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Antioxidant Activity of Fractions from Oregano Essential Oils Obtained by Molecular Distillation

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Running title: Antioxidant fraction from oregano essential oil by SPMD

ABSTRACT The objective of this study was to determine the antioxidant activity of fractions separated from oregano essential oil by short-path molecular distillation. Two residue (R1 and R2) and two distillate (D1 and D2) fractions were prepared by molecular distillation. The major components were: carvacrol, terpinen-4-ol and γ-terpinene in R1 and R2; and γ-terpinene, α-terpineol and sabinene in D1 and D2. Free-radical scavenging activity was observed in all fractions and was highest in R2 (77.2%). D1 and D2 showed a smaller amount of volatile oxidation compounds produced from sunflower oil stored at 60 ºC for 14 days. The greatest antioxidant activity was observed in D1 and D2. The thermal stability of oregano essential oil and its fractions was also analysed. R1 and R2 presented an increased carvacrol concentration and thermal stability. The short-path molecular distillation fractions can be used to prepare fractions from oregano essential oil with a higher antioxidant activity.

Keywords: essentials oils, antioxidants, oregano, distillation.

1. Introduction

Lipid oxidation is one of the main causes of chemical deterioration in food. The food oxidation process produces a rancid flavour and decreases the sensory and nutritional quality of the products making them unacceptable to consumers (Belitz, Grosch & Schielberle, 2009). Lipid oxidation occurs during storage of raw materials and/or the finished products of food, especially those that have been heat treatmed. During lipid oxidation reactions, free radicals are generated in the food matrix. These free radicals are responsible for many health problems including tumours, diabetes and cardiovascular disease (Otaegui-Arrazola, Menéndez-Carreño & Astiasarán, 2010; Fransen, Nordgren, Wang & Apanasets, 2010).

Antioxidant application is the simplest technique for reducing lipid oxidation. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxitoluene (BHT) and propyl gallate (PG), are used in many foods to decrease the oxidation process. However, their safety is questioned because they are presumed to be carcinogenic molecules. Consumers reject the addition of synthetics compounds to food because of the potential consequence of these compounds (Shearn, Fritz & Thompson, 2011).

There are several studies regarding the use of essential oils as natural compounds because they show preservative properties, such as antioxidant and antimicrobial activity (Suhaj 2006; Roginsky & Lissi, 2005; Dobravalskyte D, Venskutonis P. R. & Talou., 2012). Natural antioxidants from food plants have the following advantages: (i) they are accepted by the consumers; (ii) they are considered safe; (iii) they do not need safety tests because they are already in food; (iv) they are from natural resources, which have been used as food for a

long time; (v) they have functional properties; and (vi) they have acceptable sensory properties (Sachetti et al., 2005).

Essential oils, in general, have shown lower antioxidant activity than artificial antioxidants like BHT in food (Olmedo, Nepote, Mestrallet & Grosso, 2008; Olmedo, Asensio, Nepote, Mestrallet, Grosso, 2009; Olmedo, Nepote & Grosso, 2012a and 2012b), even when using the essential oils in higher concentrations than BHT, causing an increase in the flavour intensity in the food products which sometimes negatively affects the consumer acceptability. Oregano essential oil has been studied as an antioxidant in different kinds of food products, such as fried salted peanuts (Olmedo et al., 2008; Olmedo et al., 2009), olive oil (Asensio, Nepote & Grosso 2011; Asensio, Nepote & Grosso 2012), and coated peanuts (Olmedo et al., 2012a). Botsoglou, Govaris, Botsoglou, Grigoropoulou, & Papageorgiou (2003) reported that oregano essential oil increased the oxidative stability of breast and thigh meat during frozen storage. Kulisic, Radonic, Katalinic, & Milos (2004) determined the antioxidant activity of oregano essential oil using three different methods: the beta-carotene bleaching (BCB) test, free radical scavenging activity and the thiobarbituric acid reactive species (TBARS) assay. These authors observed that oregano essential oil showed a greater antioxidant activity with respect to the control sample. In addition, Quiroga, Riveros, Zygadlo, Grosso, & Nepote (2011) reported that different chemical compositions of oregano essential oils produced different antioxidant activity. In that research, the essential oil of the oregano varieties Cordobes and Compacto show a higher thymol content and higher radical scavenging activity and antioxidant activity when added in canola oil. For that reason, increasing the concentration of a particular molecule with high antioxidant potential in a fraction obtained from the essential oil should

increase the antioxidant power in that fraction compared with the antioxidant activity of the essential oil.

Short-path molecular distillation (SPMD) is a new technique for producing the concentration of particular compounds, on the basis of their boiling points. Molecular distillation is an appropriate method for the separation and purification of thermally unstable materials with low vapour pressures and high molecular weight, but without the danger of thermal decomposition (Shao, He, Sun & Jiang, 2009). This method is characterised by a short exposure of the liquid to be distilled to an elevated temperature and high vacuum in the distillation space. Therefore, the molecular distillation should not alter the essential oil components due to the low pressures and low dwell times (Cvengros, Mikov & Lutisan, 2000; Shao, Jiang & Ying 2007). SPMD application is very useful in the petroleum and oil areas for obtaining fractions enriched in particular compounds (Cermak, John & Evangelista, 2007; Shao et al., 2009; Kahveci & Xu, 2011). This method has been used in essential oil separation but the fractions obtained have not been studied as potential antioxidants in food (Tovar, Maciel, Pinto, Filho & Gomes, 2010; Martins, Carmona, Martinez, Sbaite, Filho & Maciel, 2012).

The objective of this study was to evaluate the antioxidant activity of fractions separated from oregano essential oil by short-path molecular distillation.

2. Materials and Methods

2.1. *Materials and extraction of the essential oil*

Leaves of oregano (*Origanum vulgare* L) were collected in April 2011 from the experimental station of the Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina. The essential oils were extracted for 1 hour in a Clevenger-type apparatus, dried with anhydrous sodium sulphate and kept in dark glass flasks at -18 ºC until molecular distillation processing.

2.2 *Fractions obtained by Short Path Molecular Distillation*

The oregano essential oil was distilled under 2 different conditions with a short path molecular distillation (SPMD) apparatus. The essential oil was put in the reception chamber (capacity, 400 ml) and the different fractions were obtained by modifying the pressure, while temperature and flow were kept constant. The first distillation was at 73 mbar, 25 ºC and 1.18 ml/min. After this first distillation, two fractions called Distillate 1 (D1) and Residue 1 (R1) were obtained. In the second distillation, R1 was distilled at 75 mbar, 25 ºC and 1.18 ml/min to obtain two new fractions called Distillate 2 (D2) and Residue 2 (R2).

2.3 C*hemical composition analysis of the essential oil and its fractions*

The composition of the oregano essential oil and its fractions obtained by SPMD were determined by gas chromatography coupled with mass spectrometry (GC-MS) using a chromatograph Perkin Elmer Clarus 600 (Palo Alto, Ca, USA). The samples were analysed using a non-polar column DB-5 (30 m x 0.25 μm). The chromatographic conditions were 40 °C initial temperature for 3 minutes, 10 °C/min for rate 1 until to 100 °C, 15 ºC/min for rate 2 until 245ºC. The injector temperature was 250 °C. The carrier gas (helium) had a flow rate of 0.9 ml/min. Ionisation was obtained by electron impact at 70 eV and

mass spectral data were acquired in the scan mode in the m/z range 35-450. The compounds were identified by their retention times (Adam, 1995), mass spectra from NIST libraries, and by co-injection authentic standards of the main components (SIGMA, St Louis, MO, USA) (Olmedo et al, 2012b). The quantification of each peak was performed by the mass reported by the mass detector. The results were expressed as a percentage (w/w).

2.4 F*ree-radical scavenging activity (test DPPH-FRSA) and total phenolic content*

The radical-scavenging activity was determined according to the method by Choi, Song, Ukeda & Sawamura (2000) using 2,2-diphenyl-1-picrylhydrazyl (DPPH). A UV-Vis diode array spectrophotometer Hewlett Packard HP 8452 A (Palo Alto, CA, USA) was used to measure the absorbance of the solutions. The activity was expressed as a percentage of DPPH inhibition (PI) using the following equation:

$PI = [(A_c - A_a)/A_c] \times 100$

where A_c and A_a were the absorbance measured at 517 nm after 30 minutes for the control and essential oil samples, respectively.

The phenol content was determined using Folin-Cicolteau reagent and gallic acid as a standard (SIGMA, St Louis, MO, USA). An aliquot of the sample (oregano essential oil and its distilled fractions) was added to 0.5 ml deionised water with 5% w/w Tween 20. This solution was mixed with 8 ml distillate water and 0.5 ml of Folin-Cicolteau Reagent. The phenol content was measured at 760 nm in a spectrophotometer after 60 min. The phenol content was expressed as mg/g of the sample (Dambolena et al., 2010).

147

148 *2.5 Accelerated oxidation test*

149 Refined sunflower oil (Natura, Aceitera General Deheza SA, General
150 Deheza, Córdoba, Argentina) was used for an oven test experiment. The
151 control and sunflower samples added to 0.02% oregano essential oil or the
152 distilled fractions of oregano essential oil were stored in an oven at 60 ºC
153 (Proestos, Boziaris, Nychas & Komaitis 2006). The samples prepared were: C =
154 100% refined sunflower oil; O = refined sunflower oil with oregano essential oil;
155 R1 = refined sunflower oil with Residue 1; R2 = refined sunflower oil with
156 Residue 2; D1 = refined sunflower oil with Distillate 1; and D2 = refined
157 sunflower oil with Distillate 2. Ten g of the samples were stored for 14 days in
158 an opened assay tube and removed from storage at days 0, 4, 7, 11 and 14.
159 The following chemical indicators of lipid oxidation were evaluated in the
160 samples: peroxide value (PV), anisidina value (AV) and conjugated dienes
161 (DC). PV was expressed as miliequivalents of active oxygen per kilogram of oil
162 (meq O_2 kg^{-1}) (AOAC, 1980); the anisidine value (AV) was evaluated following
163 the IUPAC method (IUPAC 1987) by reading the absorbance of samples at 350
164 nm; and conjugated dienes were determined according to the COI method, by
165 reading the absorbance at 232 nm (COI 2001). Also were determinate Totox
166 Value (TV) as 2PV+AV.

167

168 *2.6 Volatile oxidation compounds*

169 In addition, volatile oxidation compounds were studied to analyse the
170 antioxidant effect of the addition of distilled fraction in refined sunflower oil. For
171 this experiment, 10 g sunflower oil was put into a vial (capacity 50 ml). The

172 treatments were the same as that used in the test oven experiment. The lids
173 were put on the bottles, sealed and stored in oven at 60 ºC for 14 days.
174 Samples were removed from oven at day 0, 7 and 14. The volatile compounds
175 were analysed by GC-MS. Hexanal, heptanal, 2-heptenal and octanal were
176 identified and quantified. The volatiles compounds were captured using a solid
177 phase microextractor fibre (SPME), PDMS/DVB (Supelco, SIGMA, St Louis,
178 MO, USA). The PDMS/DVB was used because it showed good reproducibility
179 and linearity of response for the volatile compounds released by lipid oxidation
180 reactions (Brunton et al., 2000). The fibre was introduced into the vial that was
181 heated for 20 min at 130 ºC without stirring. The volatiles captured on the fibre
182 were analysed by CG-MS using a chromatograph Perkin Elmer Clarus 600
183 (Palo Alto, Ca, USA). The samples were separated in a non-polar column DB-5
184 (30 m). The chromatographic conditions were 40 °C initial temperature for 3
185 minutes, 10 °C/min for rate 1 until to 100°C, 15 ºC/min for rate 2 until 245 ºC.
186 The injector temperature was 250 °C. The carrier gas (helium) had a flow rate
187 of 0.9 ml/min. Ionisation was obtained by electron impact at 70 eV and mass
188 spectral data were acquired in the scan mode in the m/z range of 35-450. The
189 identification procedure was the same as described for the essential oil
190 composition study (section 2.3). To help the identification of the main
191 components, a co-injection of hexanal, heptanal and octanal authentic
192 standards (SIGMA, St Louis, MO, USA) were also made. Acetaldehyde
193 (SIGMA, St Louis, MO, USA) was used as an internal standard for all samples.
194 The concentration of the component was expressed as ppm (mg/l).
195
196 *2.7 Thermal stability of the fractions obtained from essential oils*

For determination of the thermal stability determination of the fractions separated by SPMD, 10 µl fractions were put into a vial (capacity 10 ml). The bottles were sealed and stored in oven at 60ºC during 14 days. Samples were removed from the oven at days 0, 7 and 14. For chemical composition analysis, a fibre SPME PDMS/DVB was placed into the vial at 70 ºC for 20 min. The volatile components captured by the fibre were determinate by GC-MS following the same procedure as described in section 2.3.

2.8 Statistical analysis

All experiments were carried out with three replications. The data were analysed using the software Infostat, version 1.1 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba). Means and standard deviations were calculated. Analysis of variance and the LSD Fisher test (α = 0.05) were used to detect significant differences between treatments. Regression equations were used to determine if the independent variables (time) had an effect on the oxidative indicators (PV, AV, CD and TV). Two principal component analyses (PCA) (Johnson and Wichern, 1998) were performed. In one PCA, the correlation matrix of the standardised (normalised) data from the chemical analysis of the oregano essential oils and fractions was used to find associations between chemical components from the oregano essential oil and chemical composition of the oregano essential oil fraction prepared by SPMD. In the other PCA, the correlation matrix of the standardized (normalized) data from chemical analysis of the essential oils and fractions that suffered thermal deterioration during storage at 60 ºC were used to find associations between chemical components from the oregano essential oil and the thermal stability of the components from the fractions prepared by SPMD.

3. Results and discussion

3.1. Chemical composition of oregano essential oil and its fractions

The chemical compositions of the studied oregano essential oil and its fractions obtained by short-path molecular distillation are shown in the Table 1. The four major components were γ-terpinene, terpineol-4-ol, carvacrol and α-terpinene for oregano essential oil, representing 66.6% of the total composition. For fraction Residue 1: γ-terpinene, terpinen-4-ol, carvacrol, and α-phellandrene, representing 67.5% of the total composition. For fraction Residue 2: carvacrol, terpinen-4-ol, γ-terpinene, and linalool, representing 76.8% of the total composition. For fraction Distillate 1: γ-terpinene, α-terpinene, sabinene, and β-phellandrene, representing 67.45% of the total composition; and for fraction Distillate 2: γ-terpinene, α-terpinene, sabinene, and β-phellandrene, representing 55.3% of the total composition. The oregano essential oil may show variation in its composition between studies. For example, Kulisic, Radonic, Katalinic, & Milos (2004) reported 35.0% thymol, 32.0% carvacrol and 10.5% γ-terpineno; while Tomaino et al. (2005) observed 48.9% carvacrol, 11.7% ρ-cimeno and 5.03% thymol. Moreover, Suhaj (2006) detected important variations in the concentration of carvacrol (0 - 12 ppm), γ-terpineno (0–13 ppm), linalyl-acetate (0–50 ppm), myrcene (0–50 ppm) and terpinen-4-ol (0–220 ppm) in the composition of oregano essential oil.

The studied oregano essential oil exhibited components with functional groups and hydrocarbons. In the residue fractions (R1 and R2), there was an increase in terpenes with an alcohol function such as carvacrol and terpinen-4-ol. These two components were less in the distillate fractions (D1 and D2), but

terpenenes such as γ-terpinene and α-terpinene increased. Differences in the component proportions occur in the fractions due to the molecular distillation process increasing compounds with low boiling points (e.g. monoterpenes) in the distillate fractions (D1 and D2), and compounds with high boiling points (such as monoterpenes with functional groups and sesquiterpenes) in residue fractions (R1 and R2). The point of separation between the residue and distillate fractions was around γ-terpinene with a boiling point at 181-182 ºC. Another difference between the distillate and residue fractions was that the residue fractions were more concentrated in a few components. The major four components represented 67.5% and 76.8% for R1 and R2, respectively, and 67.5% and 55.3% for D1 and D2, respectively.

The short path molecular distillation allowed for a different proportion of molecules of oregano essential oil in different fractions (D1, D2, R1, and R2) to be concentrated. Differences in the composition between fractions is based on the differences between boiling points of the molecules that constitute the essential oil. In this condition, a greater proportion of low boiling point compounds will be in the distilled fraction (D) with respect to the residue fraction (R). Particularly in the oregano essential oil, the cut-off point between the distilled-residue fractions was for terpenes with a boiling point between 180-200 °C. In the present study, the difference observed between fractions D1 and D2 was because D2 was distilled from the fraction R1, whose composition had less low boiling point terpenes due to these compounds being separated, mainly, in the fraction D1. Higher vacuum pressure was performed on R1 for the second distillation step that distilled greater proportion of high boiling point compounds to the distilled fraction D2. For that reason, it was a greater linalool, terpineno-4-ol, α-and terpineol concentration detected in D2. In D2, an increased amount of

terpenes with a boiling point higher than 201 °C, such as cis sabinene hydrate, was detected. The terpenes with a lower boiling point showed uneven behaviour and their distribution in the fractions responded an equilibrium between terpenes proportion in the sample used for distillation and combination of pressure-temperature used in the distillation process for each distillation step.

3.2 Determination of free-radical scavenging activity (test DPPH-FRSA) and total phenolic content

The free-radical scavenging activity (FRSA) of essential oils has been confirmed in different studies. Aromatic plants such as oregano, rosemary, laurel and thymus, among others, have shown FRSA (Sachetti et al., 2005, kulisic et al., 2004). The FRSA results from this study are presented in Table 1. Oregano essential oil showed the highest FRSA (82%). The residue fractions (R1 and R2) had a greater FRSA than the distillates. All samples showed a significant difference. The FRSA is related to the ability of molecules to hydrogen-bond-donate to another molecule. Phenols are known for this ability. R2 and D2 had a greater FRSA than R1 and D1, respectively. This higher activity could be explained, in part, because R2 and D2 had a higher phenol content than R1 and D1. However, the oregano essential oil showed a lower phenol content but higher FRSA than R2. Tomaino et al. (2005) reported 51.8% FRSA for oregano essential oils, but Kosar, Dorman & Hiltunen (2005) observed a lower value (33% FRSA). The FRSA reported by other authors are lower than the observed values in the present study for oregano essential oils and fractions.

The total phenol contents (TPC) for the oregano essential oils and fractions are shown in Table 1. R2 had the highest TPC in comparison with the other samples. Oregano and R1 did not exhibit a significance difference in the TPC. Carvacrol is a phenolic structure, and its percentage influences the TPC results. Dambolena et al. (2010) found values of 18.09 mg/g FRSA and 34.9% TPC in oregano essential oil from the La Pampa locality; and 17.46 mg/g TPC and 74.5% FRSA in oregano from the La Consulta locality. These parameters (FRSA and TPC) are indirect indicators that oregano essential oil and fractions could have a good antioxidant activity.

3.3 Accelerated oxidation test: chemical oxidation indicators

All chemical oxidation indicators increased in the refined sunflower oil samples during storage at 60 ºC. These indicators are shown in Figure 1. As a consequence of the accelerated oxidation condition (60 °C), high peroxide values were detected in the sunflower oil samples (O´Brien RD, 2009). The control samples (C) exhibited the highest peroxide value with respect to samples with the addition of oregano essential oil or its fractions obtained by SPMD. After 4 days of storage, significant differences were observed between the samples. At day 14, R1 and R2 did not show any significant difference between them, but they had a difference with respect to the distillate fractions (D1 and D2), which exhibited the lowest PV. All fractions obtained from oregano essential oil presented lower peroxide values than the whole oregano essential oils during storage.

The anisidine values observed in all samples showed a similar tendency with respect to the PV behaviour during storage at 60 ºC. After day 4, significant differences were detected between treatments. At 14 days of storage, the

control sample (C) had the highest AV followed by oregano essential oil treatment. The D2 fraction exhibited the lowest AV.

The CD observed in all samples also showed a similar behaviour, with respect to PV, during storage. At day 4, only the control sample (C) presented a significant difference with respect to the other treatments. At storage day 14, significant differences were observed between the samples. The oregano essential oil treatment showed greater CD values than the fractions obtained by SPDM (R1, R2, D1, and D2).

Therefore, the PV, AV and CD results in the sunflower oil samples during storage demonstrated that the oregano fraction treatments (R1, R2, D1, and D2) have better antioxidant effect than the oregano essential oil when added to sunflower oil. Quiroga et al. (2011) found lower oxidation indicators (PV and AV) in canola oil samples supplemented with essential oil of oregano, with respect to the control sample. These authors observed that PVs were 33 meqO_2/Kg in canola oil with oregano treatment after 12 days of storage (60 ºC storage temperature), and 43 meqO_2/Kg in the control sample.

Totox values showed the greatest values in the Control sample (C) followed by the sample with oregano essential oil (O). The residue samples (R1 and R2) exhibited higher values than distillate fractions (D1 and D2). Distillate samples D1 and D2 did not present significant differences.

The peroxide, anisidine, conjugated dienes and totox values obtained during this storage study were used to calculate the slope in lineal regression analysis where time was the independent variable (Table 2). Adjusted coefficients of determination (R^2) for the dependent variables were greater than 0.64 in all cases; therefore, it may be considered that these variables are a good indicator of the time effect. D1 and D2 exhibited a lower slope without significant

differences between them. The slopes of R1, R2, D1, and D2 did not show any significant difference in AV and CD.

3.4 Lipid oxidation in sunflower oil analysed by volatile compound formation

The volatile compounds produced by the lipid oxidation process in sunflower oil are presented in Figure 2. Hexanal, heptanal, 2-heptenal and octanal increased over 14 days of storage at 60 ºC. These type of components were produced by an advanced lipid oxidation, and they are responsible, in part, for the rancid "off flavour" in food (Grosso, Resurreccion, Walker & Chinnan, 2007). The sunflower oil treatments with addition of the fractions obtained by SPMD presented a lower number of volatiles compounds, with respect to the oregano essential oil and the control sample. At storage day 14, hexanal and 2-heptenal showed a significance difference between all fractions. The D1 and D2 treatments exhibited lower amounts of volatile compound than the R1 and R2 treatments. The heptanal and octanal contents did not show a significance difference between the fractions, with the exception of R2, where the heptanal content was higher. Boroski et al. (2012) used oregano essential oil (OEO) and oregano extract (OE) in a dairy functional beverage and measured the generation of hexanal and propanal by CG-MS after pasteurisation. Adding OE and OEO to the dairy beverage reduced propanal and hexanal formation during storage. These authors estimated the inhibition of aldehyde formation with respect to the control, and expressed it as the percentage of inhibition. In that study, OEO showed the highest percentage inhibition (0.1%) followed by OE (0.01%). Oregano essential oil in a concentration higher than 0.02% in food gives a strong odour of oregano and as a result, in some cases, a lower acceptability by consumers (Olmedo et al., 2008). This study showed that the

fractions obtained by SPMD had a greater antioxidant effect with respect to oregano essential oil, and so it should allow a decreased amount of antioxidant additive added to a particular food product.

3.5 Thermal stability of the SPMD fractions obtained from oregano essential oils

The thermal stability of the oregano essential oil (OEO) fractions obtained by SPMD was evaluated using degradation by exposing them to a heating process at 60 ºC for 14 days. Changes in the composition of the oregano essential oil and SPMD fractions were analysed by GC-MS capturing volatile compounds using a SPME. Relative percentages of sample composition only on storage days 0 and 14 are shown in the Table 3. The chemical composition of oregano essential oil and SPMD fractions changed in all samples over storage at 60 ºC. These changes were different between fractions and oregano essential oil: oregano essential oil decreased in α-terpinene, γ-terpinene, linalool, and terpinen-4-ol, but increased in σ-cymene and carvacrol; R1 and R2 showed similar changes to oregano essential oil during storage; D1 and D2 decreased in sabine, linalool and carvacrol, but D2 increased in σ-cymene and terpinen-4-ol. There were no correlations between samples because the concentration of carvacrol in oregano essential oil, R1 and R2 increased during storage, but the concentration of this component decreased in D1 and D2.

Associations between chemical components and samples (OEO, R1, R2, D1, and D2) at different storage days are shown in a biplot obtained by PCA (Fig. 3). In the PCA, OEO, R1 and R2 showed a similar association, and they were placed on the left side of the PC 1 with carvacrol. D1 and D2 were placed on the right side of PC 1 and had higher correlation with terpene molecules. Bazemore, Rouseff & Naim (2003) found that terpene-like linalool did not show

403 a difference in the concentration in orange juice before and after heat treatment.
404 Yang, Kayan, Bozer, Pate, Baker & Gizir (2007) researched the stability of five
405 essential oil components (α-pinene, limonene, camphor, citronellol, and
406 carvacrol) exposed to different temperatures (100, 150, 200 and 250 ºC) and
407 time (30 and 300 minutes). Some of these components may also be found in
408 basil and oregano essential oils. These authors observed a relationship
409 between temperature increase and degradation of the terpenes: α-pinene
410 presented the greatest deterioration, from 25% at 100 ºC to 64% at 250 ºC,
411 followed by camphor from 4% at 100 ºC to 27% at 250 ºC, and carvacrol from
412 7% at 100 ºC to 35% at 250 ºC. In the present study, carvacrol and camphor
413 exhibited higher thermal resistance than α-pinene. This thermal resistance can
414 be explained, in part, by a relationship with the boiling point of these chemical
415 compounds, which are 150, 205, and 237.7 ºC for α-pinene, camphor and
416 carvacrol, respectively. α-Pinene has a low boiling point and it showed higher
417 thermal degradation. However, carvacrol has a higher boiling point than
418 camphor, but showed higher thermal deterioration.
419

420 **4. CONCLUSION**

421 Oregano essential oil and its fractions inhibit the lipid oxidation process in
422 sunflower oil. The fractions obtained by short-path molecular distillation showed
423 greater antioxidant activity than the whole oregano essential oil, whereas the
424 distillate fractions showed better antioxidant properties than the residue
425 fractions due to the difference in their compositions. The distillate fractions (D1
426 and D2) from oregano essential oil had a higher concentration of terpenes, such
427 as α-terpinene and γ-terpinene with low boiling points and residues fractions

(R1 and R2) showed a greater concentration of terpenes with an alcohol function with a higher boiling point, such as terpinen-4-ol and carvacrol. Fractions of oregano essential oil rich in terpenes with low boiling point and without a functional group present high antioxidant property. These kind of fractions obtained from oregano essential oil by short path molecular distillation can be used as natural antioxidants for food products sensitive to lipid oxidation even in organic food.

Short-path molecular distillation constitutes an alternative to separate fractions from essential oils with higher antioxidant power that may be used for producing natural antioxidant agents.

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Figure 1. Peroxide value (PV), anisidinine value (AV), conjugated dienes (CD) and Totox value of sunflower oil with added oregano essential oil and distilled fractions during storage at 60 ºC.

Footnote of figure 1:

Abbreviations: C = sunflower oil control sample; O = sunflower oil with oregano essential oil; R1 = sunflower oil with fraction R1; R2 = sunflower oil with fraction R2; D1 = sunflower oil with fraction D1; D2 = sunflower oil with fraction D2.

Figure 2. Volatile composition used as indicators of lipid oxidation evaluated in sunflower oil (C) and sunflower oil with added oregano essential oils and fractions during storage at 60ºC.

Footnote of figure 2:

Abbreviations: C = sunflower oil control sample; O = sunflower oil with oregano essential oil; R1 = sunflower oil with fraction R1; R2 = sunflower oil with fraction R2; D1 = sunflower oil with fraction D1; D2 = sunflower oil with fraction D2.

Figure 3. Biplots of the 1st and 2nd principal components of PCA. Variables: chemical components of oregano essential oils, and oregano essential oil and fraction obtained by SPMD at different storage days (storage days 0, 7 and 14).

Footnote of figure 3:

Abbreviations: C = sunflower oil control sample; O = sunflower oil with oregano essential oil; R1 = sunflower oil with fraction R1; R2 = sunflower oil with fraction R2; D1 = sunflower oil with fraction D1; D2 = sunflower oil with fraction D2.

Table 1. Chemical Composition expressed as percentage (w/w) of terpenes separated by GC-MS, total phenolic content, and free-radical scavenging activity from oregano essential oil and its distilled fractions.

	Oregano essential oil and fractions				
	Oregano[1]	**Residue 1[1]**	**Residue 2[1]**	**Distillate 1[1]**	**Distillate 2[1]**
Essential oil component (Relative percentage)					
α-thujene	1.26 ± 0.14^{b}	1.10 ± 0.09^{b}	0.49 ± 0.13^{a}	2.82 ± 0.24^{d}	2.26 ± 0.19^{c}
α-pinene	1.09 ± 0.11^{b}	1.01 ± 0.14^{b}	0.39 ± 0.11^{a}	2.68 ± 0.17^{d}	2.12 ± 0.13^{c}
Camphene	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.0^{a}	0.20 ± 0.08^{b}	0.24 ± 0.10^{b}
Sabinene	4.62 ± 0.21^{b}	4.36 ± 0.20^{b}	2.55 ± 0.21^{a}	9.32 ± 0.11^{d}	7.03 ± 0.21^{c}
β-pinene	0.44 ± 0.14^{b}	1.65 ± 0.16^{d}	0.00 ± 0.00^{a}	1.05 ± 0.14^{c}	1.19 ± 0.12^{c}
β-myrcene	1.78 ± 0.19^{b}	0.63 ± 0.14^{a}	0.89 ± 0.18^{a}	3.67 ± 0.19^{c}	4.24 ± 0.25^{d}
α-phellandrene	0.79 ± 0.10^{b}	7.96 ± 0.23^{e}	0.38 ± 0.12^{a}	1.33 ± 0.15^{c}	1.66 ± 0.17^{d}
α-terpinene	8.54 ± 0.19^{c}	2.66 ± 0.14^{a}	5.20 ± 0.23^{b}	15.1 ± 0.22^{e}	11.8 ± 0.24^{d}
σ-cymene	2.65 ± 0.17^{b}	3.93 ± 0.22^{c}	1.77 ± 0.14^{a}	6.64 ± 0.28^{d}	6.34 ± 0.21^{d}
β-phellandrene	3.62 ± 0.22^{c}	0.00 ± 0.00^{a}	2.63 ± 0.18^{b}	6.68 ± 0.19^{d}	6.96 ± 0.27^{d}
γ-terpinene	25.1 ± 0.34^{b}	24.8 ± 0.39^{b}	19.1 ± 0.29^{a}	36.4 ± 0.31^{d}	29.7 ± 0.36^{c}
Cis sabinene hydrate	0.90 ± 0.10^{b}	0.81 ± 0.10^{b}	0.82 ± 0.22^{b}	0.42 ± 0.12^{a}	1.26 ± 0.13^{c}
Terpinolene	2.13 ± 0.14^{c}	1.84 ± 0.14^{b}	1.34 ± 0.13^{a}	2.97 ± 0.18^{d}	3.66 ± 0.17^{e}
Linalool	7.44 ± 0.25^{c}	8.84 ± 0.19^{d}	11.0 ± 0.18^{e}	2.91 ± 0.16^{a}	6.44 ± 0.23^{b}
Camphor	0.39 ± 0.12^{ab}	0.52 ± 0.12^{b}	0.55 ± 0.11^{b}	0.25 ± 0.11^{a}	1.05 ± 0.15^{c}
Terpinen-4-ol	16.7 ± 0.18^{c}	18.0 ± 0.23^{d}	23.2 ± 0.31^{e}	5.31 ± 0.17^{a}	5.94 ± 0.18^{b}
α-terpineol	2.09 ± 0.15^{c}	2.18 ± 0.18^{c}	2.79 ± 0.22^{d}	0.38 ± 0.10^{a}	1.39 ± 0.11^{b}
Thymol methyl ether	1.87 ± 0.11^{c}	1.61 ± 0.13^{b}	1.68 ± 0.14^{bc}	0.82 ± 0.13^{a}	2.24 ± 0.14^{d}
Carvacrol	16.2 ± 0.17^{c}	16.8 ± 0.19^{d}	23.5 ± 0.17^{e}	0.76 ± 0.11^{a}	3.06 ± 0.18^{b}
β-caryophyllene	0.83 ± 0.17^{c}	0.61 ± 0.12^{bc}	0.74 ± 0.12^{bc}	0.11 ± 0.05^{a}	0.51 ± 0.12^{b}
Germancrene D	1.03 ± 0.11^{d}	0.72 ± 0.18^{c}	0.88 ± 0.15^{cd}	0.05 ± 0.02^{a}	0.34 ± 0.11^{b}
Total composition	99.5	99.9	99.9	99.8	99.5
Phenol Content[2] (mg/g)	11.1 ± 0.06c	11.1 ± 0.09c	13.1 ± 0.07d	2.36 ± 0.11a	6.17 ± 0.07b
FRSA[3] Percentage	84.1 ± 1.14e	74.0 ± 0.18c	77.2 ± 0.61d	54.6 ± 0.79a	64.4 ± 0.61b

[1] Values with different letter in the same row are significantly different (n = 3, LSD Fisher, α = 0.05.
[2] Phenol Content expressed as mg/g on dry weight.
[3] FRSA%: expressed as percentage of inhibition.

Table 2. Regression coefficients and adjusted R^2 for the dependent variables: peroxide (PV), p-anisidine (AV) values, conjugated dienes (CD) and Totox value (TV) of sunflower oil samples evaluated during storage.

Dependent variable	samples	Regression[a]			
		β_0	β_1[b]	ANOVA	R^2
CD	C	3.3861	0.5936	C	0.96
	O	3.2694	0.3934	B	0.94
	D1	3.4683	0.3219	A	0.95
	D2	3.4539	0.3064	A	0.83
	R1	3.5528	0.3200	A	0.96
	R2	3.2896	0.3678	B	0.94
AV	C	6.3155	0.0512	C	0.82
	O	6.2954	0.0351	B	0.64
	D1	6.2882	0.0285	AB	0.71
	D2	6.3140	0.0220	A	0.74
	R1	6.3856	0.0270	A	0.82
	R2	6.3075	0.0217	A	0.83
PV	C	-0.8202	6.3036	D	0.96
	O	2.7504	3.8371	C	0.96
	D1	2.2212	2.7173	A	0.96
	D2	1.8886	2.7979	A	0.95
	R1	1.9531	3.1892	B	0.90
	R2	0.3130	3.2849	B	0.92
TV	C	4.6736	12.6584	D	0.96
	O	11.7945	7.7131	C	0.96
	D1	10.2919	6.4053	A	0.90
	D2	6.8938	6.6025	A	0.92
	R1	10.7305	5.4631	B	0.96
	R2	10.0911	5.6178	B	0.95

Samples: sunflower oil. C = sunflower oil control sample; O = sunflower oil with oregano essential oil; D1 = sunflower oil with distillated fraction 1; D2 = sunflower oil with distillated fraction 2; R1 = sunflower oil with residue fraction 1; and R2 = sunflower oil with residue fraction 2.

[a] Regression equations: $Y = \beta_0 + \beta_1 X$; where Y = dependent variable (PV, AV, CD, and TV; β_0 = a constant that it is equal the value of Y when the value of X = 0; β_1 = coefficients of X; X = independent variable (time); R^2 = adjusted determination coefficient. For all regression equations, the p value was <0.0001.

[b] ANOVA and LSD Fisher test: The slope (β_1) of each variable and sample followed with different letters in the same column are significantly different at $\alpha = 0.05$.

Table 3. Chemical composition expressed as relative percentage of oregano essential oil and fraction obtained by SPMD from samples of thermal stability test during 14 days of storage at 60ºC.

	Orégano[1]		Residue 1[1]		Residue 2[1]		Distillate 1[1]		Distillate 2[1]	
Compounds	**0**	**14**	**0**	**14**	**0**	**14**	**0**	**14**	**0**	**14**
α-tujene	0.66±0.21	0.59±0.21	0.51±0.17	0.34±0.12	0.36±0.18^{b}	0.00±0.00^{a}	1.58±0.19^{a}	1.93±0.15^{b}	1.25±0.21	1.58±0.14
α-pinene	0.57±0.17	0.55±0.17	0.49±0.12	0.40±0.19	0.34±0.19^{b}	0.00±0.00^{a}	1.48±0.22^{a}	2.06±0.21^{b}	1.19±0.20	1.56±0.19
sabinene	2.57±0.19^{b}	1.07±0.18^{a}	1.92±0.18^{b}	0.69±0.14^{a}	1.51±0.12^{b}	0.40±0.11^{a}	5.18±0.23^{b}	3.56±0.24^{a}	4.66±0.16^{b}	3.69±0.24^{a}
β-pinene	0.41±0.22	0.34±0.14	0.35±0.21	0.35±0.15	0.28±0.14^{b}	0.00±0.00^{a}	0.81±0.21	1.15±0.14	0.74±0.12	0.95±0.15
beta Myrcene	1.23±0.34	1.33±0.19	1.07±0.20	0.82±0.19	0.80±0.12^{b}	0.47±0.13^{a}	2.96±0.19^{a}	3.54±0.19^{b}	2.61±0.18^{a}	3.35±0.22^{b}
α-phellandrene	0.45±0.28	0.42±0.12	0.34±0.18^{b}	0.00±0.00^{a}	0.29±0.11^{b}	0.00±0.00^{a}	0.73±0.22	0.59±0.17	0.72±0.14	0.80±0.13
α-terpinene	4.27±0.11^{b}	2.47±0.19^{a}	2.99±0.11^{b}	1.71±0.17^{a}	2.59±0.19^{b}	1.09±0.14^{a}	6.87±0.17^{b}	5.11±0.23^{a}	7.05±0.26	6.78±0.17
σ-cymene	1.94±0.17^{a}	10.4±0.11^{b}	3.07±0.19^{a}	10.6±0.31^{b}	3.23±0.19^{a}	6.79±0.21^{b}	6.60±0.13^{a}	22.8±0.34^{b}	4.50±0.20^{a}	17.1±0.28^{b}
β-phellandrene	2.36±0.19	2.22±0.16	2.82±0.16^{b}	2.15±0.21^{a}	2.49±0.17^{b}	1.59±0.19^{a}	5.33±0.13^{a}	6.95±0.27^{b}	4.96±0.18^{a}	6.74±0.19^{b}
γ-terpinene	10.4±0.11^{b}	3.29±0.24^{a}	6.54±0.29^{b}	1.39±0.17^{a}	6.30±0.21^{b}	0.66±0.17^{a}	14.9±0.22^{b}	14.3±0.24^{a}	16.1±0.32^{a}	18.0±0.39^{b}
cis sabinene hydrate	1.52±0.22^{b}	0.79±0.12^{a}	1.95±0.18^{b}	0.77±0.21^{a}	1.69±0.24^{b}	0.80±0.20^{a}	1.50±0.17^{b}	0.98±0.15^{a}	1.45±0.26^{b}	0.59±0.20^{a}
terpinolene	1.06±0.14	0.96±0.18	0.95±0.12^{b}	0.73±0.14^{a}	0.96±0.19^{b}	0.46±0.20^{a}	1.79±0.17^{a}	2.17±0.19^{b}	1.88±0.17	2.02±0.11
linalool	13.1±0.11^{b}	7.45±0.26^{a}	16.4±0.31^{b}	8.62±0.19^{a}	15.8±0.17^{b}	8.91±0.17^{a}	11.8±0.21^{b}	4.84±0.27^{a}	12.1±0.28^{b}	6.44±0.24^{a}
camphor	1.82±0.19^{b}	0.81±0.13^{a}	2.67±0.24^{b}	1.29±0.17^{a}	2.43±0.21^{b}	1.47±0.17^{a}	0.61±0.17	0.87±0.16	0.75±0.19	0.91±0.15
terpinen-4-ol	29.5±0.17^{b}	22.6±0.13^{a}	2.67±0.25^{a}	25.4±0.15^{b}	26.5±0.32	26.1±0.38	14.8±0.14	14.9±0.21	14.7±0.28^{a}	19.9±0.36^{b}
α-terpineol	5.23±0.21^{b}	3.37±0.18^{a}	5.45±0.16^{b}	4.69±0.19^{a}	5.20±0.27	5.57±0.20	2.87±0.19^{b}	2.39±0.17^{a}	2.69±0.17^{b}	2.03±0.17^{a}
thymol metil ether	1.77±0.21^{b}	1.18±0.17^{a}	1.92±0.19^{b}	0.89±0.14^{a}	2.01±0.22^{b}	0.69±0.17^{a}	0.83±0.14^{a}	1.38±0.14^{b}	0.89±0.13	1.06±0.12
carvacrol	20.4±0.25^{a}	39.2±0.12^{b}	22.1±0.24^{a}	38.6±0.31^{b}	20.4±0.29^{a}	43.9±0.24^{b}	18.3±0.17^{b}	6.88±0.19^{a}	19.4±0.31^{b}	5.18±0.28^{a}
β-carophyllene	0.47±0.14	0.60±0.16	3.43±0.19^{b}	0.62±0.18^{a}	3.34±0.28^{b}	0.67±0.17^{a}	0.45±0.14^{a}	2.62±0.24^{b}	0.89±0.18	0.74±0.11
Germancrene D	0.25±0.12	0.46±0.17	0.44±0.12	0.45±0.17	3.56±0.21^{b}	0.49±0.13^{a}	0.36±0.10^{a}	0.87±0.11^{b}	1.45±0.15^{b}	0.60±0.17^{a}

[1] Values with different letters in the same row are significantly different (LSD Fisher; α = 0.05, n = 3). Values without letter do not show significant differences between samples.

Figure 1.

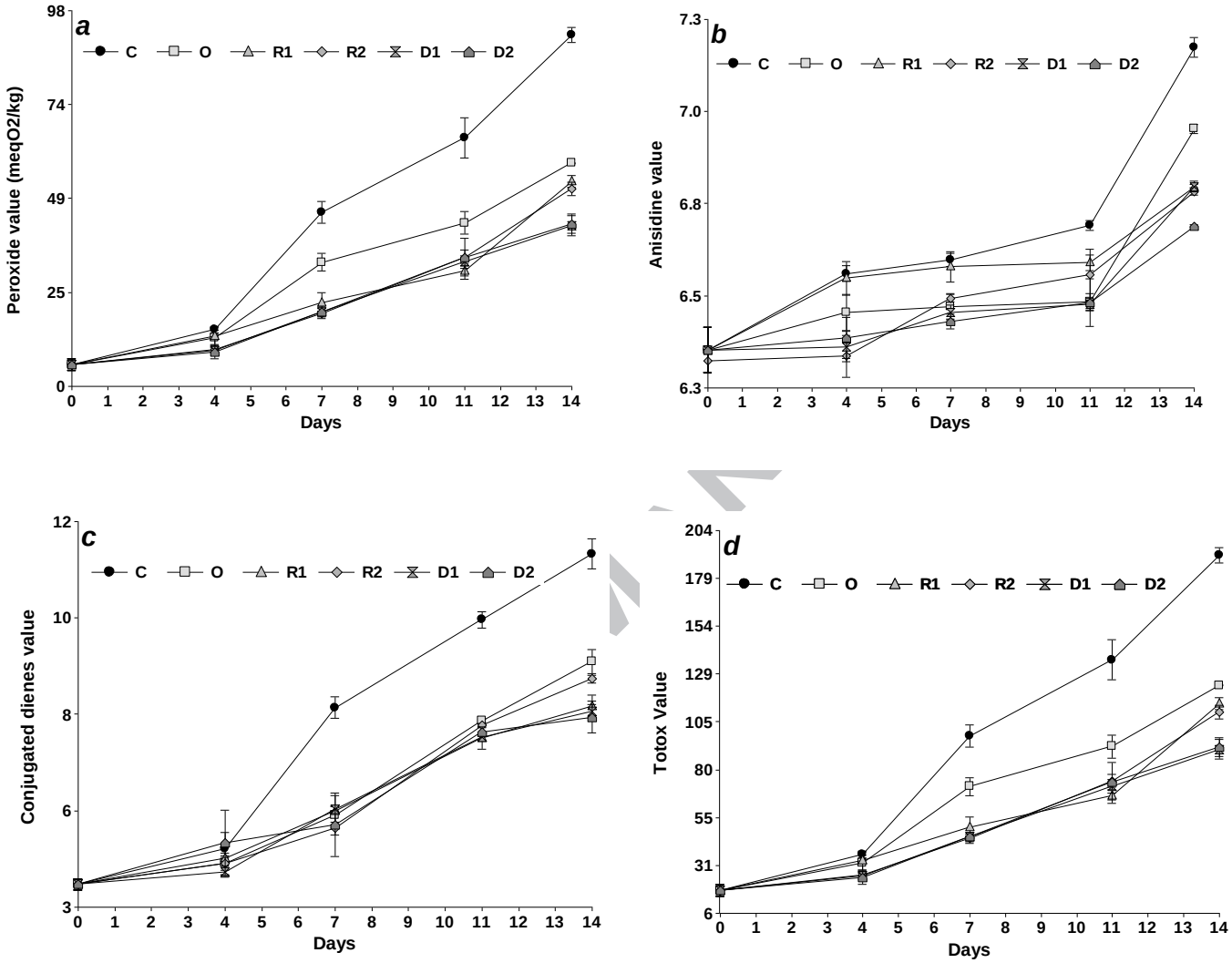


Figure 2.

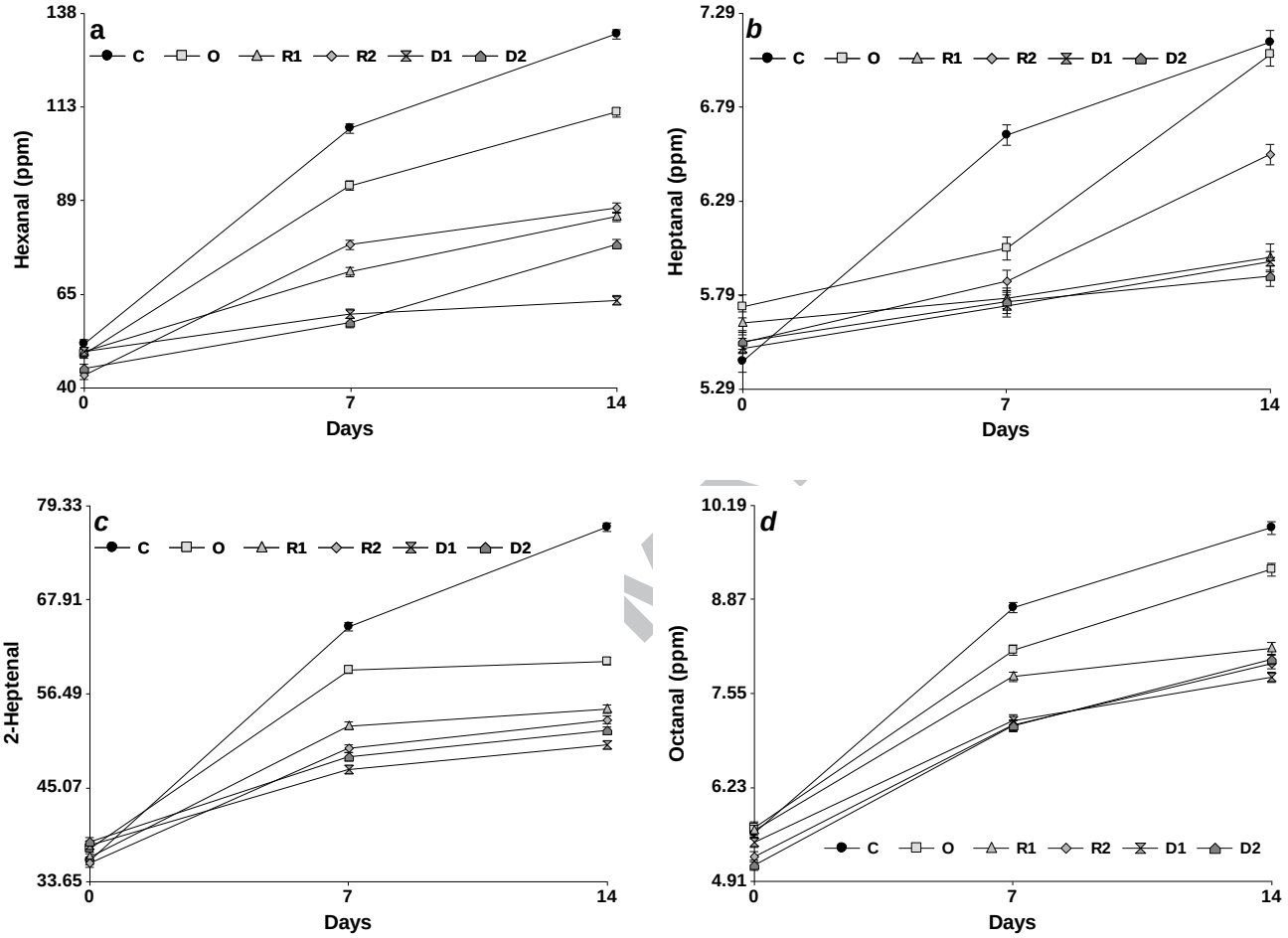
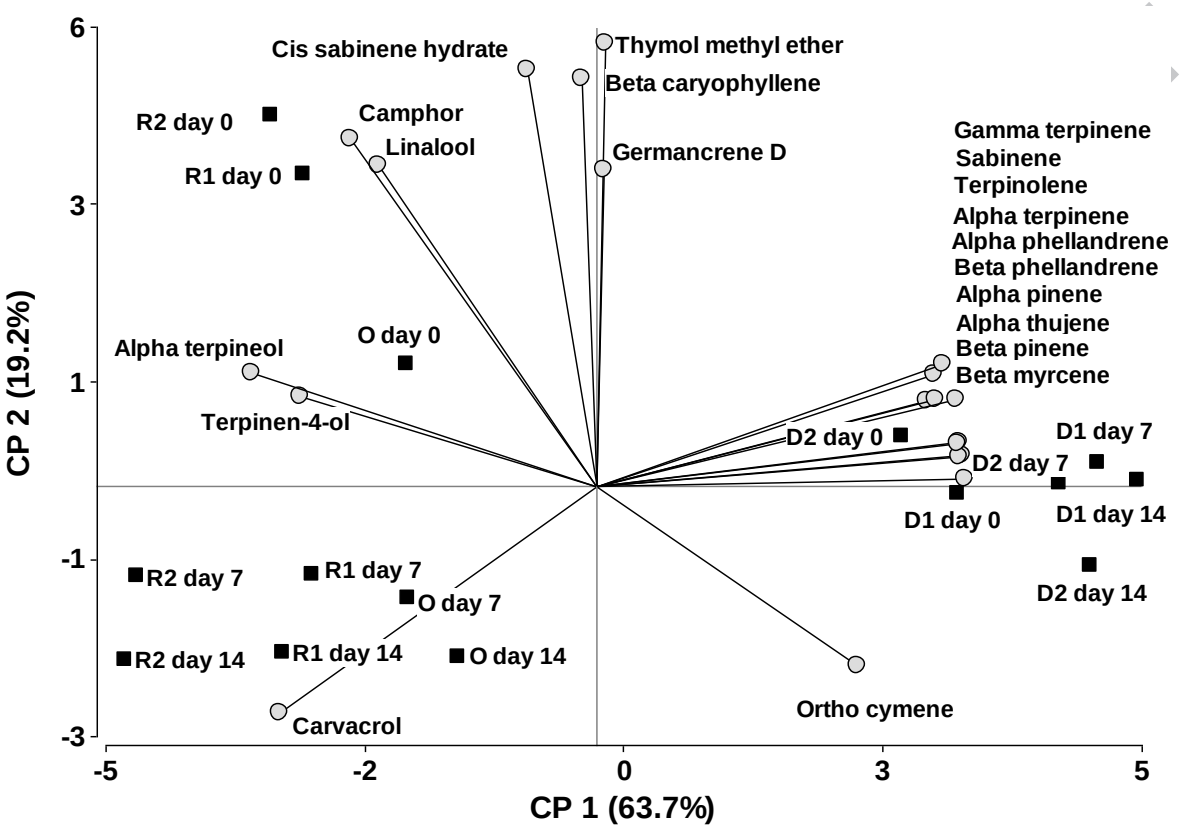


Figure 3.



Highlights

- Oregano essential oil fractions can be separated by SPMD without molecular damages.
- SPMD fractions have higher antioxidant activity than oregano essential oils.
- Oregano essential oil fractions decrease oxidation reaction in sunflower oil.
- Oregano essential oil fractions can be tested as a preserving agent for foods.