Industrial Crops and Products 69 (2015) 21–28

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Thermal stability and antioxidant activity of essential oils from aromatic plants farmed in Argentina

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ARTICLE INFO

Article history:
Received 7 October 2014
Received in revised form 2 February 2015
Accepted 3 February 2015

Keywords:
Essential oils
Antioxidants
Stability
Volatiles

ABSTRACT

Food industries are looking for natural antioxidant to replace synthetic because these last ones are questioned due to healthy reasons. Essential oils are natural products that can have antioxidant activity, but their composition and antioxidant activity could change for thermal storage condition. The objective of this study was to evaluate the antioxidant effect and thermal stability of rosemary, oregano and laurel essential oils (EO). The major components of the essential oils were terpineol (E) Beta (55.5%), terpinen-4-ol (15.9%), and thymol (12.9%) in oregano; camphor (35.7%), verbenone (26.6%), and β-caryophyllene (15.8%) in rosemary EO; and linalool (45.0%), sabinene (31.9%), and methyl eugenol (14.3%) in laurel EO. The volatile composition of the EO changed during the thermal stability study. The antioxidant activity of the essential oils was analyzed measuring free-radical scavenging activity (FRSA) and total phenolic content; and performing a storage study of sunflower oil measuring the formation of peroxide and volatile oxidation compounds. The FRSA showed that laurel (61.74%), oregano (59.97%), and rosemary (48.23%) EOs showed better percentage inhibitions than BHT (8.76%). In the storage study of sunflower oil, samples with 0.10% oregano EO, 0.02% oregano EO, 0.02% laurel EO, and 0.10% rosemary EO showed better antioxidant properties, exhibiting less peroxide and anisidine values during storage. Also, 0.02% oregano EO in the storage study showed reduced formation of volatile compounds like hexanal, 2-heptenal, and 2,4-decadienal. The studied EOs have antioxidant activity and constitute natural potential agents that could be used as antioxidants in food products. Also, the studied EOs are compounds that change under high temperature conditions during storage, which could affect their potential antioxidant activity.

1. Introduction

The oxidation process introduces a rancid flavor and decreases the sensory and nutritional qualities of food products, making them unacceptable to consumers. Lipid oxidation occurs in stored raw materials and/or finished food, especially when these products have suffered heating treatment (Tomaino et al., 2005). Then, unhealthy free radicals start to be generated in the food. Health problems like tumors, diabetes, and cardiovascular disease, among others, are related to free radicals formed in food that undergoes deterioration during storage (Fransen et al., 2010). In addition, the shelf-life of the food product decreases because of the perception of off-flavor derived from compounds like hexanal, heptanal, and other volatile derivatives originating from the oxidation of lipid components (Belitz et al., 2009).

Adding antioxidants to foods is a technique to reduce lipid oxidation. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are used in many foods to retard the oxidation process. However, their food safety is questioned because of the potential risk of these compounds to health (Tomaino et al., 2005; Shearn et al., 2011).

Essential oils from aromatic plants show preservative properties as antioxidants and antimicrobials and many of them are obtained from edible sources (Suhaj, 2006). Many researchers have reported on the antioxidant activities of natural products like essential oils (Sacchetti et al., 2005; Kulisic et al., 2004; Proestos et al., 2006; Tepe et al., 2006). The inclusion of essential oils as antioxidants has been researched in different kinds of food like peanut products (Olmedo et al., 2012a,b), cheese cream (Olmedo et al., 2013), and cooking oil (Olmedo et al., 2014), among other products.

Essential oils (EO) from different parts of plants like seeds or leaves have shown different antioxidant properties. Essential oils

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http://dx.doi.org/10.1016/j.indcrop.2015.02.005
0926-6690/

from leaves typically present greater antioxidant activity (López-Mejia et al. 2014; Nikolic et al., 2014). The antioxidant activity of an essential oil is related to its chemical composition (Guimaraes et al., 2010). Olmedo et al. (2014) reported that fractions obtained by short path molecular distillation show different antioxidant activities because of differences in their chemical composition.

Argentina is a country with diverse climates that allows many species of aromatic plants to be farmed. About 8000 tons of spices are produced annually. The main crops are oregano, mint, and chamomile. The most consumed spices in Argentina are oregano, rosemary, and bay leaf. The objective of this study was to evaluate the antioxidant effect and thermal stability of rosemary, oregano and laurel essential oils obtained from aromatic plants farmed in Argentina.

2. Materials and methods

2.1. Materials and extraction of essential oils

Leaves of oregano (*Origanum vulgare* L.), rosemary *(Rosmarinus officinalis* L.), and laurel (*Laurus nobilis* L.) were collected in April, 2012 from the experimental station of the Facultad de Ciencias Agropecuarias, Universidad Nacional of Córdoba, Córdoba, Argentina. The essential oils (EO) were obtained by hydrodistillation using Clevenger type apparatus according to Olmedo et al. (2012a). Leaves (50 g) of laurel, oregano, and rosemary were distilled. After distillation, the essential oil was recovery and kept in dark glass flasks with sodium sulfate in a freezer at −18 °C.

2.2. Essential oils analysis

The essentials oils were analyzed with a PerkinElmer Clarus 600 GC–MS (Shelton, Connecticut, USA) coupled with an ion trap mass detector (MS) and non polar capillary column Elite-ms5 (methylpolysiloxane, 5% phenyl, 30 m, 0.25 mm id, and 0.25 μm coating thickness). The chromatographic conditions were 40 °C initial temperature during 3 min; rate of 10 °C/min until to 100 °C; a second rate of 15 °C/min until 245 °C. The injector temperature was 250 °C. The carrier gas (helium) had a flow rate of 0.9 mL/min. Ionization was obtained by electron impact at 70 eV and mass spectral data was acquired in the scan mode in the *m*/*z* range from 35 to 450. The retention index of chemical compounds were determined with homologous *n*-alkane hydrocarbons in the same conditions that the essential oils were analyzed. The identification was realized by comparing mass spectra, their retention time, retention index and comparing with libraries NIST and Adams. Also the main components were identified by coinjection in the GC–MS of pure standards (SIGMA, USA). The quantification of each peak was performed by the mass reported by the mass detector. The results were expressed as relative percentage of mass detected by the mass detector (Olmedo et al., 2014).

2.3. Thermal stability of essential oils

Glass flask (capacity 10 mL) with 10 μL of each essential oil were sealed with a rubber lid and stored in oven at 60 °C during 28 days. Samples were analyzed at 0, 14, and 28 storage days. A solid phase micro-extraction fiber (SPME) of polydimethylsiloxane/divinylbenzene (PDMS/DVB) was used to capture the volatiles compounds. Then, the SPME fiber was introduced in the flask and was heated at 70 °C during 20 min. Previous tests were carried out to find out the best procedure conditions to capture higher amount of volatile compounds. Finally, the fiber was removed from the flask and injected in the CG-MS. The gas chromatography conditions and compound identification and quantification were performed according to procedure described in Section 2.2 (Olmedo et al., 2014).

2.4. Free-radical scavening activity (FRSA) and total phenolic content

The free-radical scavenging activity of the essential oils was determined using 2,2-dipheny-l-1-picrylhydrazyl radical (DPPH) (Aldrich, Milwakee, WI, USA) according to Choi et al. (2000). Absorbance of solutions was measured at 517 nm with spectrophotometer (PerkinElmer Lambda 25 UV/vis Spectrometer, Bucks, United Kingdom) after 30 min. The radical-scavenging activity was expressed as percentage of DPPH inhibition (Tepe et al., 2006).

Phenol content was determined by Folin–Cicolteau reagent and the concentration calculated using gallic acid as standard (SIGMA, St Louis, MO, USA). The reaction was carried out with 10 μL of each essential oil. Phenol content was measured at 760 nm using a spectrophotometer (Hewlett Packard HP 8452 A, Palo Alto, CA, USA). Phenol content was expressed as mg/g (Dambolena et al., 2010).

2.5. Accelerated oxidation test (oven test)

Refine sunflower oil samples (Natura, Aceitera General Dehesa, General Cabrera, Córdoba, Argentina) added with 0.02% and 0.10% essentials oils of laurel (SL 0.02% and SL 0.10%, respectively), oregano (SO 0.02% and SO 0.10%, respectively), and rosemary (SR 0.02% and SR 0.10%, respectively), were stored in oven at 60 °C (Proestos et al., 2006). Butyl hydroxy toluene (BHT) at 0.02% in refined sunflower oil was used as a comparative reference. The samples were stored for 28 days and were removed for analysis every 7 days. Peroxide value (PV) and *p*-anisidine (AV) as chemical indicators of lipid oxidation were evaluated in the samples (Olmedo et al., 2014).

2.6. Volatile oxidation compounds

The antioxidant effects of the essential oils were also analyzed by volatile oxidation compounds formed in refined sunflower oil during the storage. For this assay, 10 g sunflower oil was put into glass flask (50 mL) and stored in the same condition that accelerated test oxidation described in the point 2.5. The samples were control (pure sunflower oil); 0.02% rosemary, laurel, and oregano essential oil in sunflower oil; and 0.02% BHT in sunflower oil. Samples were removed from storage at day 0, 14, and 28. The volatiles compounds were captured using a solid phase micro extraction fiber (SPME) of PDMS/DVB (Supelco, Sigma, St Louis, MO, USA) that was introduced into the glass flask and heated for 20 min at 130 °C. After that, the fiber was injected for 1 min in the GC–MS injector. The volatiles captured for the fiber were analyzed using a chromatograph PerkinElmer Clarus 600 (Palo Alto, Ca, USA). The samples were separated in a non-polar column DB-5 (30 m). The chromatographic conditions were same as described in Section 2.2. To help the identification of the main components, co-injection of authentic standards (SIGMA, St Louis, MO, USA) were made. Acetaldehyde (SIGMA, St Louis, MO, USA) was running as an internal standard in all samples. The concentration of the component was expressed as ppm (mg/L) (Olmedo et al., 2014).

2.7. Statistical analysis

The experiments were replicated three times. The data was analyzed using Infostat software, version 1.1 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba). Means and standard deviations were calculated. Analysis of variance and LSD Fisher test ($\alpha = 0.05$) were used to detect significant differences between treatments. Regression equations were used to determine if the

Table 1
Chemical composition, free radical scavenging activity (FRSA), and total phenol content from oregano essential oil and its volatile composition (VC) from the thermal stability study analyzed in samples stored during 28 days at 60 °C.

Retention index	Components	EO g/100 g ± SD[a]	VC day 0 g/100 g ± SD[a]	VC day 14 g/100 g ± SD[a]	VC day 28 g/100 g ± SD[a]	Methods of identification[b]
923	α-Thujene	1.3 ± 0.1	0.7 ± 0.1 a	0.9 ± 0.1 b	0.8 ± 0.1 b	GCMS
933	α-Pinene	1.1 ± 0.1	0.6 ± 0.1 a	0.8 ± 0.1 b	0.7 ± 0.1 b	GCMS-Co
973	Sabinene	4.6 ± 0.2	2.6 ± 0.1 c	2.0 ± 0.1 b	1.6 ± 0.1 a	GCMS-Co
980	β-Pinene	0.4 ± 0.1	0.4 ± 0.1 a	0.5 ± 0.1 a	0.5 ± 0.1 a	GCMS-Co
991	β-Myrcene	1.8 ± 0.2	1.2 ± 0.1 a	1.4 ± 0.1 ab	1.4 ± 0.1 b	GCMS
1005	α-Phellandrene	0.8 ± 0.1	0.5 ± 0.1 a	0.5 ± 0.1 a	0.6 ± 0.1 b	GCMS
1018	α-Terpinene	8.5 ± 0.2	4.3 ± 0.2 c	3.5 ± 0.1 b	3.4 ± 0.1 a	GCMS
1020	*ortho*-Cymene	2.7 ± 0.2	1.9 ± 0.1 a	12.5 ± 0.1 b	13.1 ± 0.1 c	GCMS
β-1031	β-Phellandrene	β-	2.4 ± 0.1 a	3.1 ± 0.1 b	3.5 ± 0.1 c	GCMS
1059	γ-Terpinene	β-	10.4 ± 0.1 c	4.2 ± 0.1 b	3.9 ± 0.1 a	GCMS-Co
1069	*cis*-Sabinene hydrate	0.9 ± 0.1	1.5 ± 0.1 a	Trace	Trace	GCMS-Co
1084	Terpinolene	2.1 ± 0.1	1.1 ± 0.1 b	0.9 ± 0.1 a	0.9 ± 0.1 a	GCMS
1098	Linalool	7.4 ± 0.2	13.1 ± 0.1 c	6.3 ± 0.1 b	5.7 ± 0.1 a	GCMS
1143	Camphor	0.4 ± 0.1	1.8 ± 0.1 c	1.6 ± 0.1 b	1.3 ± 0.1 a	GCMS
1177	Terpinen-4-ol	16.7 ± 0.2	29.5 ± 0.1 c	25.3 ± 0.1 a	26.0 ± 0.1 b	GCMS-Co
1189	α-Terpineol	2.1 ± 0.2	5.2 ± 0.1 a	5.7 ± 0.1 b	5.7 ± 0.1 b	GCMS
1205	*trans*-Piperitol	Trace	Trace	1.0 ± 0.1 b	0.8 ± 0.1 a	GCMS
1235	Thymol methyl ether	1.9 ± 0.1	1.8 ± 0.1 c	1.1 ± 0.1 b	1.0 ± 0.1 a	GCMS
1298	Carvacrol	16.2 ± 0.2	20.4 ± 0.1 a	25.1 ± 0.1 b	26.34 ± 0.2 c	GCMS-Co
1418	β-Caryophyllene	0.8 ± 0.2	0.5 ± 0.1 a	1.2 ± 0.1 c	1.1 ± 0.1 b	GCMS
1509	β-Bisabolene	1.0 ± 0.1	0.3 ± 0.1 a	1.0 ± 0.1 c	0.9 ± 0.1 b	GCMS
1576	Spathulenol	Trace	Trace	1.1 ± 0.1 b	0.9 ± 0.1 a	GCMS
	Total	99.5	99.9	99.6	100.0	
	FRSA[c] percentage	60.0 ± 2.5 b				
	Phenol[d] content (mg/g)	10.0 ± 0.1 b				

[a] Values with different letter in the same raw are significantly different ($n = 3$, LSD Fisher, $\alpha = 0.05$).
[b] GCMS: peak identifications are based on MS comparison with file spectra. Co: peak identifications are based on standard comparison with relative retention time.
[c] FRSA: expressed as percentage of inhibition.
[d] Phenol content expressed as mg/g of essential oil.

independent variables (time) had an effect on the indicators of lipid oxidation (PV, AV and CD). Principal components analysis (PCA) was utilized for correlation between concentration of different essential oil and oxidation variables (Jonhson and Wichern, 1998).

3. Results and discussion

3.1. Chemical composition of essential oils

The major compounds in oregano essential oil (Table 1) were γ-terpinene (25.1%), terpinen-4-ol (16.7%), and carvacrol (16.2%) (Table 1). Other studies have shown different percentages of these compounds in oregano EO. Kulisic et al. (2004) reported 35.0% thymol, 32.0% carvacrol, and 10.5% γ-terpineno. Tomaino et al. (2005) found 48.9% carvacrol, 11.7% *ρ*-cimeno, and 5.0% thymol. Suhaj (2006) observed important variations in the concentration of carvacrol (between 0 and 12 ppm), γ-terpineno (0 at 13 ppm), linalyl-acetate (0–50 ppm), myrcene (0–50 ppm), and terpinen-4-ol (0–220 ppm). Dambolena et al. (2010) also reported variations in the concentrations of the main components among oregano species, where monoterpene like *trans*-sabinene hydrate oscillated between 27.7% and 36.7% and thymol between 17.7 and 30.75%.

The major components of rosemary EO (Table 2) were 1,8-cineole (22.2%), β-myrcene (21.5%), and α-pinene (11.0%). Sacchetti et al. (2005) found verbenone (21.8%), camphor (14.6%), bornyl acetate (12.3%), and borneol (10.4%) in rosemary essential oil. This chemical composition reported by those authors is very different with respect to the values observed in this research. Suhaj (2006) detected variations in the components of rosemary essential oil, where camphene oscillated between 0 and 145 ppm and terpinel-4-ol between 0 and 40 ppm. The variations in rosemary essential oil among different studies can be explained by differences in farming conditions, genetic characteristic of plant material, phenological stage of plant materials used for essential oil extraction, environmental factors as climate or soil conditions, or parameters of extraction (distillation time, pressure, and/or temperature) that could significantly affect the chemical composition of rosemary EO.

The major components of laurel EO (Table 3) were 1,8-cineole (42.1%), linalool (11.9%), and α-terpineol acetate (9.3%). Laurel EO also showed variation in its chemical composition between researches. Flamine et al. (2007) reported high percentages of 1,8-cineole (35.7%), *trans*-Sabinene hydrate (9.7%), α-terpineol acetate (9.3%), methyl eugenol (6.8%), and sabinene (6.5%). If it is considered as the three major components in oregano, rosemary, and laurel EOs, the cumulative percentages exhibited by them were 58%, 55%, and 63%, respectively.

3.2. Thermal stability of oregano, rosemary and laurel essential oils

The volatile composition of the EO changed during the thermal stability study. In oregano essential oil, terpinen-4-ol and linalool decreased from 29.5% to 26.0% and from 13.1% to 5.7% after 28 storage days, respectively (Table 1). At the same time, carvacrol increased from 20.4% to 26.4%. Also, *ortho*-cymene showed a percentage increased from 1.94% to 13.13% during the thermal stability study.

In rosemary essential, 1,8-cineole and camphor increased their percentages from 16.6% to 21.8% and from 12.7% to 18.7% after 28 storage days, respectively. In contrast, β-myrcene decreased from 14.1% to 12.3%.

In laurel essential, 1,8-cineole increased from 32.3% to 52.0% during 28 days of storage linalool and terpineol acetate decreased from 11.7% to 5.6% and from 13.2% to 12.6%, respectively. Yang et al. (2007) determined the degradation of 5 terpenes: alpha pinene, limonene, camphor, citronellal, and carvacrol at 100, 150, 200, and 250 °C during 30 and 300 min. All terpenes showed degradation under the different experimental conditions. Those authors found that camphor, citronellal, and carvacrol showed slower degradation in comparison with α-pinene and limonene. Differences in

Table 2
Chemical composition, free radical scavenging activity (FRSA), and total phenol content from rosemary essential oil and its volatile composition (VC) from the thermal stability study analyzed in samples stored during 28 days at 60 °C.

Retention index	Components	EO g/100g ± SD[a]	VC day 0 g/100g ± SD[a]	VC day 14 g/100g ± SD[a]	VC day 28 g/100g ± SD[a]	Methods of identification[b]
923	α-Tujene	0.9 ± 0.1	0.6 ± 0.1 a	0.7 ± 0.1 a	0.6 ± 0.1 a	GCMS
933	α-Pinene	11.0 ± 0.2	7.6 ± 0.1 c	5.6 ± 0.1 b	5.4 ± 0.1 a	GCMS-Co
952	Camphene	3.6 ± 0.2	2.4 ± 0.1 b	1.7 ± 0.1 a	1.7 ± 0.1 a	GCMS
980	β-Pinene	2.4 ± 0.1	1.4 ± 0.1 b	1.1 ± 0.1 a	1.0 ± 0.1 a	GCMS-Co
991	β-Myrcene	21.5 ± 0.2	14.1 ± 0.1 b	12.8 ± 0.1 a	12.3 ± 0.1 a	GCMS
1018	α-Terpinene	1.5 ± 0.1	0.7 ± 0.1 a	Trace	Trace	GCMS
1023	β-Cymene	3.0 ± 0.1	1.7 ± 0.1 a	4.1 ± 0.1 b	4.2 ± 0.1 b	GCMS
1032	1,8-Cineole	22.2 ± 0.2	16.6 ± 0.1 a	22.0 ± 0.1 b	21.8 ± 0.1 b	GCMS
1059	γ-Terpinene	3.4 ± 0.2	1.3 ± 0.1 c	0.3 ± 0.1 a	0.5 ± 0.1 b	GCMS-Co
1098	Linalool	2.4 ± 0.1	2.5 ± 0.1 a	3.5 ± 0.1 b	3.4 ± 0.1 b	GCMS
1143	Camphor	10.1 ± 0.1	12.7 ± 0.1 a	19.6 ± 0.1 c	18.7 ± 0.1 b	GCMS
1145	Isopulegol	0.8 ± 0.1	0.9 ± 0.1 a	Trace	Trace	GCMS
1156	Isoborneol	1.3 ± 0.1	1.9 ± 0.1 b	1.3 ± 0.1 a	2.9 ± 0.1 c	GCMS
1177	Terpinen 4 ol	3.6 ± 0.2	2.7 ± 0.1 a	3.0 ± 0.1 b	3.3 ± 0.1 c	GCMS-Co
1182	Terpineol	2.3 ± 0.1	4.6 ± 0.1 a	6.4 ± 0.1 b	6.5 ± 0.1 b	GCMS
1204	Verbenone	1.3 ± 0.1	3.9 ± 0.1 a	9.6 ± 0.1 c	9.2 ± 0.1 b	GCMS
1228	Citronellol	1.8 ± 0.1	Trace	0.6 ± 0.1 a	1.5 ± 0.1 b	GCMS
1285	Bornyl acetate	Trace	0.9 ± 0.1 a	1.0 ± 0.1 a	0.9 ± 0.1 a	GCMS
1298	Carvacrol	3.3 ± 0.1	1.5 ± 0.1 a	Trace	Trace	GCMS-Co
1401	Eugenol methyl ether	Trace	2.8 ± 0.1 b	1.9 ± 0.1 a	1.9 ± 0.1 a	GCMS
1404	γ-Caryophyllene	Trace	10.3 ± 0.1 b	1.5 ± 0.1 a	1.4 ± 0.1 a	GCMS
1418	β-Caryophillene	2.3 ± 0.2	1.6 ± 0.1 c	0.9 ± 0.1 a	1.1 ± 0.1 b	GCMS
1454	α-Caryophyllene	0.7 ± 0.1	3.9 ± 0.2 c	0.5 ± 0.1 b	0.4 ± 0.1 a	GCMS
1573	Oxide caryophyllene	Trace	1.9 ± 0.1 c	1.7 ± 0.1 b	1.5 ± 0.1 a	GCMS
	Total	99.5	98.5	100.0	100.0	
	FRSA[c] percentage	48.3 ± 1.0 a				
	Phenol[d] content (mg/g)	8.0 ± 0.1 a				

[a] Values with different letter in the same raw are significantly different ($n = 3$, LSD Fisher, $\alpha = 0.05$).
[b] GCMS: peak identifications are based on MS comparison with file spectra. Co.: peak identifications are based on standard comparison with relative retention time.
[c] FRSA: expressed as percentage of inhibition.
[d] Phenol content expressed as mg/g of essential oil.

their boiling points could explain the differences in thermal degradation. In the present study, no relation was observed between the thermal stability behaviors of molecules in different essential oils. For example, α-pinene: the relative percentage of α-pinene decreased in rosemary EO but increased in oregano and rosemary EO. These differences could be responsible for the relationships among different components in the oregano, rosemary, and laurel EOs.

Table 3
Chemical composition, free radical scavenging activity (FRSA), and total phenol content from laurel essential oil and its volatile composition (VC) from the thermal stability study analyzed in samples stored during 28 days at 60 °C.

Retention index	Components	EO g/100g ± SD[a]	V day 0 g/100g ± SD[a]	V day 14 g/100g ± SD[a]	V day 28 g/100g ± SD[a]	Methods of identification[b]
933	α-Pinene	3.4 ± 0.1	1.8 ± 0.1 a	3.3 ± 0.1 b	3.7 ± 0.1 c	GCMS-Co
952	Camphene	0.5 ± 0.1	Trace	Trace	Trace	GCMS
973	Sabinene	6.4 ± 0.1	3.9 ± 0.1 c	1.9 ± 0.1 a	2.1 ± 0.1 b	GCMS-Co
980	β-Pinene	3.1 ± 0.1	1.9 ± 0.1 b	1.4 ± 0.1 a	1.3 ± 0.1 a	GCMS-Co
991	β–Myrcene	1.4 ± 0.1	0.5 ± 0.1 a	Trace	Trace	GCMS
1011	Carene	0.6 ± 0.1	Trace	Trace	Trace	GCMS
1032	1,8-Cineole	42.1 ± 0.2	32.3 ± 0.1 a	48.0 ± 0.2 b	52.0 ± 0.1 c	GCMS
1059	γ-Terpinene	0.8 ± 0.1	0.4 ± 0.05 a	Trace	Trace	GCMS-Co
1084	Terpinolene	0.6 ± 0.1	0.4 ± 0.1 a	Trace	Trace	GCMS
1098	Linalool	11.9 ± 0.1	11.7 ± 0.1 c	5.8 ± 0.1 b	5.6 ± 0.1 a	GCMS
1156	Isoborneol	1.3 ± 0.1	Trace	Trace	Trace	GCMS
1177	Terpinen 4 ol	3.4 ± 0.1	6.1 ± 0.1 c	4.1 ± 0.1 b	3.5 ± 0.1 a	GCMS-Co
1189	α-Terpineol	7.4 ± 0.2	10.0 ± 0.1 c	5.6 ± 0.1 b	4.9 ± 0.1 a	GCMS
1237	Pulegone	0.8 ± 0.1	Trace	Trace	Trace	GCMS
1285	Bornyl acetate	0.8 ± 0.1	0.6 ± 0.1 a	0.7 ± 0.1 a	0.6 ± 0.1 a	GCMS
1323	Methy geranate	0.8 ± 0.1	1.3 ± 0.1 c	1.2 ± 0.1 b	1.0 ± 0.1 a	GCMS
1328	α-Terpineol acetate	9.3 ± 0.2	13.2 ± 0.1 c	13.0 ± 0.1 b	12.6 ± 0.1 a	GCMS
1356	Eugenol	0.9 ± 0.1	3.1 ± 0.1 c	2.8 ± 0.1 b	2.3 ± 0.1 a	GCMS
1401	Eugenol methyl ether	3.3 ± 0.2	10.2 ± 0.1 b	10.0 ± 0.1 b	8.8 ± 0.1 a	GCMS
1576	Spathulenol	1.0 ± 0.1	2.3 ± 0.1 b	2.3 ± 0.1 b	1.5 ± 0.1 a	GCMS
	Total	99.9	99.9	99.9	99.9	
	FRSA[c] percentage	61.7 ± 0.5 b				
	Phenol[d] content (mg/g)	10.5 ± 0.1 c				

[a] Values with different letter in the same raw are significantly different ($n = 3$, LSD Fisher, $\alpha = 0.05$).
[b] GCMS: peak identifications are based on MS comparison with file spectra. Co: peak identifications are based on standard comparison with relative retention time.
[c] FRSA: expressed as percentage of inhibition.
[d] Phenol content expressed as mg/g of essential oil.

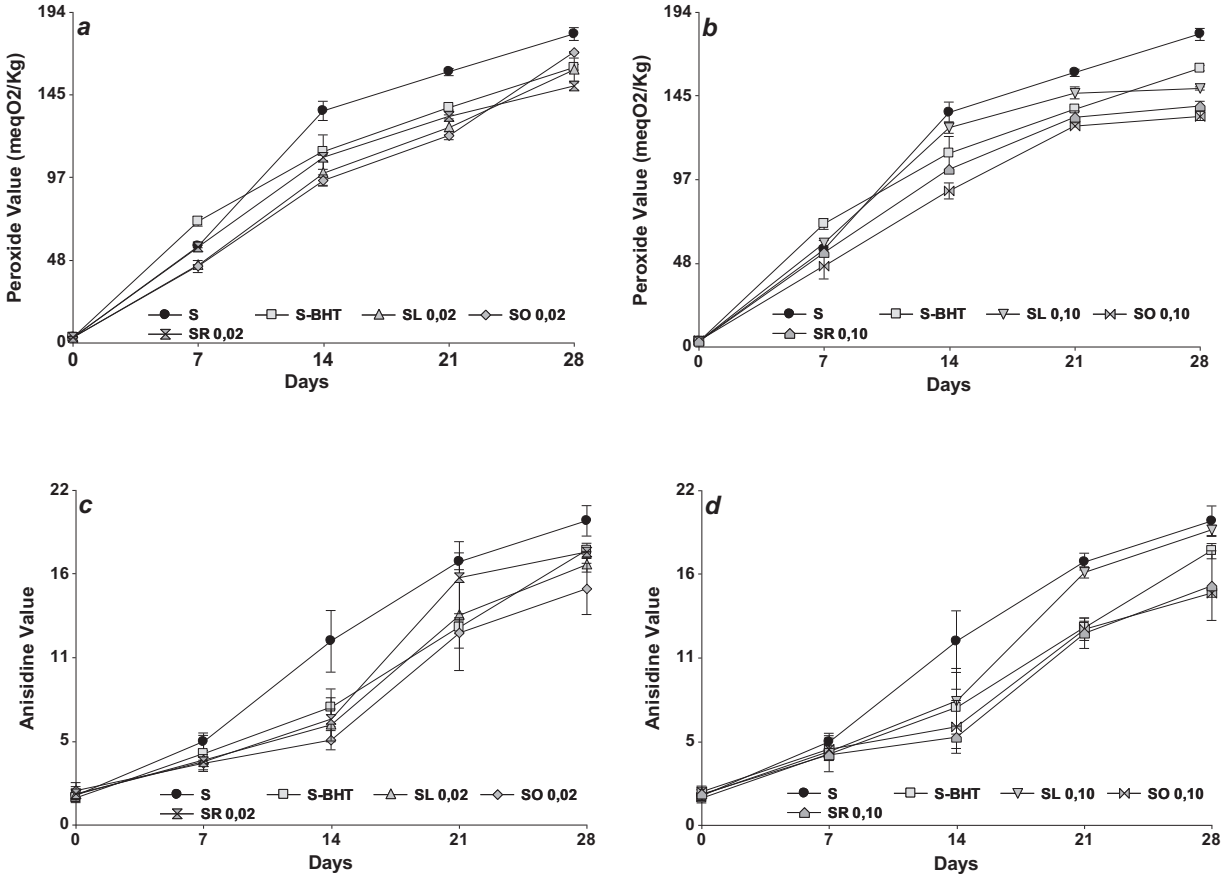

Fig. 1. Peroxide (PV) and anisidinine (AV) values from sunflower oil samples stored at 60 °C. Samples: S (sunflower oil), S-BHT(sunflower oil with 0.02% BHT), SL 0.02 (sunflower oil with 0.02% laurel EO), SL 0.10 (sunflower oil with 0.10% laurel EO), SO 0.02 (sunflower oil with 0.02% oregano EO), SO 0.10 (sunflower oil with 0.10% oregano EO), SR 0.02 (sunflower oil with 0.02% rosemary EO), and SR 0.10 (sunflower oil with 0.02% rosemary EO).

3.3. *Free-radical scavenging activity (FRSA) and total phenolic content (TPC)*

Essential oils from aromatic plants like laurel, thymus, oregano, and rosemary have free-radical scavenging properties (Sachetti et al., 2005; Kulisic et al., 2004). Oregano and laurel EOs (Tables 1 and 3) showed the strongest FRSA activity. Tomaino et al. (2005) found lower values of FRSA for oregano essential oils (51.8%) with respect to those detected in this study. But Dambolena et al. (2010) reported higher values (75%) for *O. vulgare ssp. Vulgare* variety Compacto. Sacchetti et al. (2005) observed higher FRSA (63.8%) in rosemary EO with respect to the result found for rosemary EO. Also, Sacchetti et al. (2005) reported the free-radical scavenging activity of Trolox, a synthetic antioxidant, as less than (28.2%) that of rosemary EO, a natural antioxidant. In laurel essential oil, Kosar et al. (2005) found a FRSA value of 55% compared to the result reported in this study, although, their values were greater than those detected in the essential oils from oregano and rosemary. Kosar et al. (2005) found decreases in FRSA values for laurel (55%), oregano (33%), and rosemary (20%) EOs similar to those found in this study.

Free-radical scavenging activity is mediated by a donor electron molecule (antioxidant). Phenols are H-donor molecules and, therefore, the total phenolic content can be related to FRSA. The oregano EO exhibited a phenolic content of 10.0 mg GAE/g (Table 1). The laurel EO exhibited the greatest total phenolic content (10.48 mg GAE/g) and also showed the highest FRSA (Table 3). The phenol content in in laurel EO was similar to that found in oregano EO; both EOs also showed the highest FRSA values. Ouchikn et al. (2011) reported higher value of total phenolic content in laurel leaves (29.94 mg GAE/g). Finally, rosemary EO (Table 2) exhibited the lowest total phenolic content (8.03 mg GAE/g). In terms of the chemical composition of essential oils, there are certain molecules with phenolic structure like carvacrol whose concentrations affect the total phenolic content. But the total phenolic content is not explainable simply based on phenol molecules, due to the fact that the Folin–Ciocalteu reagent measures reducing agents, and essential oils present different kind of molecules with reducing capacity (Lester et al., 2012). The chemical composition of laurel EO did not show phenol structures. This EO had a higher total phenolic content because this EO presents molecules with reducing capacity that, probably, are responsible for this EO's antioxidant capacity. Sha et al. (2013) studied the total phenolic content in infusions from 223 different medicinal plants. They showed that TPC can differ between plants, and individual TPC values can vary widely. For example, *Pyrola Calliantha* H. showed a low TPC with 0.19 mg GAE/g, while *Salvia miltiorrhiza* had a much larger TPC with 98.88 mg GAE/g. Skotti et al. (2014) found that the correlation coefficient (R) between phenolic content (TPC) and antioxidant activity obtained from the DPPH assay (FRSA) was 0.979. This indicates that a higher TPC correlates to a higher FRSA.

3.4. *Accelerated oxidation test (oven test)*

The control sample (S) exhibited the highest peroxide values during storage (Fig. 1a and b). In general, the S-BHT sample presented greater PVs than the samples with added EO at day 28 of storage. The samples SO 0.01% showed the best antioxidant activity

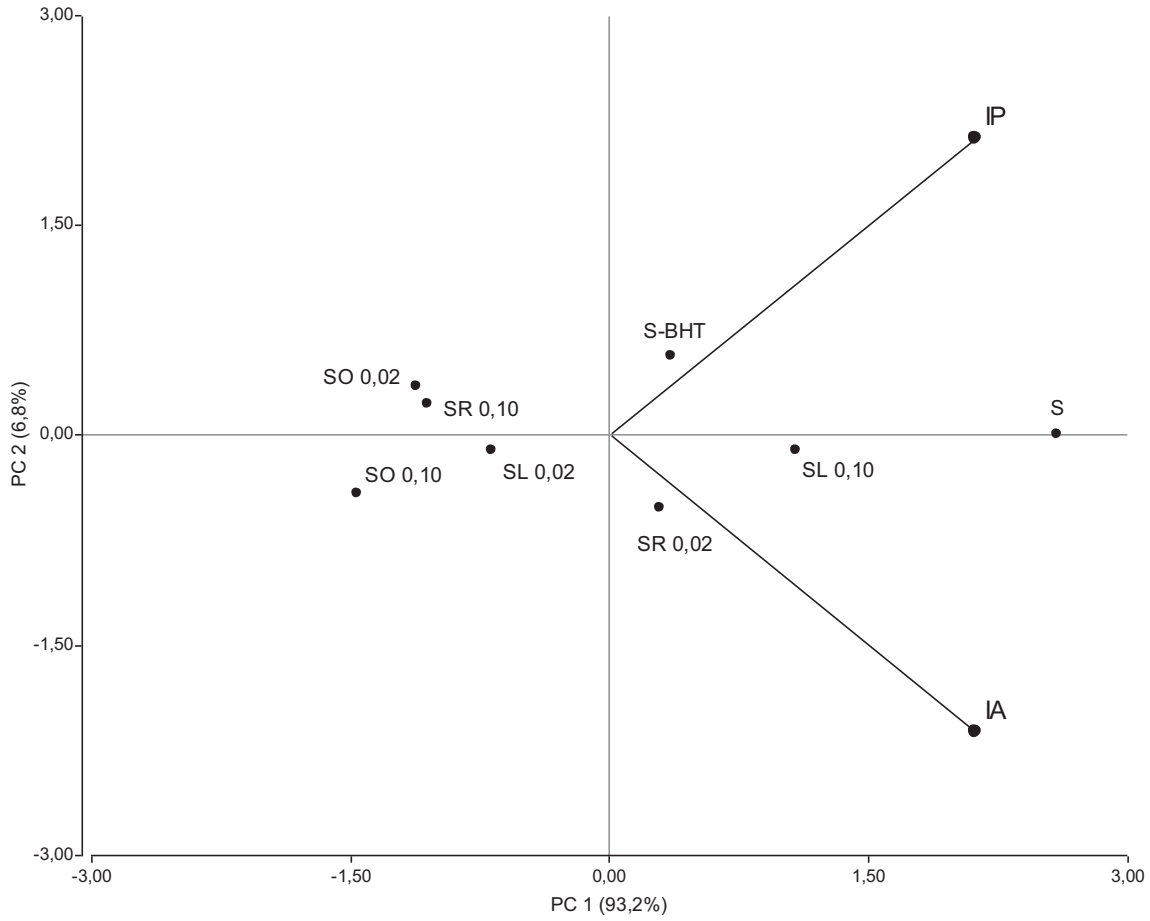

Fig. 2. Biplot of first and second principal components from PCA. Variables: peroxide and anisidine values, and sunflower oil (S) and sunflower oil samples during storage at 60 °C. Samples: S (sunflower oil), S-BHT (sunflower oil with 0.02% BHT), SL 0.02 (sunflower oil with 0.02% laurel EO), SL 0.10 (sunflower oil with 0.10% laurel EO), SO 0.02 (sunflower oil with 0.02% oregano EO), SO 0.10 (sunflower oil with 0.10% oregano EO), SR 0.02 (sunflower oil with 0.02% rosemary EO), and SR 0.10% (sunflower oil with 0.02% rosemary EO).

in sunflower oil during storage. The samples SO 0.10%, SO 0.02%, and SR 0.10% exhibited lower slopes in the linear regression equation.

With respect to anisidine analysis, the control sample (S) presented the highest values (Fig. 1c and d). The samples SR 0.02% had higher AV than S-BHT, SO 0.02%, and SL 0.02%. The samples SO 0.02% exhibited the lowest AV during storage. The samples SL 0.10% had greater anisidine values than the other antioxidants (BHT and EOs). The samples SO 0.02%, SO 0.10%, and SR 0.10% presented the lowest anisidine values. The results of this storage study evidence that oregano, rosemary, and laurel EOs are good natural antioxidants for sunflower oil, and probably for other edible vegetable oils. Even so, these natural antioxidants are better than the synthetic antioxidant BHT in some assays.

The peroxide and anisidine values were used to calculate the slopes of linear regression equations. Samples SR 0.10% and SO 0.10% had the lowest slopes in terms of peroxide values, without significant differences between them, but with significant differences with respect to the other samples. The slopes of AV in the regression equations showed a similar behavior to that of PV. The samples SR 0.10%, SO 0.10%, and SO 0.02% exhibited the lowest slopes. For all regression equations, the *p*-values were less than 0.0001.

3.5. Principal components analysis

In the present research, a principal components analysis (PCA) was made to detect which treatment showed the highest antioxidant property (Fig. 2). The first and second components explained 93.2% and 6.8% of the variability, respectively. The peroxide and anisidine values were placed on the right side of the first component, so samples located on this side indicate a higher degree of lipid oxidation. In contrast, samples placed on opposite sides correspond to treatments suffering less oxidation deterioration, which means higher antioxidant activity. The samples SO 0.10%, SO 0.02%, SL 0.02%, and SR 0.10% were located on the negative side of the first component and opposite to the lipid oxidation indicators (PV and AV). Therefore, these samples demonstrated better antioxidant activity. According to their locations in the biplot, samples S-BHT, SL 0.10%, and SR 0.02% showed lower antioxidant properties. The study of Frutos and Hernandez-Herrero (2005) reported similar antioxidant behavior in rosemary extract. That study performed an oxidation accelerating test with 4 different concentrations of rosemary extract (0, 2, 4, and 6 g/L) and evaluated chemical oxidation indicators using the thiobarbituric acid test and peroxide values in sunflower oil samples. Those authors determined that a concentration of 4 g/L had greater antioxidant activity than the other concentrations. On the other hand, Simic et al. (2003) established an assay to evaluate the antioxidant activity of laurel extracts, measuring levels of peroxide in the samples. Those authors found 60.5%, 64.6%, and 59.4% peroxide formation inhibition for concentrations of 2, 3, and 5 mg/100 g sample. Also in that study, the highest concentration of essential oil used showed less antioxidant activity. Before using an essential oil as a natural antioxidant, it should be tested in each food product at different concentrations in order to determine the concentration with the best antioxidant activity.

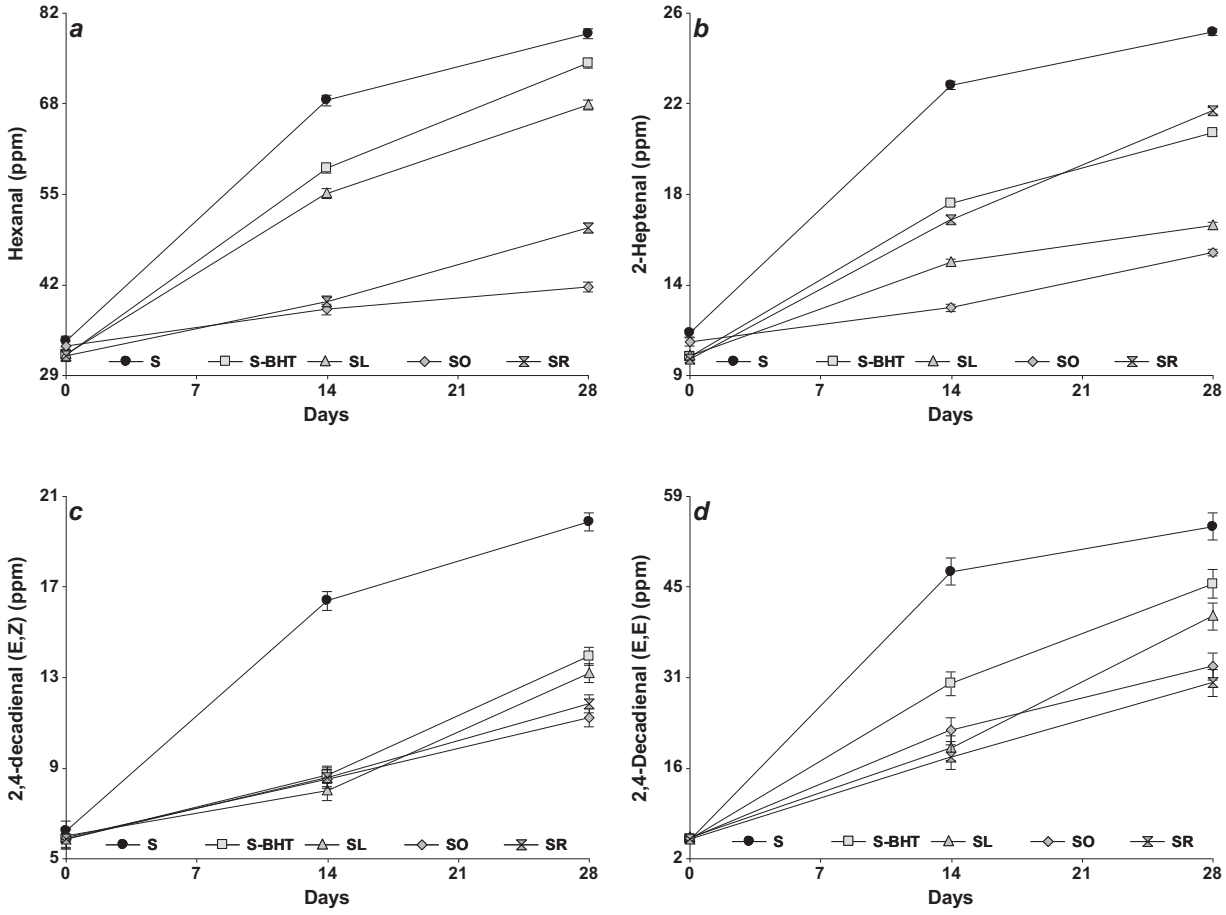

Fig. 3. Concentration changes of volatile oxidation compounds evaluated in sunflower oil samples: control sample (C) and sunflower oil added with 0.02% BHT (S-BHT), and 0.02% laurel (SL), oregano (SO), and rosemary (SR) essential oils during storage at 60 °C.

3.6. Volatile oxidation compounds

Odor-active monocarbonyl compounds are generated during the autoxidation of fatty acids. Sunflower oil has 63% linoleic acid (18:2) and 18% oleic acid (18:1) making it susceptible to autoxidation reactions. The main volatile compounds formed by autoxidation from these unsaturated fatty acids are 5100 ppm hexanal, 450 ppm 2-heptenal (E), 250 ppm 2,4-decadienal (E,Z), and 150 ppm 2,4-decadienal (E,E) for each 1 g oil (Belitz et al., 2009). In this study, hexanal, 2-heptenal, 2,4-decadienal (E,E), and 2,4-decadienal (E,Z) increased during storage in all samples (Fig. 3). The control samples had the highest content of all of these volatile oxidation compounds. The sunflower oil samples with essential oils exhibited lower volatile compounds with respect to Control samples at the end of storage; and the volatile content was similar to S-BHT. The oregano essential oil was the best antioxidant, decreasing the formation of volatiles in comparison with the other studied essential oils. Abdalla and Roozen (1999) found that different natural products like oregano, sage, and other aromatic extracts confer antioxidant protection and oxidative stability to sunflower oil. In another study (Olmedo et al., 2014), it was reported that sunflower oil samples supplemented with oregano essential oil and fractions obtained by short pat molecular distillation (SPMD) showed that volatile compound formation was lower in these samples with respect to the control sample. The essential oil fractions with low boiling point molecules like α and γ-terpinenes had greater antioxidant activity. In the present study, laurel and rosemary exhibited many molecules with low boiling points in their chemical compositions, which probably drove to decrease the formation of oxidative volatile compounds.

4. Conclusions

The oregano, laurel, and rosemary essential oils decrease the oxidative process in sunflower oil during accelerated oxidation. Also, these essential oils show higher free-radical scavenging activity (FRSA) than the reference synthetic antioxidant (BHT). The laurel and oregano essentials oils exhibit higher antioxidant activity and phenolic content. Also, the essentials oils prevent the formation of volatile oxidation compounds in sunflower oil during storage, meaning their sensory properties are retained longer. The studied essential oils are also thermolabile compounds that change under high temperature conditions during storage, which could affect their potential antioxidant activity.

Oregano, laurel, and rosemary essential oils are potential antioxidant agents for food. The antioxidant properties of essential oils depend on several factors like the hydrophilic-lipophilic blend in a food product, the storage condition (temperature), the chemical composition of the product, among others. Before adding an essential oil to a food product, it is essential to assay it to determine its real antioxidant capacity in that particular food. In addition, essential oils have a strong flavor that can change some sensory attributes in the finished product.

Acknowledgements

We thank to the Consejo de Investigaciones Cientificas y Tecnicas (CONICET) and Secretaria de Ciencia y Tecnologia de la Universidad Nacional de Cordoba (SECYT-UNC).

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