

# Quality characters, chemical composition and biological activities of oregano (*Origanum* spp.) Essential oils from Central and Southern Argentina



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## ABSTRACT

The objective of this work is to assess the sensory, chemical, physicochemical profiles and the biological activities of *Origanum* spp. essential oils from central Argentina (Córdoba province) and new production areas in the southern Argentinian provinces of Rio Negro and Neuquén. Chemical (GC/MS), physicochemical (refractive index, density), sensory profiles (color, aroma) and biological activities (four antioxidants tests, anti-yeast and roundworm lethality assays) of four different oregano essential oils ('Compacto', 'Cordobes', 'Criollo', and 'Mendocino') were determined. Essential oils were characterized by high amounts of *trans*-sabinene hydrate (17.9–27.2%), terpinen-4-ol (6.2–11.1%),  $\gamma$ -terpinene (7–9.8%), thymol (12.1–17.4%) and lower amounts of carvacrol (0.1–3.5%). Oils showed varied tints of yellow (light, mid to dark yellow). Physical/chemical properties of oils showed that these parameters can be used to differentiate these oils. The aroma of the oils was characterized as herbaceous, with medicinal, resinous, minty and coniferous notes. Essential oils from central Argentina ('Mendocino' from Córdoba) showed higher acceptance aroma rates. An antioxidant profile showed that the types from southern provinces were the most active (9.2–10.4, arbitrary scale up to 12) than the same oregano-types from central Argentina. A similar trend was observed for anti-yeast and roundworm lethality assays. These higher biological activities were associated with lower aroma and higher color acceptances. The oils from central Argentina having better aroma profiles can be used for food (flavor) and non-food purposes (fragrances), while the oils from the southern provinces having higher bioactivities can be used as new sources of antioxidants and biocides for pharmaceutical, food (preservation) and veterinary industries. This study used a comprehensive science based approach to increase interest in Argentinian natural products and to contribute to develop trade and quality control standards in support of their commercialization.

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## 1. Introduction

Argentina has a rich and remarkable history of using and producing herbs, spices and medicinal plants (HSMP). These plants are collected from the wild or grown as commercial crops (e.g. oreganos) and used to prepare many commercial products, including herbal teas, spices, beverages, over the counter medications and

pharmaceutical products (Farías et al., 2010; Juliani et al., 2007). In Argentina, oregano is one of the most important commercial HSMP crops covering more than 80% of the national cultivation area and generating export markets for 1200 tons/year with a value of \$2.5 million of US dollars (Di Fabio, 2000; Ferran et al., 2011). Production areas of oregano and other HSMP in Argentina include the provinces of San Juan, Mendoza (Cuyo region), Córdoba and San Luis provinces (Traslasierras region). Minor areas of production are located in northwestern provinces of Salta and Jujuy.

Currently, there is an increased interest by consumers in new sources of aromas, flavors and biological activities (e.g. natural antioxidants and biocides). Essential oils are economically important products derived from HSMP, they are complex mixtures of components and composed by hydrocarbons with or without oxygen (monoterpenes, sesquiterpenes, and phenylpropanoids). Some notable properties of essential oils and their components are their

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dual lipophilicity and hydrophobicity, an important feature for many applications in the food, medicinal and fragrance industries (Gaspar and Leeke, 2004; Juliani et al., 2008).

Oregano types exhibited several therapeutic benefits (diaphoretic, antiseptic, antispasmodic, and tonic) and are used in alternative, homeopathic or naturopathy medicines around the world (Souza et al., 2007). Oregano essential oils have been used as flavoring agents in foods and beverages, fragrances, fungicides and insecticides, pharmaceutical and veterinary, and industrial products (Rehder et al., 2004). Herbs collectively known as oreganos belong to several plant species and genera, some examples include Mexican oregano (*Lippia graveolens*) and Cuban oregano (*Plectranthus amboinicus*) (Kintzios, 2002).

In addition, *Origanum* species are used in many products other than foods and thus can be considered as industrial crops in their own right as many uses and applications have been identified throughout the years (cosmetics, perfumeries and toiletries, aromatherapy, pharmaceutical preparations, among others) (Kintzios, 2004; Shylaja, 2004). Local *Origanum* spp. in central Argentina have been used in traditional veterinary medicine for decades (Martínez and Luján, 2011). Thus, *Origanum* spp. and their essential oils are currently the subject of intense research in veterinary medicine (Vucinik et al., 2012).

Most of *Origanum* species are found in the Mediterranean region, *Origanum vulgare* L. is distributed not only across the Mediterranean but also in Euro-Siberian and Irano-Turanian regions, nowadays oregano is growing worldwide for research, recreational and commercial purposes (Spada and Perrino, 1997). *Origanum* species shared a similar aroma profile, described as spicy, phenolic and minty, as many of them contain thymol and carvacrol in varying amounts (Meyers, 2005). The most popular oregano species is *O. vulgare* with many subspecies including the 'Greek' oregano (*O. vulgare* subsp. *hirtum* Ietsw.) which has been considered as one of the best quality oreganos. Oregano's aroma is due to the presence of essential oils that are accumulated in leaf trichomes. *Origanum* species show high variability in their essential oils, both in term of quality and quantity. To illustrate this phenomenon, some populations of the same species grown in Greece (Athos) showed high levels of thymol (77–85%), while others (Peloponnesse) were high in carvacrol (92–96%) (Kokkini, 1997).

In Argentina, the increased demand of oregano has stimulated the development of new production areas and thus generating new commercial opportunities for small holders. Four oregano-types, 'Compacto', 'Cordobes', 'Criollo' and 'Mendocino' are commercially produced and processed as a dried raw ingredient (Asensio et al., 2012).

The Cuyo region generates most of the national oreganos with Mendoza leading the production (50%) followed by San Juan (15%). In the Traslasierras region, the province of Córdoba is a major producer (25%) (Farías et al., 2010). In recent years, oregano production has extended to southern (Neuquén, Chubut) and eastern provinces (Buenos Aires, Santa Fe and Entre Ríos).

Commercial oregano crops have been grown for decades, thus they have been selected to meet requirements of Argentinian consumers. Many researchers have characterized the essential oils from Argentinian oregano species, with most of the studies focusing on the chemistry (by gas chromatography) and biological activities, with few efforts to characterize physical and sensorial properties of essential oils a key aspect to support their commercialization.

Argentinian oreganos are either hybrids or subspecies of *O. vulgare*. The 'Mendocino' type is a hybrid between *O. vulgare* and *O. majorana* L. (*Origanum x majoricum* Camb.). The type 'Compacto' has been classified as *O. vulgare* subsp. *vulgare* while 'Criollo' and 'Cordobes' varieties as the subspecies *hirtum* (Torres et al., 2010).

The chemical composition of these four varieties ('Cordobes', 'Criollo', 'Mendocino' and 'Compacto') grown in Argentina were

described. Similar oil composition in four varieties were found with higher amount of *trans*-sabinene hydrate (27–38%), with 'Criollo' showing the lowest level of this latter compound (6.3%) and higher amount of  $\gamma$ -terpinene (18.2%). With all oregano types containing high levels of thymol (14.9–30.8%). The types 'Compacto' and 'Cordobes' showed low levels of carvacrol (less than 1%) while 'Criollo' and 'Mendocino' higher levels (less than 7.6%) (Dambolena et al., 2010; Quiroga et al., 2011).

Another study of four Argentinian commercial oregano was conducted in the provinces of the northeast indicated that the variety '27-09', 'Peruvian' and 'native' clones corresponded to the hybrid *Origanum x majoricum* and one 'green Spanish' belong to *O. vulgare* subsp. *viridulum* Nyman. The chemical composition of the essential oils showed that the hybrids were rich in *trans*-sabinene hydrate (8.0, 5.3 and 14.0%, respectively), Terpinen-4-ol (11.0, 12.0, and 15.5%), thymol (27.3, 28.0, and 24.9%) and low levels of carvacrol (4.0, 4.7, and 0.4%). These oregano types also contained higher levels of  $\gamma$ -terpinene (17.2, 14.4, and 9.7%). The green Spanish type showed the highest levels of this latter compound (30.6%), low levels of *trans*-sabinene hydrate (0.2%), high levels of carvacrol methyl ether (9%), low levels of thymol (0.5%) and high amounts of carvacrol (23.6%) (Farías et al., 2010).

In view of the increasing importance of oregano production in Argentina, this work sought to characterize the sensory, chemical, physicochemical profiles and biological activities of *Origanum* spp. essential oils from two distinct production regions of central (Córdoba province) and southern Argentina (new production areas in the provinces of Rio Negro and Neuquén). Although other studies have been conducted on the chemistry of Argentinian essential oils, this research included oils from new production regions and aimed to further characterize their essential oils (sensorial, chemical, physicochemical, and biological).

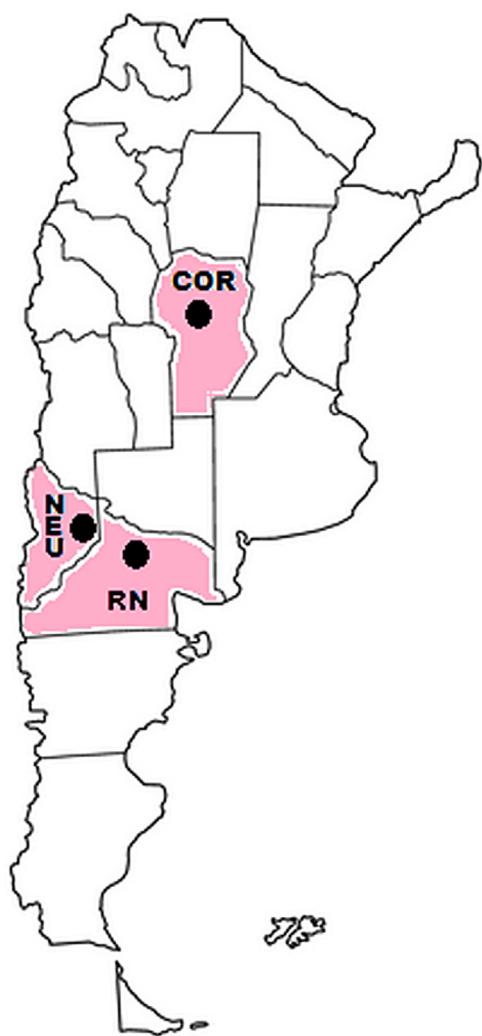
## 2. Materials and methods

### 2.1. Plant material

Aerial parts (leaves, stems and inflorescences) of four oregano-types (a) *Origanum x majoricum*, 'Mendocino'; (b) *O. vulgare* subsp. *vulgare*, 'Compacto'; (c) *O. vulgare* subsp. *hirtum* clone 'Cordobes'; and (d) *O. vulgare* subsp. *hirtum* clone 'Criollo') were harvested (three subsamples of ten plants for each oregano-type) at the end of the growing season (April 2012) and dried under the shade. Samples were collected from the Experimental Station of the "Facultad Ciencias Agropecuarias" (National University of Córdoba), Córdoba (COR) (central Argentina, 30°30'0"S, 64°30'0"W, 432 m of altitude above sea level, 17 °C average annual temperature and 600 mm annual rainfall). The oregano-types 'Cordobes' and 'Criollo' are only cultivated in Córdoba. Samples of 'Compacto' and 'Mendocino' were also collected from Chañar, Neuquén (NEU) province (south Argentina, 38°37'49"S, 68°17'49"W, 396 m of altitude, 14 °C average annual temperature and 180 mm annual rainfall), and form Alto Valle, Rio Negro (RN) province (39°01'00"S, 67°40'00"W, 241 m, 15.5 °C average annual temperature and 240 mm. annual rainfall) (Fig. 1).

### 2.2. Essential oil extraction and Gas chromatography analysis

Essential oils were extracted from each subsample by steam distillation using a (Clevenger-type) apparatus, with each distillation running for two hours. Essential oils were analyzed using a gas-liquid chromatography (Agilent 6890 gas chromatograph, Wilmington, DE, USA) (CG) coupled to a mass spectrometry detector (MSD) and Flame Ionization Detector (FID). Two capillary columns were used for each detector (HP-5 column, 30 m



**Fig. 1.** Location of growing sites (black dots) of Argentinian oregano-types in Central (COR, Córdoba) and Southern (NEU, Neuquén, RN, Rio Negro) provinces.

long, 0.25 mm internal diameter, and 0.25 mm coating thickness). 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 35–450. The temperature program was 60 °C for 5 min and from 60 °C to 200 °C with a rate of 4 °C/min. For the MDS run, the injector and detector were maintained at 200° and 280 °C, respectively. FID temperature was kept at 220 °C. Retention indices (RI) were calculated using a series of n-alkanes (8–20 atoms of carbon) in both columns connected to MSD and FID. The compounds were identified by comparing their RI and mass spectra with those from literature (Adams, 1995) and libraries (Wiley275.L). The main components were further identified by co-injection of authentic standards (Sigma®, St. Louis, Mo., U.S.A.). The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to equal one (Juliani et al., 2008).

### 2.3. Sensory profile, consumer's acceptance and physicochemical analysis

Descriptive analysis was performed to determine the sensory profile by a total of nine trained panelists. All panelists showed perfect score in taste sensitivity test and ability to identify 5 of 7 commonly found food flavors (Meilgaard et al., 2010). The descriptive test procedures describe by Meilgaard (2010) were used to

train the panel, a “hybrid” method of the Flavor Profile Method with a five point grade scale (1 = not detectable; 2 = just detectable; 3 = slightly strong; 4 = moderate strong; 5 = strong) was used by the panelists to evaluate the samples (Gacula, 1997; Meilgaard et al., 2010). A sheet of paper with list of definitions and descriptors were given in each evaluation session. Essential oils (0.5 mL) were place in slides of absorbent paper (10 cm long × 0.5 cm wide) coded with three-digit random numbers. Samples were evaluated in partitioned booth under fluorescent light at room temperature. A completely randomized block design was used for testing samples.

The consumer's acceptance test was performed according to Meilgaard et al. (2010) following a nine point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely to evaluate overall acceptance from the samples. For sample evaluation, essential oil samples were served as in the descriptive analyses. Panelists evaluated color and odor preferences of a maximum of four samples every week.

Oil density was measured at 23 °C using a 2 mL pycnometer. Refractive index was determined with a Fisher Scientific Abbe Benchtop Refractometers at 23 °C.

### 2.4. Antioxidant activity

#### 2.4.1. ABTS assay

A modified method of Re et al. (1999) was used. Briefly, diluted ABTS reagent (990 µL) (Sigma®, St. Louis, MO) in ethanol (absorbance 0.7 ± 0.02) were added to 10 µL of essential oils, and standard Trolox (Sigma®, St. Louis, MO). The concentration of volatile oils giving the same percentage of absorbance inhibition of the radical cation at 734 nm (HP 8453 UV-Visible Spectrophotometer) as 1 mM Trolox was calculated in terms Trolox equivalent antioxidant activity (TEAC). The TEAC of essential oils was expressed as mM Trolox/mL essential oil.

#### 2.4.2. FRAP method

The ferric reducing power (FRAP) measures the ability of antioxidants to reduce iron in aqueous medium in relation to ascorbic acid. In a reaction tube, 10 µL of essential oil, or blank (water) and 990 µL of FRAP reagent (ferric chloride and TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) in acetate buffer (pH 3.6) were placed. The absorbance was measured after 5 min at 593 nm in a spectrophotometer. A calibration curve with serial dilutions of ascorbic acid was done in order to determine ascorbic acid equivalent antioxidant activity (mM ascorbic acid/me of essential oil, AEAC) (Benzie and Strain, 1996).

#### 2.4.3. β-Carotene bleaching assay

Ten mg of β-carotene (Sigma®, St. Louis, MO) was dissolved in 10 mL chloroform. The carotene-chloroform solution, 0.2 mL, was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma®, St. Louis, MO) and 200 mg Tween 40 (Sigma®, St. Louis, MO). Chloroform was removed using a rotary evaporator and distilled water was added to the residue, slowly with vigorous agitation to form an emulsion. This emulsion (5 mL) was added to a tube containing 0.2 mL of the sample solution and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically at 470 nm over a 60 min period. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation (de Oliveira et al., 2012).

#### 2.4.4. ORAC

All reagents and sample dilutions were made in 75 mM PBS (Phosphate buffered saline), pH 7. Tween 40 was used to emulsify the essential oils. Stock solutions of fluorescein 1 mM and

Trolox 2.5 mM were prepared and aliquots were stored at -20 °C. Fluorescein was diluted to 6 nM and 150 µL and was added to each well. Next, 25 µL Trolox standard (50, 25, 12.5, 6.25, 3.125 µM) and samples were added to each well in triplicate. The plate was pre-incubated at 37 °C for 30 min. A fresh solution of 127 mM AAPH (2,2'-Azobis(2-methylpropionamidine) dihydrochloride) was prepared and 25 µL added to each well after pre-incubation. Fluorescence readings were recorded every minute for 75 minutes with a 485 nm, 20 nm bandpass, excitation filter and a 528 nm, 20 nm bandpass, emission filter (Synergy HT Multi-Detection Microplate Reader, Bio-Tek, Winooski, VT). The net area under the curve (AUC) was calculated by subtracting the AUC of the blank from the standard or sample. A regression line was generated by plotting the concentration of Trolox by net AUC. To determine the antioxidant capacity of compounds with known concentrations over a desired range, the net AUC for each compound was calculated as explained above and plotted against the concentration. To determine Trolox equivalents of each sample, range the ratio of the slope (m) of the linear regression analysis of the compound to the slope of the linear regression of Trolox was used: TE (range of concentrations)= $m_{\text{compound}}/m_{\text{Trolox}}$  (Brescia, 2012; Prior et al., 2003).

## 2.5. Biocide tests

### 2.5.1. Anti-yeast test

A modified method (Sanchez and Konigsberg, 2006) was used to assess yeast viability. Yellow MTT (5 mg/mL) (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) was added for detecting live yeast (active dry yeast, *Saccharomyces cerevisiae*) as the dehydrogenase from the yeast mitochondria changed the MTT to purple formazan, while in killed yeasts MTT remained yellow (Kuhn et al., 2003; Miyamoto et al., 2002). Euconazole (300 mg/mL) was used as positive control. Sucrose (5%) and dry yeast (5%) were added to sterile tap water and transferred (200 µL) to 48-well plate. First, 50 µL of controls (positive and negative) and essential oils were added in each well in triplicate, 200 µL of the yeast solution and then incubated for 2 h at 30 °C. Then 20 µL of MTT were added and incubated for two more hours. A Synergy HT Multi-Detection Microplate Reader (Bio-Tek, Winooski, VT) was used measure change of color at 570 nm.

### 2.5.2. Roundworm lethality assay

The modified method of Li et al. (2005) was used to test nematocidal activity of essential oils. A growing medium was prepared by simmering tap water (160 mL) and commercially available oatmeal (Quaker Original, 40 g) for ten minutes. After cooling down to room temperature, 10 mL of worm culture was transferred into the fresh oatmeal medium and 5 g of commercial dehydrated yeast was added. Roundworms were allowed to grow and multiply for 7 days at room temperature in the new media before screening. In a 96 wells-plate 100 µL worm suspension (*Panagrellus redivivus*) were added to each well, 5 µL positive control (CuSO<sub>4</sub>, 160 mg/mL), negative control (water) and essential oils solutions (10, 15, 20 mg/mL) were added and incubated for 4 h. Active and inactive worms were counted under a dissecting microscope, worms not responding to physical stimuli were considered dead. Percentage of lethality was calculated. The lethal Concentration 50 (LC50) was calculated from the curve obtained by plotting the percentage of lethality vs. the EO concentrations. All treatments were replicated three times.

## 2.6. Statistical analysis

Experiments were carried out three times and results were expressed as mean±standard deviations. Analysis of variance

(ANOVA,  $\alpha=0.05$ ) and LSD Fisher's multiple range test were performed to determine significant differences between means. Principal component analysis (PCA) (Souza et al., 2011; Cruz et al., 2012) was performed on the correlation matrix of the standardized (normalized) data from descriptive analysis. Associations between essential oils and biological activity tests were assessed by PCA. Data were analyzed using the InfoStat software (Di Renzo et al., 2012), version 2013 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba).

## 3. Results and discussion

### 3.1. Sensory and chemical profile

The color of the Argentinian oregano essential oils showed varying tints of yellow (Table 1). The 'Compacto' oregano-types were characterized by mid to dark yellow colors, 'Cordobes' and 'Criollo' were mid yellow while 'Mendocino' types were light yellow (Table 1). The dark yellow was significantly the most liked oil, the 'Compacto' type from Rio Negro showed an hedonic scale of 6.5 (with 9 extremely liked and 5 neutral), followed by mid yellow samples of 'Criollo' (6.2) and 'Compacto' from Córdoba (5.8) (Fig. 2).

Studies to characterize the aroma profile revealed that the oils showed significant variability, with all samples having acceptance levels higher than the neutral value (higher than 5) (Figs. 2 and 3). The spider diagram revealed the oils were characterized mostly as herbaceous, with medicinal, resinous, minty and coniferous notes (Fig. 3a–c). The 'Mendocino' oregano-types shared a similar sensory profile, the type from Córdoba and Neuquén showed mostly medicinal notes followed by herbaceous and minty notes, and slight woody and coniferous notes (Fig. 3c). Rio Negro essential oil was characterized by an expanded aroma profile, also exhibiting medicinal notes, but also high herbaceous, resinous and coniferous notes. The 'Compacto' types were characterized by herbaceous, with lower minty, woody and coniferous notes (Fig. 3b). The 'Criollo' type from Córdoba was the most diverse oil in terms of aroma notes, as it also showed herbaceous and mint notes with lower floral, coniferous and even citrus notes. The 'Cordobes' essential oil notes were similar to those of the 'Criollo' type. This latter oil in addition to the minty and herbaceous notes, also exhibited green, resinous, woody and coniferous notes (Fig. 3a).

The sensory profile of the main component of the oregano oils, sabinene hydrate, could explain the herbaceous characters of the oils described in this study as the aroma profile of this component has been characterized as herbal type, with woody, terpene (coniferous) and minty notes (Mosciano, 1997). The Argentinian oregano types showed similar chemical profiles, with all the varieties dominated by *trans*-sabinene hydrate, with the 'Compacto' (23.4–27.2%), and 'Mendocino' (24.3–28.1%) showing the highest values followed by 'Cordobes' (22.9%), and 'Criollo' (17.9%) (Table 2). Similar results with higher levels of *trans*-sabinene hydrate (27–38%) were reported for these varieties. In the 'Criollo' type lower values of *trans*-sabinene hydrate (6.3%) with varying levels of sabinene (3.6–4.5%) were observed (Dambolena et al., 2010; Quiroga et al., 2011). Dambolena et al. (2010) found higher levels of  $\gamma$ -terpinene in the 'Compacto' type (15.5%) as opposed to the other three types showing lower levels (4.4–5.9%). In addition, Quiroga et al., 2011 observed high levels of this component in the 'Compacto' (12.4%) but also in the 'Criollo' (18.2%). In this study, two of the 'Compacto' types from Córdoba and Neuquén showed significantly higher levels of  $\gamma$ -terpinene (Table 2).

In the present study, two types showed lower levels of thymol ('Mendocino' and 'Compacto' from Córdoba, 12.1 and 14.4%, respectively), with the rest of the varieties showing higher and significant levels (16.6–18.6%) (Table 2). Quiroga et al. (2011) reported higher

**Table 1**

Color and physicochemical properties of the essential oils of four oregano-types ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Rio Negro (RN).

Type	'Compacto' <sup>a,b</sup>			'Cordobes' <sup>a,b</sup>		'Criollo' <sup>a,b</sup>		'Mendocino' <sup>a,b</sup>		
Province	COR	NEU	RN	COR	COR	COR	COR	NEU	RN	
Color	Mid yellow	Mid yellow	Dark yellow	Mid yellow	Mid yellow	Light yellow	Light yellow	Light yellow	Light yellow	
RI	1.478 ± 0.02	a	1.481 ± 0.001	b	1.486 ± 0.03	a	1.476 ± 0.02	a	1.479 ± 0.003	a
Density	0.907 ± 0.0034	a	0.914 ± 0.003	b	0.914 ± 0.003	b	0.898 ± 0.0034	ab	0.916 ± 0.0034	b

<sup>a</sup> Compacto: COR (Córdoba), NEU (Neuquén), RN (Río Negro); Cordobes: COR (Córdoba); Criollo (Córdoba); Mendocino: COR (Córdoba), NEU (Neuquén) and RN (Río Negro).

<sup>b</sup> The same letter in the row means that there are not significant differences at  $\alpha = 0.05$  ( $n = 3$ , LSD Fisher)

levels of thymol in 'Compacto' and 'Cordobes' (26.6–29.7%, respectively) and lower levels in 'Criollo' and 'Mendocino' (14.9–17.7%). Dambolena et al. (2010) found the higher levels in 'Criollo' and 'Cordobes' (30.1–26.1%), and lower levels in 'Compacto' (20.5%), with lowest levels in 'Mendocino'.

Quiroga et al. (2011) described the higher levels of carvacrol in 'Criollo' and 'Mendocino' (7.6–4.2%, respectively), while Dambolena et al. (2010) found the highest levels in the 'Mendocino' type (3.6%). This work showed a similar trend with this latter variety showing higher levels of carvacrol (1.8, 3.5 and 3.4% for Córdoba, Neuquén and Río Negro respectively), though 'Compacto' types from Neuquén and Río Negro also showed higher levels (Table 2).

For the hybrid *Origanum × majoricum*, a different profile with two types dominated by thymol (27–28%), terpinen-4-ol (11–12%),  $\gamma$ -terpinene (14–17%) with lower levels of *trans*-sabinene hydrate (5–8%) was reported (Farías et al., 2010).

The oils showed lower and varying levels of  $\gamma$ -terpinene, a constituent that also exhibits herbaceous and citrus notes (Mosciano, 1993). The amounts of this component in 'Compacto' and 'Criollo' oreganos were slightly higher (7.3–9.8%, 8%, respectively), while in 'Mendocino' (7–7.5%) and 'Cordobes' (7%) were slightly lower (Table 2). The monoterpene  $\gamma$ -terpinene could explain the herbaceous and citrus on the 'Criollo' type (Fig. 3a). The oils also contained terpinen-4-ol being described as a monoterpene with citrus, minty, coniferous and woody notes (Mosciano, 1998). All the oreganos 'Compacto', 'Cordobes', 'Criollo', and 'Mendocino' showed similar values (7.8–11, 6.2, 9.5, 6.6–11.1%, respectively). The alcohol terpineol with floral notes was also present in lower values in all oils (1.5–2.7%).

No direct correlation between the sensory and chemical profiles was detected, as several of the individual components shared similar aroma notes (e.g. sabinenes and terpinenes with herbaceous characters). These essential oils contained several chemical components found in lower percentages which also contribute to the oils overall aroma notes (Fig. 3, Table 2).

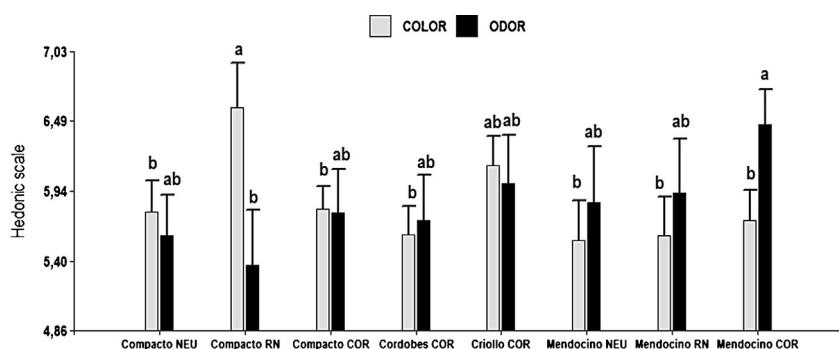
The acceptability test showed the 'Mendocino' type were the most liked, particularly the one from Córdoba (hedonic value 6.5).

and followed by the oil from Mendoza (hedonic value 6) (Fig. 2). The 'Criollo' type also showed higher hedonic values (5.8). The high acceptability score of the 'Mendocino' from Córdoba could be related with its lowest amounts of thymol (12.1%) and slightly higher levels of carvacrol (1.8%). The 'Compacto' oregano-type oil was the least liked, the Río Negro type exhibited a value of 5.3, closer to the neutral value (5) and the Neuquén type a hedonic value of 5.6 (Fig. 2).

### 3.2. Physical and chemical properties

Physicochemical properties are important to characterize essential oils as their values are affected by the chemical composition of the oils (Juliani et al., 2006, 2008). Argentinian essential oils showed narrow variations in their refractive indices (RI) ranging from 1.476 to 1.486 (Table 1). The only sample showing significant differences was the 'Compacto' from Río Negro. 'Mendocino' exhibited the lowest RI (1.476–1.479), while the 'Compacto' highest values (1.478–1.486), with 'Criollo' and 'Cordobes' presenting intermediate values (1.48) (Table 1). These results suggest that RI can be used to differentiate these oils.

Density of essential oils is another important physical property to assess their quality. In oregano oils dominated by either carvacrol or thymol, the density will be close to the individual components as thymol and carvacrol are phenols having densities closer to water (0.965 and 0.976). The density of the 'Mendocino' oil from Córdoba was the lowest (0.898), while density of 'Compacto' (Córdoba) was slightly higher (0.907). These densities were associated with chemical profiles lower in phenols (carvacrol and thymol, 14%) (Tables 1 and 2). 'Cordobes' and 'Criollo' also showed lower levels of phenols (18.8 and 17.4, respectively) and associated with slightly higher densities (0.905 and 0.908, respectively). The Southern Argentinian oils exhibited higher levels of phenols (19.4–21%), their densities were also higher ('Mendocino', and 'Compacto', 0.914–0.916) (Tables 1 and 2). This research also suggests density can be used as one of the characters to differentiate among varieties and growing locations.



**Fig. 2.** Acceptability tests for color and aroma using a 9-point hedonic scale of the four oregano-types essential oils ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Río Negro (RN). The same letter means no significant differences at  $\alpha = 0.05$  ( $n = 3$ , LSD Fisher). Hedonic scale, 1 = dislike extremely, 5 neutral, 9 like extremely.

**Table 2**

Relative composition of the essential oils of four oregano-types ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Rio Negro (RN).

RI	Oregano-types	'Compacto'			'Cordobes'			'Criollo'			'Mendocino'		
		Compound	COR <sup>a,b,c</sup>	NEU <sup>a,b,c</sup>	RN <sup>a,b,c</sup>	COR <sup>a,b,c</sup>	COR <sup>a,b,c</sup>	COR <sup>a,b,c</sup>	COR <sup>a,b,c</sup>	NEU <sup>a,b,c</sup>	RN <sup>a,b,c</sup>		
854	Hexenal	0.1±0b	0.1±0.01a	0.0±0.02a	0.0±0a	0.0±0a	0.1±0a	0.0±0a	0.0±0a	0.0±0.02a			
931	α-Thujene	0.7±0.01a	0.7±0.03a	0.7±0.02a	0.9±0.02c	0.8±0b	0.9±0d	0.7±0.01a	0.9±0.01a	0.9±0.01a			
939	α-Pinene	0.8±0.01d	0.5±0.02b	0.4±0.04a	0.8±0.02d	0.8±0d	0.6±0.05c	0.4±0.01a	0.5±0.01a	0.5±0.01a			
954	Camphene	0.2±0.02c	0.1±0a	0.0±0.02a	0.4±0.01e	0.5±0.01d	0.1±0b	0.0±0.0a	0.0±0.0a	0.0±0.06a			
977	Sabinene	4.5±0.03b	0.36±0.19a	4.0±0.11ab	3.9±0.07ab	3.6±0.02a	4.5±0.02b	4.1±0ab	4.0±0.01ab				
980	β-Pinene	1.8±0.01e	0.4±0.01a	0.4±0a	1.4±0.01c	5.9±0.76d	1.0±0b	0.4±0a	0.5±0.01a				
987	3-Octanone	0.5±0d	0.0±0a	0.0±0.01a	1±0b	0.1±0.01b	0.3±0.15c	0.0±0.01a	0.1±0.01a				
992	Myrcene	1.8±0.01a	1.4±0.05a	1.6±0.4a	1.8±0.03a	1.7±0.02a	1.7±0.15a	1.6±0.01a	1.7±0a				
996	3-Octanol	0.2±0d	0.0±0a	0.0±0a	0.1±0b	0.1±0.01b	0.1±0.07c	0.0±0.01a	0.1±0.06a				
1006	α-Phellandrene	0.2±0a	0.2±0.02b	0.2±0.03b	0.2±0a	0.2±0.02b	0.2±0a	0.2±0.01a	0.2±0.01a	0.2±0.02b			
1013	δ-3-Carene	0.1±0b	0.0±0a	0.0±0a	0.0±0b	0.0±0.01a	0.0±0.02a	0.0±0.35a	0.0±0.02a				
1020	δ-Terpinene	2.9±0.02a	3.9±0.15b	3.7±0.48b	2.6±0.04a	3.3±0.03b	3.0±0.03a	3.6±0.02b	3.8±0.03b				
1028	Orto-Cymene	5.6±0.03d	2.3±0.15b	2.4±0.07b	5.1±0.06c	6.3±0.02e	7.8±0.02d	2.2±0.07a	2.4±0.05b				
1033	δ-Phellandrene	1.8±0.01a	1.8±0.08a	1.9±0.29a	0.19±0.02a	1.9±0.01a	2.0±0.04a	1.9±0.03a	2.0±0.05a				
1036	1,8-Cineole	0.1±0.01b	0.1±0a	0.0±0.02a	0.1±0c	0.1±0c	0.1±0.02b	0.0±0.01a	0.0±0.04a				
1040	Z-Ocimene	1.4±0.01b	0.7±0.02a	0.7±0.14a	2.8±0.03e	2.5±0.01d	1.6±0.16b	0.7±0.11a	0.7±0.71a				
1051	E-Ocimene	0.2±0c	0.1±0.01a	0.1±0.02a	0.3±0d	0.3±0d	0.2±0b	0.1±0.11a	0.1±0a				
1063	γ-Terpinene	9.8±0.04d	8.3±0.27c	7.3±0.2b	7.1±0.05b	8.0±0.02b	7.5±0.01b	7.0±0.06a	7.5±0.01b				
1071	cis-Sabinene hydrate	1.8±0a	2.9±0.09b	3.3±0.88b	3.3±0.04b	3.0±0.01b	3.4±0.4b	3.4±0.17b	3.2±0.23b				
1090	Terpinolene	1.0±0a	1.3±0.06b	1.2±0.22b	0.8±0a	1.1±0b	1.0±0a	1.2±0.07c	1.3±0.07b				
1100	trans-Sabinene hydrate	27.2±0.09c	23.4±0.16b	25.7±0.73c	22.9±0.08b	17.9±0.06a	28.1±0.05d	27.6±0.02c	24.3±0.81c				
1113	Menthatriene	0.0±0.01a	0.1±0b	0.1±0.03b	0.9±0d	0.9±0.01d	0.3±0.03c	0.1±0b	0.1±0.35b				
1125	Sabina ketone dehydro	0.8±0a	1.1±0.1c	1.2±0.08c	0.8±0a	1.0±0.01b	0.8±0a	1.1±0.03c	1.2±0.83c				
1130	Campholenal	0±0a	0.03±0.01d	0.02±0.03c	0.0±0a	0.0±0.01a	0.0±0.08a	0.02±0.1b	0.02±0b				
1132	1-Terpineol	0±0a	0±0a	0±0.01a	0±0a	0±0a	0±0a	0.0±0.05a	0.0±0.08a				
1144	cis β Terpineol	0.4±0.02a	0.7±0.09b	0.7±0.11b	0.4±0a	0.6±0.03b	0.4±0b	0.6±0.03b	0.8±0.05b				
1154	Karahanaenona	0.1±0.01a	0.1±0a	0.0±0.11a	0.0±0a	0.1±0.02a	0.0±0.07a	0.0±0a	0.1±0.08a				
1170	Borneol	0.5±0.01c	0.2±0.02a	0.2±0.06a	1.5±0.01d	1.8±0.01d	0.4±0.37b	0.2±0.08a	0.2±0.03a				
1181	Terpinen-4-ol	7.8±0.02a	11.0±0.79b	10.9±0.17b	6.2±0.01a	9.5±0.04b	6.6±0.02a	10±0.01b	11.1±0.02b				
1187	Cymen-8-ol	0.1±0a	0.1±0.01a	0.1±0.07a	0.1±0a	0.1±0.01a	0.1±0.03a	0.0±0.08a	0.10±0.06a				
1192	α Terpineol	1.5±0.01a	2.4±0.07c	2.7±0.37d	2.3±0.01c	2.6±0.01cd	2.0±0.06b	2.6±0d	2.7±0.16d				
1197	Dihydro carvone	0.2±0.07a	0.4±0.02b	0.4±0.03b	0.2±0a	0.3±0.01a	0.3±0.57a	0.4±0.02b	0.4±0b				
1206	Trans dihydro carvone	0.0±0a	0.1±0b	0.1±0.03b	0.0±0a	0.0±0a	0.1±0.04b	0.1±0.01b	0.1±0.01b				
1209	γ-Terpineol	0.2±0.01a	0.3±0.05c	0.3±0.06c	0.2±0.01a	0.3±0b	0.2±0.71a	0.3±0.27b	0.4±0.39c				
1237	Thymol methyl ether	3.7±0.01c	0.5±0.01a	0.2±0.57a	0.3±0.01a	0.3±0a	1.1±0b	0.2±0.09a	0.2±0a				
1247	Carvacrol methyl ether	1.5±0.01c	1.2±0.02a	1.2±0.04a	1.3±0.01b	1.3±0.01b	1.7±0d	1.2±0.06a	1.2±0.03a				
1258	Linalool acetate	0.0±0a	2.0±0.01c	2.5±0.71c	1.1±0.01b	1.1±0b	1.8±0.23c	2.5±0.01c	2.4±0c				
1286	α Terpinen-7-al	0.0±0a	0.0±0a	0.0±0a	0.0±0a	0.0±0a	0.0±0.77a	0.0±0a	0.0±0a				
1289	Bornyl acetate	0.0±0a	0.2±0a	0.0±0a	0.1±0c	0.2±0d	0.1±0.01b	0.0±0a	0.0±0a				
1292	Thymol	14.4±0.12a	16.6±0.51b	17.2±0.23b	18.6±0.43b	17.1±0.26b	12.1±0.03a	17.4±0b	16.9±0.27b				
1301	Carvacrol	0.1±0a	2.8±0.11c	3.4±0.77	0.2±0.01a	0.2±0.01a	1.8±0.02b	3.5±0c	3.4±0.01c				
1389	β-Bourbonene	0.2±0.01b	0.0±0b	0.0±0a	0.1±0.01b	0.2±0b	0.1±0.01a	0.0±0a	0.0±0.11a				
1425	E-Caryophyllene	1.0±0.0a	2.0±0.06ab	2.1±0.11ab	2.8±0.05ab	2.9±0.05b	2.2±0.01ab	2.1±0.01ab	2.1±0.11ab				
1434	β Copaene	0.1±0b	0.1±0b	0.0±0a	0.0±0.01b	0.1±0b	0.0±0a	0.0±0.01a	0.0±0.05a				
1460	α Humulene	0.1±0.01a	0.2±0b	0.2±0.08b	0.1±0.01a	0.1±0a	0.2±0.01	0.2±0.01b	0.2±0.17b				
1479	γ Gurguyene	0.0±0a	0.2±0b	0.0±0a	0.0±0a	0.0±0a	0.0±0a	0.0±0.01a	0.0±0.07a				
1486	Germacrene D	1.5±0.01b	0.4±0.01a	0.0±0.68a	1.2±0.03b	1.0±0.01b	0.3±0.01a	0.0±0a	0.1±0.02a				
1500	Bicyclogermacrene	1.4±0.01b	1.3±0.06a	1.3±0.03a	1.7±0.02d	1.4±0.02a	1.5±0c	1.4±0.01a	1.2±0a				
1511	Bisabolene beta	0.3±0.01a	0.2±0.01a	0.1±0.23a	0.7±0.01b	0.7±0.02b	0.2±0a	0.1±0.03a	0.1±0.03a				
1527	δ Cadinene	0.0±0.01a	0.1±0a	0.0±0.07a	0.1±0.04a	0.1±0.01a	0.0±0.03a	0.0±0.01a	0.0±0.03a				
1588	Caryophyllene oxide	0.2±0a	0.2±0.02a	0.2±0.11a	0.4±0.02a	0.5±0.01b	0.3±0.25a	0.1±0.01a	0.2±0.05a				
1643	Aromadendrene epoxide-allo	0±0a	0.5±0a	0.1±0.03a	0.1±0.01a	0.1±0.01b	0.1±0.01a	0.0±0.02a	0.0±0.03a				

<sup>a</sup> Compacto: COR (Córdoba), NEU (Neuquén), RN (Rio Negro); Cordobes: COR (Córdoba); Criollo (Córdoba); Mendocino: COR (Córdoba), NEU (Neuquén) and RN (Rio Negro).

<sup>b</sup> The same letter in the row means that there are not significant differences at  $\alpha=0.05$  ( $n=3$ , LSD Fisher).

<sup>c</sup> 0.0 are 'traces' of less than 0.01%.

### 3.3. Biological activities

In this study, the two major groups of assays to determine antioxidant activity in essential oils were used to generate an antioxidant profile based on two main sets of assays (Tables 3 and 5). These methods are based on (1), the single electron transfer (SET) reaction, displayed through a change in color as the oxidant is reduced (ABTS assay and ferric reducing antioxidant power, FRAP, and carotene blanching assay), and (2) hydrogen atom transfer (HAT), which measure the activity of the antioxidant to scavenge peroxyl radicals in the oxygen radical absorbance capacity (ORAC) (Huang et al., 2005).

**ABTS assay.** The antioxidant capacity of the oregano essential oils was first evaluated using the ABTS radical species (Table 3). The 'Compacto' type from Neuquén showed the highest antioxidant activity, followed by 'Criollo' from Córdoba, and 'Compacto' from Rio Negro, with oils showing significant differences (0.234, 0.210, 0.206 mM Trolox/mg of essential oils, TEAC, respectively). 'Mendocino' (Córdoba) was the sample with the lowest antioxidant capacity (0.163 TEAC). No relationship between the antioxidant activity of essential oils and their main components was observed. The correlation between *trans*-sabinene hydrate and TEAC was low ( $R^2=0.26$ ), the highest correlation was observed for thymol and terpinen-4-ol ( $R^2=0.42$ ). The 'Compacto' types from Rio Negro and

**Table 3**

Antioxidant activity of the essential oils of four oregano-types ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Río Negro (RN) determined by ABTS, FRAP and ORAC methods.

Type	'Compacto' <sup>a,b</sup>			'Cordobes' <sup>a,b</sup>		'Criollo' <sup>a,b</sup>		'Mendocino' <sup>a,b</sup>		
Province	COR	NEU	RN	COR	COR	COR	NEU	RN		
TEAC <sup>c</sup>	0.175 ± 0.0045a <sup>1</sup>	0.234 ± 0.0026c	0.206 ± 0.0025b	0.174 ± 0.0027a	0.210 ± 0.0039b	0.163 ± 0.0017a	0.177 ± 0.0083a	0.184 ± 0.0017a		
AEAC <sup>d</sup>	0.137 ± 0.044c	0.172 ± 0.145d	0.147 ± 0.043c	0.154 ± 0.021c	0.184 ± 0.641d	0.072 ± 0.251a	0.155 ± 0.677c	0.118 ± 0.121b		
ORAC <sup>e</sup>	1.155 ± 0.035d	1.659 ± 0.017h	1.708 ± 0.008g	1.064 ± 0.004b	1.393 ± 0.021f	1.281 ± 0.017e	1.024 ± 0.067a	1.094 ± 0.044c		

<sup>a</sup> Compacto: COR (Córdoba), NEU (Neuquén), RN (Río Negro); Cordobes: COR (Córdoba); Criollo (Córdoba); Mendocino: COR (Córdoba), NEU (Neuquén) and RN (Río Negro).

<sup>b</sup> The same letter in the row means that there are not significant differences at  $\alpha = 0.05$  ( $n = 3$ , LSD Fisher).

<sup>c</sup> TEAC (Trolox equivalent antioxidant capacity mM Trolox/mg essential oil).

<sup>d</sup> AEAC (Ascorbic acid antioxidant capacity mM Asc. Ac./mg essential oil).

<sup>e</sup> ORAC (TE: Trolox equivalents of each sample at a range of concentrations).

Neuquén had high amounts of  $\delta$ - and  $\gamma$ -terpinene (3.71%, 3.94% and 7.32%, 8.33%, respectively), *trans*-sabinene hydrate (25.68%, 23.35%), and oxygenated monoterpenes such as terpinen-4-ol (10.88%, 10.96%) and thymol (17.23%, 16.59%). These results suggested that several of the components of the essential oils are contributing to the TEAC, as many of these components are known for possessing electron scavenging antioxidant properties, due to the number of conjugated double bonds and the phenolic functional group (Müller et al., 2011).

**FRAP method.** Essential oils significantly reduced the ferric di-TPTZ complex used in this assay. The reducing power assay is one of the effective means to evaluate the ability of antioxidants to donate electrons. 'Criollo' (Córdoba) and 'Compacto' (Neuquén) were the samples exhibiting higher ascorbic acid equivalent antioxidant capacity (0.185 and 0.1726 mM/mg of oil, AEAC, respectively), while 'Mendocino' (Córdoba) showed the lowest value (0.072 AEAC) coinciding with lower levels of electron-donating phenols thymol and carvacrol (14%). The ferric reducing activity is mainly influenced by the number of conjugated double bonds (CDB) system and hydroxyl functions for phenolic compounds like vitamin E, phenolic acids and flavonoids (Müller et al., 2010). In the present study, weak correlations were observed between this assay and *trans*-sabinene hydrate ( $R^2 = 0.43$ ), and thymol/terpinen-4-ol ( $R^2 = 0.39$ ). In contrast to other tests that measure total antioxidant power, the FRAP assay is simple, fast, inexpensive, and robust (Prior et al., 2005).

The ability of antioxidants to scavenge free radicals (ABTS) and to reduce iron (FRAP) were closely related ( $R^2 = 0.68$ ), as 'Criollo' (Córdoba) and 'Compacto' (Neuquén) also showed the highest TEAC values (Table 3). Thus, compounds should react in similar manner in TEAC and FRAP assays, which should lead to a good correlation between their results (Prior et al., 2005). The reaction conditions between these assays differ, especially the pH-value (FRAP: 3.6; TEAC: 7.4). TEAC and FRAP may give comparable relative values, but FRAP values are usually lower than TEAC for a given series of antioxidant compounds. FRAP values showed poor relationships when compared to other antioxidant tests (Prior et al., 2005).

**$\beta$ -Carotene bleaching assay.** The antioxidant activity of oregano essential oils showed variation in their ability to prevent bleaching of molecules, a characteristic that increased with the amount of essential oil. 'Mendocino' type from Neuquén significantly inhibited bleaching by 89.2% (20  $\mu\text{g}/\text{mL}$  of essential oil), followed by 'Compacto' from Río Negro (RN) (79.3%) and Córdoba (COR) (75.3%) (Fig. 4). The transfer of hydrogen atom that prevents peroxidation is the underlying basis of  $\beta$ -carotene/linoleic acid bleaching test. Compounds containing hydrogen atoms in the allylic and/or benzylic positions will show better activity in this test because of relatively easy removal of hydrogen from these functional groups by peroxy radical during test reaction (Safaei-Ghomie et al., 2009).

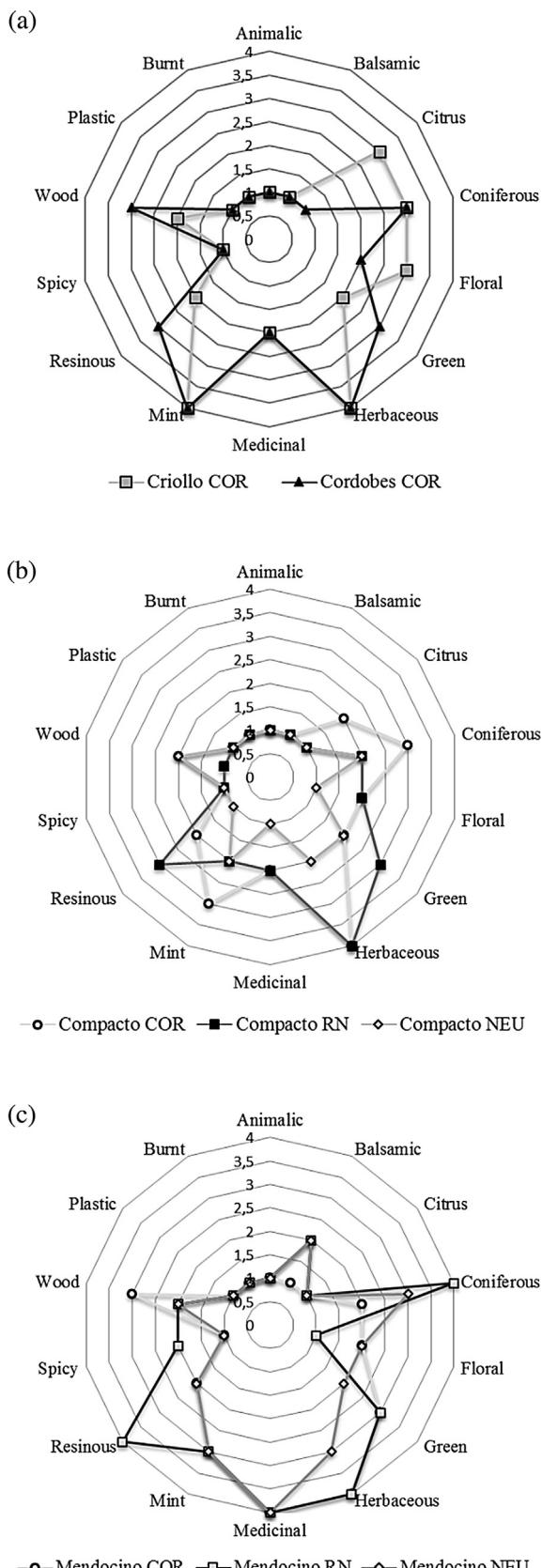
**ORAC.** The ORAC is also an *in vitro* assay, though the one with the most biological significance (Cavar et al., 2012). The ORAC uses an

area-under-curve (AUC) technique and thus combines both inhibition time and inhibition degree of free radical action produced by an antioxidant into a single quantity. ORAC assay was used to determine the antioxidant behavior of essential oils of *Origanum* species. Significant differences were observed in the 'Compacto' type from Río Negro (1.708 Trolox Equivalents, TE) followed by the same type from Neuquén (1.659 TE). The oil with the lowest ORAC value was 'Mendocino' from Neuquén (1.024 TE), the others oils showed intermediate ORAC values (1.094–1.281 TE). It was observed a high correlation between the ABTS and ORAC assays ( $R^2 = 0.80$ ), there were also weak correlations between ORAC values and the percentages of a given components or group of components, the highest observed correlation between ORAC values and  $\alpha$  and  $\gamma$  terpinenes was only 0.32.

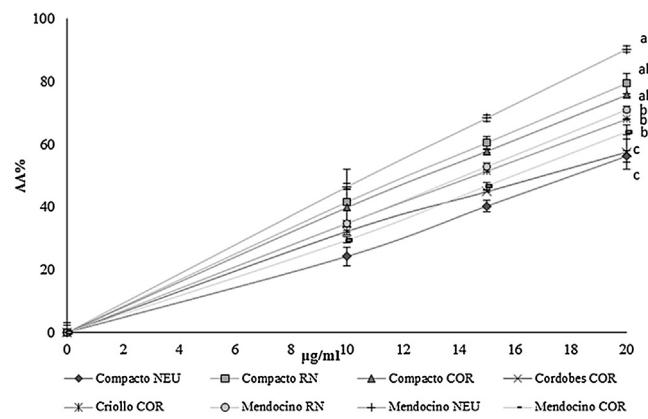
An antioxidant profile was obtained by assigning a value of one to the lowest antioxidant value and three to the highest for all the four antioxidant assays (with a possible maximum value of antioxidant score of 12) to test the eight essential oils (Table 5). The results showed that the most bioactive oil was the 'Compacto' type from southern provinces (Neuquén and Río Negro with values of 9.2 and 10.4, respectively), followed by 'Criollo' (9.9) from central Argentina (Córdoba). Within the 'Mendocino' types, higher values of the antioxidant score was also observed in the samples from Southern Argentina (6.9–8.1), with the lowest value in the sample of Córdoba (5.2). The 'Cordobes' type from Córdoba also showed lower values (6) (Table 5, Fig. 1).

Assays based on a HAT mechanism are preferred over a SET reaction because the peroxy radical is the predominant free radical found in lipid oxidation of foods and biological systems. It has been reported that no single assay provides enough information of the antioxidant activity, thus multiple assay should be performed to generate an "antioxidant profile".

**Anti-yeast test.** The oregano essential oils showed significant levels of anti-yeast activity. Significant differences between the concentrations (5, 10 and 15 mg/mL) (Fig. 5) were observed in the samples 'Compacto' and 'Criollo', with some of these samples showing higher activities (15 mg/mL essential oil) than the positive control. The 'Compacto' oils (Córdoba and Neuquén) showed the highest activity and were significantly different from the others in this concentration. Other studies have shown a strong cidal effect (total elimination of the microbial initial inoculum) of *O. vulgare* extracts and hydrosols (Sagdic et al., 2002). The functional groups of the components play an important role for anti-microbial activity. For instance, phenolic components are capable of disrupting the microbial membrane, thus penetrating inside the cell, where they interact with cellular metabolic mechanisms (Baydar et al., 2004). Several studies have shown an association between the chemical composition and the anti-yeast activity of essential oils (Abdel-Mallek et al., 1994; Van Vuuren et al., 2009). Essential oil of *O. vulgare* subsp. *vulgare* 'Compacto' has been reported to be rich in phenolic compounds including carvacrol, thymol and other monoterpenes such as  $\gamma$ -terpinene,  $\alpha$ -terpinene,



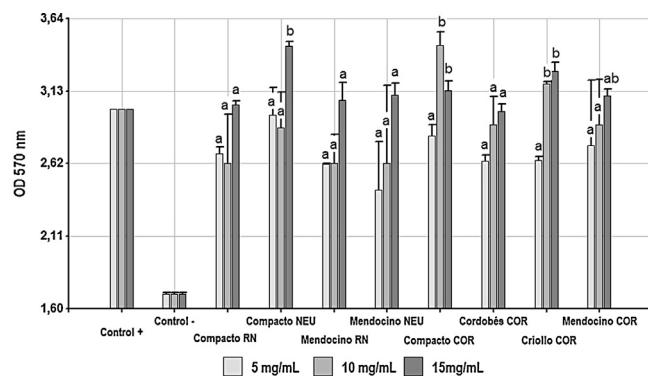
**Fig. 3.** Spider diagram sensory profile of the four oregano-types essential oils ('Cordobés', 'Criollo', A, 'Compacto', B, and 'Mendocino', C) from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Rio Negro (RN).



**Fig. 4.** Carotene bleaching assay of the four oregano-types essential oils ('Compacto', 'Cordobés', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Rio Negro (RN). AA% = Antioxidant Activity of oregano essential oil as inhibition percentage of linoleic acid. The same letter between essential oils slopes means no significant differences them at  $\alpha = 0.05$  ( $n = 3$ , LSD Fisher).

p-cimene,  $\gamma$ -terpineol, sabinene, myrcene, caryophyllene, geranacrene, and spathulenol, which are believed to be responsible for its prominent antimicrobial activity (Souza et al., 2007). In this study, anti-yeast activity of 'Compacto' oils from Neuquén and Córdoba were associated with high levels of terpinen-4-ol (10.96 and 7.76%, respectively),  $\gamma$ -terpinene (8.33 and 9.8%), and thymol (16.59 and 14.39%). Reports have demonstrated that the activity of the essential oils is related to their composition and the structural configuration and functional groups of their constituents (Bozin et al., 2006). The screening of Argentinian oregano types confirms the anti-yeast activity of these essential oils and supports their use as alternative natural antimicrobial compounds.

**Roundworm lethality assay.** In this study, a free-living nematode was used as a model organism to determine the lethality of plant extracts to determine clues against multicellular organisms. This species is advantageous as it is easy to grow and maintain, and poses no threats to human health. The response of this lethality immersion assay varied according to the type and concentration of the essential oil (Fig. 6). South Argentinian oregano oils were more active in this test than the essential oils from central Argentina (Córdoba province). At 10 mg/mL, the 'Compacto' oil from Rio Negro showed 66.7% mortality followed by 'Compacto' from Neuquén (52.5%) and 'Mendocino' from Rio Negro (52.38%). 'Mendocino' and 'Compacto' (Neuquén and Rio Negro) had 100% lethality when the concentration was 15 mg/mL. Nematicidal



**Fig. 5.** Optical density (OD) of yeast media treated with MTT of the four oregano-types essential oils ('Compacto', 'Cordobés', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Rio Negro (RN). High optical density showed low dehydrogenase activity characterized of dying yeasts as affected by the oils. The same letter between the same essential oil concentration means no significant differences between essential oils at  $\alpha = 0.05$  ( $n = 3$ , LSD Fisher).

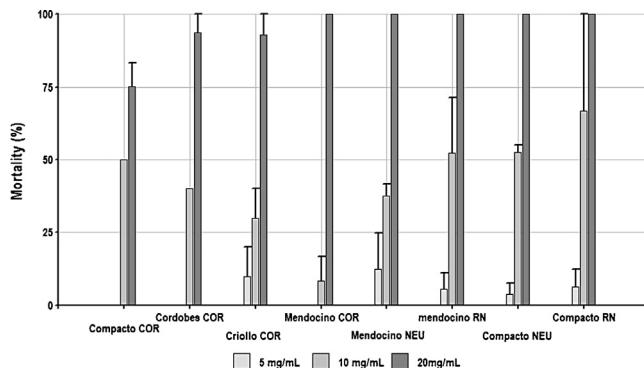
**Table 4**

Nematicidal activities of the essential oils of four oregano-types ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Rio Negro (RN) against *Panagrellus redivivus* using the immersion bioassay.

Type	'Compacto' <sup>a,b</sup>			'Cordobes' <sup>a,b</sup>			'Criollo' <sup>a,b</sup>			'Mendocino' <sup>a,b</sup>		
Province	COR	NEU	RN	COR	COR	COR	NEU	RN	COR	NEU	RN	
Slope	4.64a	6.17c	5.83b	6.13c	5.63b	7.02d	5.89c	6.08c				
LC 50	13.46d	11.32b	10.36a	12.54c	12.68c	13.65d	11.67b	11.22b				

<sup>a</sup> Compacto: COR (Córdoba), NEU (Neuquén), RN (Río Negro); Cordobes: COR (Córdoba); Criollo: COR (Córdoba); Mendocino: COR (Córdoba), NEU (Neuquén) and RN (Río Negro).

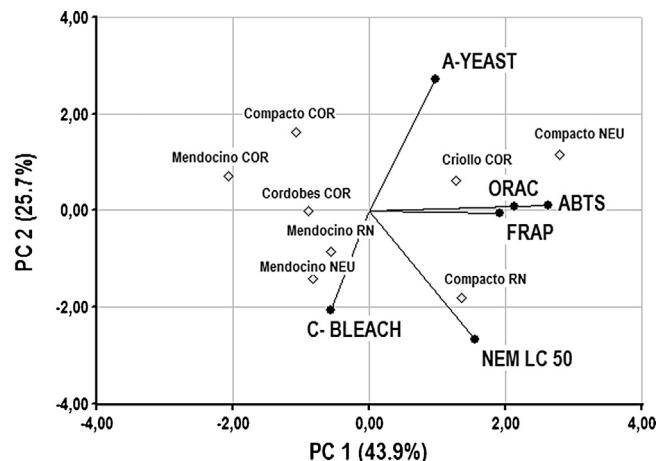
<sup>b</sup> The same letter in the row means that there are not significant differences at  $\alpha = 0.05$  ( $n = 3$ , LSD Fisher).



**Fig. 6.** Mortality percentage of nematodes (5–20 mg/mL of essential oil) with three different concentrations of the four oregano-types essential oils ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Río Negro (RN).

activity was also evaluated comparing LC 50 values (Table 4), 'Compacto' (Río Negro) was the most effective nematicide (10.36 mg/mL) followed by 'Mendocino' (Río Negro and Neuquén) (11.2, 11.7 mg/mL, respectively). A weaker nematicidal activity was also observed in 'Mendocino' and 'Compacto' type from Córdoba (LC50, 13.7 and 13.5 mg/mL, respectively). Plant essential oils are potential nematicides because some of them are selective, are degraded to nontoxic products, and are innocuous to non-target organisms and the environment (Chitwood, 2002). Reports of nematicidal activity of plant products and their mode of action are limited and further studies are needed to evaluate systemic actions of essential oils against nematodes (Jourand et al., 2004; Kong et al., 2006).

In this study a limited number of assays were run to characterize the biocidal properties of Argentinian essential oils, a trend in the bioactivity score was also observed. The 'Compacto' oils from southern Argentinian provinces and the 'Criollo' (Córdoba) were the most active against these two organisms with a biocidal score of 4.8 and 4 (maximum of 6, for Neuquén, Río Negro, Córdoba, respectively). For the 'Mendocino' type, the oils from Neuquén and Río Negro were more active than the oils from Córdoba (3.3, 3.3 and 2.3, respectively), with the lowest score observed for the 'Cordobes' type (also from Córdoba) (Table 5). The total score followed the trend of the antioxidant and biocides scores, with the 'Compacto' oils



**Fig. 7.** Principal Component Analysis (PCA) biplot obtained from the first and second principal components (PC). Euclidean distance variables: A-YEAST (Anti-yeast activity), C-BLEACH (Carotene Bleaching assay), NEM LC 50 (Nematode Lethal Concentration 50), FRAP (Ferric Reducing Power), ABTS (ABTS essay) and ORAC (oxygen radical absorbance capacity). Treatments: four oregano-types essential oils ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Río Negro (RN).

from Neuquén and Río Negro showing the highest values (14–14.4), followed by the 'Criollo' type. The Southern Argentinian types showed higher values than the Córdoba type (Table 5, Fig. 1).

The results of the biological activity tests were used to create a Principal Component Analysis (PCA) biplot obtained from the first and second principal components (PC) (Fig. 7). The two principal components explained the 69.6% variability (PC 1 representing the 43.9%) of the essential oils considering their antioxidant and biological activities.

The PCA confirmed the strong association within the FRAP, ABTS and ORAC antioxidant tests, and the association of these antioxidant assays with the 'Compacto' (NEU, RN) and 'Criollo' (COR) essential oils (Fig. 7). This observation coincides with the fact that these oils exhibited the highest values in the bioactivity scores (Table 5).

Anti-yeast and nematicidal activities were located to the right of PC1 and in different quadrants, both showing positive correlations with the antioxidant tests ORAC, ABTS and FRAP, suggesting that

**Table 5**

Biological activity scores for antioxidant and bioassays for the four oregano-types essential oils ('Compacto', 'Cordobes', 'Criollo' and 'Mendocino') from the Argentinian provinces of Córdoba (COR), Neuquén (NEU) and Río Negro (RN).

Type	'Compacto'			'Cordobes'			'Criollo'			'Mendocino'		
Province	COR	NEU	RN	COR	COR	COR	NEU	RN	COR	NEU	RN	
Antioxidant activity score <sup>1</sup>	7.3	9.2	10.4	6.0	9.9	5.2	8.1	6.9				
Biocidal activity score <sup>2</sup>	3.8	4.8	4.0	3.0	4.0	2.3	3.3	3.3				
Total score <sup>3</sup>	11.1	14.0	14.4	9.0	13.9	7.5	11.4	10.2				

The score results from the conversion of a given activity in values from 1 (low activity) to 3 (high activity) then values for a given bioactivity (for antioxidant activity four tests, for biocidal two tests) are added up to generate a score (for antioxidants the total possible score is 12, for biocidal 6 and for the total score 18).

the mechanism of reaction (either SET or HAT) are needed for the bioactivity (Fig. 7).

The PCA also showed that anti-yeast (A-YEAST) and nematicidal activities (N LC50) were weakly correlated. 'Criollo' from Córdoba and 'Compacto' from Neuquén were the closest treatments to A-YEAST, while the 'Compacto' from Rio Negro was closely associated with the nematicidal activity (NEM LC 50).

The Carotene bleaching activity (C-BLEACH) was placed on the left side of the plot, showing that this activity did not correlate well with the other antioxidant tests, as the carotene bleaching assay assessed different antioxidant mechanisms. The 'Mendocino' type from Neuquén and Rio Negro provinces were highly related with this test (Fig. 7).

Another important trend was the weak association of the essential oils from Córdoba ('Compacto', 'Mendocino' and 'Cordobes') with the bioactivity assays, while the essential oils from the Southern provinces (Rio Negro and Neuquén) showed higher associations. These results were in agreement with the bioactivity scores (Table 5).

#### 4. Conclusion

Argentinian oregano essential oils showed variations in their sensory, chemical, and physicochemical profiles and biological activities. All these parameters were affected by the oregano-type and production region. The oregano essential oils were characterized by their aroma, showing that the essential oils from central Argentina ('Mendocino' and 'Criollo') were among the most liked essential oils. This study demonstrated that the chemical composition of these four types is quite stable thus important features to consider these varieties as unique Argentinian types. Sensory, physical and chemical properties (e.g. refractive index and density) served to differentiate the oils according to their origins. Overall biological activity scores and PCA showed that the essential oils from Southern Provinces were more active than the central Argentinian types (Córdoba). These higher biological activities were linked to lower aroma and higher color acceptances.

This study suggests that oils from Central Argentina can be used in the food, flavor and fragrance industries, while the oils from southern Argentinian provinces can provide new sources of antioxidants and biocides for use in the food (preservation), pharmaceutical and possibly veterinary industries. Development of trade and quality control standards (sensory, physical and chemical) are the initial steps to support the commercialization process. This study sought to increase the interest in Argentinian oregano essential oils in support of the development of local, regional and international trade as a vehicle for rural economic growth in central and the south regions of the country.

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