5.09 and 7.26 μg mL^{-1} in *C. antarctica* and *P. fluorescens* lipases, respectively, Table 2).

Other authors found anti-lipase activity of different extracts from aromatic plants. The IC_{50} results in the inhibitory effect of extracts from *Monarda punctata* on lipase from mice plasma were the following: acetone extract, 2000 μg mL^{-1}; water extract, 5600 μg mL^{-1}; ether extract, 180 μg mL^{-1}; carvacrol, 706.02 μg mL^{-1}; and orlistat, 44.6 μg mL^{-1} (Yamada *et al*., 2010), the inhibitory effects of methanolic extract from leaves of sage against pancreatic lipase (IC_{50}: 94 μg mL^{-1}). Carnosic acid and carnosol, compounds isolated from the extract, substantially inhibited pancreatic lipase activity with the following IC50: 12 and 4.4 μg mL^{-1}, respectively (Ninomiya *et al*., 2004). A new coumaroyl triterpene, 3-*O*-trans-*p*-coumaroyl actinidic acid, and five known triterpenes, ursolic acid, 23-hydroxyursolic acid, corosolic acid, asiatic acid and betulinic acid, were isolated from ethyl acetate-soluble extract of the roots of *Actinidia arguta*. These compounds were evaluated for inhibitory activities on pancreatic lipase (PL). 3-*O*-trans-*p*-coumaroyl actinidic acid possessed the highest inhibitory activity on PL (IC_{50} = 9.45 μg mL^{-1}) followed by ursolic acid (IC_{50} = 7.22 μg mL^{-1}). 23-Hydroxyursolic acid, corosolic acid, asiatic acid and betulinic acid also showed significant PL inhibitory activity (IC_{50} values ranging from 9.58 to 37.31 μg mL^{-1}, Jang *et al*., 2008).

Correlation analysis

Pearson correlation coefficients among the chemical variables of the oregano and *Lippia* essential oils are presented in Table 3. Total phenolic compounds showed negative correlation with DPPH and lipase IC_{50}. DPPH and lipase IC_{50} were positively correlated between them. The increment of phenolic content in the essential oils was related with higher scavenging and anti-lipase activity.

Conclusions

Origanum vulgare L. essential oil had phenols like thymol and carvacrol, and *L. turbinata* Griseb. essential oil exhibited high concentration of terpenes like limonene and 1,8-cineole. All of these components could be responsible for the antioxidant activity detected in

Table 3 Pearson correlation coefficients among the chemical variables: DPPH inhibitory concentration 50% (DPPH-IC_{50}) and the lipase inhibitory concentration 50% (L-C-IC_{50}: activity on lipase from *Candida antarctica*, and L-P-IC_{50}: activity on lipase from *Pseudomonas fluorescens*) of oregano and *Lippia* essential oils

	DPPH IC_{50}	L-C-IC_{50}	L-P-IC_{50}
L-C-IC_{50}	0.89*		
L-P-IC_{50}	0.92*	1.00**	
Total phenol content	−0.82*	−0.99**	−0.97**

Significant coefficient at $*P < 0.05$, $**P < 0.01$.

these essential oils. Oregano essential oil had higher total phenolic compounds, radical scavenging activity on DPPH and anti-lipase activity than *Lippia* essential oil. However, these essential oils did not show significant differences in their antioxidant activity measured by the Rancimat test.

Origanum vulgare L. and *L. turbinata* Griseb. essential oils could be used as natural antioxidants and anti-lipase additives on food products with high lipid content as an alternative for replacing the synthetic ones that are questioned for health issues. However, safety profile experiment should be performed on the essential oil before to use as an additive in food products.

Acknowledgments

This work was supported by Erasmus Mundus (External Cooperation Window), SECYT-UNC and CONICET.

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