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

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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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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