Research Article

Antioxidant Activity of Essential Oils Extracted from *Aloysia triphylla* and *Minthostachys mollis* that Improve the Oxidative Stability of Sunflower Oil under Accelerated Storage Conditions

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/ ejlt.201700374].

Received: August 30, 2018 / Revised: February 20, 2018 / Accepted: February 27, 2018

Abstract

This research determined the antioxidant properties of the essential oils from *Aloysia triphylla* (AT) and *Minthostachys mollis* (MM), their antioxidant activity in sunflower oil during accelerated storage (60 °C), and how this storage condition affected the volatiles profile of these essential oils. The main constituents of AT essential oil were neral (27.3%), spathulenol (25.6%), and geranial (24.4%), whereas pulegone (53%) and menthone (29.5%) predominated in MM. Both essential oils presented minor modifications in the chemical compositions after storage at 60 ºC for 14 days. The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and monitoring the chemical and volatile oxidation indicators during accelerated storage of sunflower oil. The DPPH results showed 48.2 and 24.6% free radical inhibition for AT and MM, respectively. In the accelerated oxidation test, the samples with 0.02% essential oil showed the best antioxidant properties and were comparable to butylated hydroxytoluene. Based on these results, AT and MM essential oils show potential use as antioxidants in foods with high lipid content.

Practical applications: Lipids are susceptible to oxidation associated with off-flavor generation, which decreases their quality and nutritional properties. Natural antioxidants can be used as an alternative to replace synthetic antioxidants, like BHT, TBHQ or BHA. The results of this research demonstrated that the essential oils obtained from two aromatic-culinary plants (AT and MM) have antioxidant activity and constitute potential additives for preserving the quality of lipid-rich foods. Deciphering the antioxidant mechanism of these essential oils will promote interest in their use by the food industry. The current investigation shows the dynamics of lipid oxidation indicators in a lipid food matrix supplemented with these natural antioxidants.

Keywords**:** *Aloyzia*, *minthostachys*, antioxidants, oxidation, volatile profile.

Short title: *Aloysia triphylla* and *Minthostachys mollis* antioxidant

Abbreviations:

AT: *Aloyzia Triphylla*

MM: *Minthostachys mollis*

DPPH: 2,2-diphenyl-1-picrylhydrazyl

TPC: Total phenolic Content

FRSA: Free radical scavenging activity

SPME: Solid phase micro-extraction.

PDMS/DVB: Polydimethylsiloxane/divinylbenzene.

SA (0.02%, 0.10%, 0.20%): sunflower oil with different concentration of AT.

SM (0.02%, 0.10%, 0.20%): sunflower oil with different concentration of MM.

S: Sunflower oil control.

BHT: Butylated hydroxytoluene.

SBHT: Sunflower oil with BHT.

PV: Peroxide Value.

AV: Anisidine Value

CD: Conjugated dienes.

PCA: Principal components analysis.

LSD: Lest significant difference.

R^2: Coefficient of determination.

IC_{50}: Half maxima inhibitory concentration.

CP: Component principal.

EO: Essential oil

EOs: Essential oils

1. Introduction

Lipid oxidation is one of the major causes of food deterioration and shelf-life reduction in the food industry. The oxidation process results in rancidity and decrements in the nutritional quality, color, flavor, texture, and safety of food [1]. Lipid-rich foods are particularly susceptible to oxidation. However, other factors also affect lipid oxidation of food products, such as processing and storage conditions (temperature, time, light, oxygen, the presence of pro-oxidants/catalysts), as well as the content of unsaturated fatty acids and their distribution in the triacylglycerol molecule [2]. Volatile compounds that evolve during oxidation are responsible for off-flavors, such as hexanal and heptenal, which are among the main compounds generated [3].

Moreover, lipid oxidation can generate free radicals, which contribute to diseases, like cancer and cardiovascular disease [4]. One of the simplest and most effective approaches to decrease lipid oxidation is to incorporate antioxidants. In this context, natural antioxidants are preferred due to the potential toxic health effects of synthetic additives [4]. Consequently, there is great interest in the use of spices and aromatic herbs because they are perceived as safe and contribute important sensory attributes [5]. Additionally, these common food ingredients exhibit antioxidant and antimicrobial properties, which, in some instances, are more efficacious than their synthetic counterparts [5].

Several non-volatile components, like carnosol, quercetin, caffeic acid, and rosmarinic acid, are well-known for their free radical scavenging efficacy [6]. Some essential oils (EOs) also have potential as natural agents for food preservation [7]. For instance, previous researchers have documented the antioxidant activity effect of oregano EO, which is rich in thymol and carvacrol, added to peanut products [8, 9], dairy beverages [10], meat [11], and cheese [12]. However, although the antioxidant properties of EOs from aromatic spices, like oregano, rosemary, and laurel, are reported [5, 13], there is no similar information available, to date, on the EOs from *Aloysia triphylla* (AT) or *Minthostachys mollis* (MM) in food.

AT is used in traditional medicine for its sedative action and to counter depression. An infusion of its aerial parts provides antipyretic, antispasmodic, digestive, and diuretic effects [14]. MM is used as a digestive, carminative, and antispasmodic, whereas a decoction affords benefits against rheumatism and muscle pain [15]. Both species grow

in the mountain region of Cordoba in Argentina, where they are among the most intensively harvested aromatic plants. These plants are used to prepare infusions. After harvesting, these herbs are processed and sold by industrial and artisanal production facilities.

The interest in EOs as natural sources of antioxidants is attributed to their diverse composition of terpenes and other volatile compounds. Auto-oxidation is a complex process that typically involves multiple mechanisms. Thus, various antioxidant methods that include both indirect (e.g., 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total phenolic content (TPC)) [16] and direct (oxidation) approaches have been developed. Several different tests are recommended to measure the antioxidant activity of EOs [17].

The objective of this research was to determine the antioxidant properties of the EOs from AT and MM, their antioxidant activity in sunflower oil under accelerated storage condition (60 °C), and how this storage condition affects the volatile profile of these EOs.

2. Materials and methods

2.1. EOs extraction

Leaves (50 g) of AT and MM were collected from the experimental station of the Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba (UNC), Córdoba, Argentina (May 2014). The EOs were obtained by hydro-distillation with water vapor, using a Clevenger-type apparatus [18], and were stored in glass vials at -18 °C in darkness [8].

2.2. EOs composition

The EOs were studied by gas chromatography-mass spectrometry (GC-MS), using a Perkin Elmer Clarus 600 gas chromatograph (Shelton, Connecticut, USA) coupled to an ion trap mass detector. The separation was achieved on a non-polar Elite-ms5 capillary column (methylpolysiloxane, 5% phenyl; 30 m × 0.25 mm internal diameter × 0.25 µm coating thickness). The EOs were analyzed under the following chromatographic conditions: 40 ºC for 3 min; 10 ºC/min to 100 ºC, and finally, 15 ºC/min to 245 ºC, where was held for 2 min. The injector was set at 250 ºC. Helium was used as the carrier gas, with a flow rate of 0.9 mL/min. The mass spectra were acquired in the scan mode in the 35–450 *m/z* range by electron-impact ionization at 70eV. Homologous *n*-alkane

hydrocarbons series were used to establish the retention index of the chemical compounds. Components of the EOs were identified by comparing the mass spectra, retention time, and retention index to standard reference data, according to Adams [19] and the data provided by the NIST library. Standard components (Sigma, St. Louis, USA) were also co-injected into the GC system. The concentration of each peak was expressed as a relative percentage of the mass detected by the mass detector [17].

2.3. TPC and DPPH free radical scavenging activity (FRSA)

The indirect methods to determine antioxidant activity in the EOs were DPPH FRSA and TPC [13]. The TPC was measured using the Folin-Ciocalteu reagent, with gallic acid (Sigma, St Louis, MO, USA) as a standard and 10 µL EO. Absorption was measured at 760 nm in a spectrophotometer (Hewlett Packard HP 8452 A, Palo Alto, CA, USA). The results were expressed as mg/g phenol in EO [17].

The DPPH (Aldrich, Milwaukee, WI, USA) FRSA method was conducted according to Choi et al. [20]. The reaction mixture contained ethanol (40 µL), 40 µL of 0.5% w/w Tween 20, Tris-HCl at pH 7.4 (900 µL), EO (10 µL), and 1000 µL of 500 µM DPPH (to give 250 µM DPPH in the final mixture). The reaction was carried out for 30 min and the spectrophotometric absorbance measured at 517 nm. The FRSA was expressed as a percentage of DPPH inhibition, calculated according to the following equation: inhibition (%) = $[(A_c - A_a)/A_c] \times 100$, where A_c and A_a are the absorbance values of the control and EO sample, respectively.

2.4. Change in the volatiles profile of the EOs at 60 °C

The volatiles profile of each EO was determined at 60 ºC. An aliquot (10 µL) of EO was placed in a glass flask (capacity 10 mL), sealed with a rubber lid, and stored in an oven at 60 ºC for 14 days. Samples of the EOs were removed at 0, 7, and 14 days of storage. The volatile compounds were detected using a solid-phase microextraction (SPME) fiber [1] of polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Supelco, Sigma, St Louis, MO, USA). The fiber was introduced into the glass flask, then, heated at 70 ºC for 20 min. Next, the fiber was injected into the GC-MS apparatus. The compound identification and quantification were performed under the same condition described in section 2.2. Three different kinds of SPME fibers were previously tested, to establish which one retained the major volatiles [13].

2.5. Accelerated oxidation test

Chemical oxidation (antioxidant capacity). Refined sunflower oil (7 g) (Natura, Aceitera General Deheza, Córdoba, Argentina) was combined with 0.02, 0.10, and 0.20% (w/w) AT and MM EOs (termed SA 0.02, 0.10, and 0.20, and SM 0.02, 0.10, and 0.20, respectively) [17]. Sunflower oil alone (control, S) and sunflower oil with 0.02% butylated hydroxytoluene (BHT) (termed SBHT) were also analyzed. The samples were stored in an oven at 60 °C, according to Olmedo et al. [17]. The experiments were carried out in triplicate. The samples were removed at 0, 2, 4, 7, 9, 11, and 14 days. Peroxide value (PV), anisidine value (AV), conjugated dienes (CD), and totox values were evaluated as lipid oxidation indicators, according to Olmedo et al. [13].

Volatile compounds. Volatile oxidation compounds from refined sunflower oil were determined by GC-MS. Refined sunflower oil (10 g) was put into a glass flask (capacity 50 mL), sealed, and stored at 60 °C under the same condition as given by Olmedo et al. [17] and used to assess the chemical oxidation. The studied samples were S (control), SBHT (0.02% BHT), SA (0.02, 0.10, and 0.20% AT EO), and SM (0.02, 0.10, and 0.20% MM). The samples were removed at 0, 7, and 14 days of storage. The volatile oxidation compounds were captured by the SPME fiber of PDMS/DVB that was inserted into the glass flask and heated at 130 ºC for 20 min. Next, the fiber was introduced into the GC-MS injector for 1 min. The chromatographic conditions used were the same as described in section 2.2. Only the main volatile oxidation compounds were identified, which was achieved by co-injection of the standards (hexanal, 2-heptanal, 2,4-decadienal (*E*,*Z*), and 2,4-decadienal (*E*,*E*)) (Sigma, St Louis, MO, USA). Acetaldehyde (Sigma, St Louis, MO, USA) was injected in all samples as the internal standard. The compounds were expressed as ppm (mg/L) [13].

2.6. Statistical analysis

Triplicate experiments were performed in all instances. The statistical analysis was made using Infostat software, version 1.1 (Facultad de Ciencias Agropecuarias, UNC). Means and standard deviations were calculated, and analysis of variance (ANOVA) was used to detect significant differences between sampling days, with Fisher's least

significant difference (LSD) test applied to identify significant differences ($\alpha = 0.05$) between means. Principal component analysis

was used for correlation between chemical and volatile oxidation indicators, and different treatments.

3. Result and discussion

3.1. Chemical composition of EOs

The major compounds in MM EO (Table 1) were pulegone (53.1%), menthone (29.5%), and *cis*-dihydrocarvone (4.7%). Gillij et al. [21] reported a similar composition of 51.2% pulegone, 29.5% menthone, and 10.1% limonene. Valladares et al. [22] found that the two major components, pulegone and menthone, denoted more than 70% of the total EO. Banchio et al. [23] showed that MM EO had more than 50 components, but many of them were only present in trace amounts. The present study detected 12 components that characterized 99.2% of the total EO. In agreement with Gillij et al. [21] and Van Barem et al. [15], pulegone and menthone represented more than 70% MM EO from Cordoba, Argentina.

The predominant components in AT EO (Table 2) were neral (27.3%), limonene (25.6%), geranial (24.4%), and myrcene (6.6%). Duarte et al. [24] also found limonene (18.8%) and geranial (21.8%) as the key components. Likewise, Texeira Ruarte et al. [25] noted the major components in AT EO included geranial (21.8%), limonene (18.8%), *trans*-geraniol (8.3%), and neral (6.5%). Moreover, Carnat et al. [26] determined 40% citral (geranial + neral) while limonene, cineole, geraniol, β-caryophyllene, and spathulenol were present at more than 25% of total. Therefore, the dominant components in all three works are similar. In comparison to Carnet et al. [26], however, in the current study, the citral content was slightly higher, at 51.7% (27.3% neral + 24.4% geranial), whereas limonene and β-caryophyllene were also detected at high percentages but, conversely, no geraniol, cineole, and spathulenol, were evident. These last authors [26] documented a low citral content because no neral was detected.

3.2. Indirect analysis of antioxidant activity: TPC and FRSA

EOs from aromatic plants present antioxidant properties [13, 17]. The AT EO had a high TPC (8.3 mg/g), and this is comparable to the maximum TPC values reported in aromatics [14, 17]. For instance, laurel, oregano, and rosemary EOs had TPCs of 10.5, 10.0, and 8.0 mg/g, respectively [17]. Vinha et al. [14] found that AT had a TPC of 8.7 mg/g and this value concurred with that found in the present study. MM EO showed a lower TPC (5.7 mg/g) than AT EO.

Both, AT and MM EOs showed FRSA, respectively. Particularly, AT EO exhibited 48.2% DPPH inhibition while it was lower in MM EO (24.6% DPPH inhibition). The literature contains many studies and references about the antioxidant activity of EOs, but studies about the kinds of aromatic plants investigated in the current work are deficient regarding the antioxidant determinations. EOs like oregano (60.0%), laurel (61.7%), and rosemary (48.3%) have shown more than 40% free radical inhibition [17]. Among these examples, rosemary EO had a similar FRSA to AT EO. Ricci et al. [27] studied the DPPH activity of EO from *Teucrium marum* and reported IC_{50} values (concentration where 50% free radicals are inhibited) of 13.13 µg/mL compared to 86.63 µg/mL for BHT. However, for inhibition of 5-lipooxygenase-catalyzed lipid peroxidation of linolenic acid, these same authors observed IC_{50} values of 12.48 µg/mL for the EO and 3.86 µg/mL for BHT, being this last one the more effective antioxidant. These results highlight the variability in the FRSA test, according to the preparation of the analysis (concentration, time, among other experimental variables) [28].

Phenolic substances exert a potent antioxidant activity due to their multiple hydroxyl groups. In general, a higher phenolic content is correlated with a higher antioxidant activity; however, some studies have reported no such relation [29]. From the chemical composition of the AT and MM EOs, no molecules with a phenolic structure were observed, to justify the TPC values obtained. Although the Folin-Ciocalteu reagent is used to determine TPC, molecules besides phenols give a positive test because this reagent also measures non-phenolic reducing substances [30]. Olmedo et al. [13] established a correlation between FRSA and TPC for various fractions separated from oregano EO by short-path molecular distillation. The residue fractions, with a greater concentration of phenols, had a higher FRSA compared to the distillate fractions but in an accelerated oxidation test with sunflower oil, the distillate fractions showed more potent antioxidant properties than the residue fractions.

Although both FRSA and TPC are useful for exploratory antioxidant activity, these preliminary tests can show variability and an incorrect appreciation about the antioxidant activity. Foti [28] thoroughly reviewed the use of the DPPH test. Amorati et al. [31]

comprehensively surveyed the antioxidant activity of EOs and the methods to establish this activity. The authors [28] provided theory and practice for the antioxidant mechanism. The DPPH and TPC tests give some conclusions on the antioxidant activity of EOs. The DPPH assay determines the H-donor ability, but it is not related to any specific antioxidant activity. For that reason, experimental variables (e.g., solvent, concentration, time) are crucial to establishing the conclusion of the DPPH test, and the results must be repeatable to enable a comparison with other researches. Among other methods, the TPC does not provide a radical reaction. This test only indicates some reducing ability of a possible antioxidant compound. However, in contrast to the DPPH and TPC assays, an accelerated oxidation test (direct method) is a specific oxidation analysis of a food product and provides a more accurate measure of its antioxidant activity [32].

3.3. Volatile profile change of the EOs at 60 °C

Heat treatment affected the percentage of the individual components in AT and MM EOs during storage at 60 ºC, particularly, the volatiles composition. In MM EO, sabinene, linalool, and menthone decreased from 0.4% to trace amounts, 0.9 to 0.5%, and 28.4 to 26.8% during 14 days of storage, respectively. Simultaneously, pulegone, carvone, and piperitone increased from 54.2 to 55.4%, 2.7 to 3.1%, and 1.9 to 2.2% (Table 1), respectively.

Under equivalent conditions (60 °C, 14 days), four components decreased during storage of AT EO, including α-pinene (from 0.7 to 0.3%), myrcene (from 4.3 to 3.8%), *p*-cymene (from 1.3 to 0.7%), and *cis*-limonene oxide (from 0.3% to trace amount). At the same time, five components increased, including neral (from 28.6 to 30.4%), geranial (from 25.4 to 26.2%), germacrene D (trace amount to 0.4%), β-bisabolene (from 0.4 to 0.7%), and limonene (from 27.8 to 28.8%) (Table 2).

The changes in the composition of the EOs were minor in comparison to other similar studies. Olmedo et al. [17] reported that linalool decreased 7% (from 13.1 to 5.7%) in oregano EO, compared to 0.4% (from 0.9 to 0.5%) in the present study. The difference can be explained, in part, because the authors evaluated the samples after storage at the same temperature but at 28 days, which was double the storage used in the current experiment. Yang et al. [33] showed that five terpenes (α-pinene, limonene, camphor, citronella, and carvacrol) decreased during storage (100–250 ºC for 30–300 min). The EOs presented subtle differences in the extent of molecular modifications because most

of the molecules that decreased in percentage had a Kováts index less than 1200, with some exceptions, such as limonene (Kováts index, 1031).

Other authors evaluated short-term storage. For instance, Abderrahim et al. [34] obtained the changes in AT phenols composition at 70, 80, and 90 °C for 8 h. During short-term storage of cardamom, clove bud, lavender, pine, and rosemary EOs, Turek and Stintzing [35] noticed that temperatures above 38 °C generated dominant alterations in the volatiles profiles. Notably, there were decreased amounts of terpenic hydrocarbons, such as β-caryophyllene, β-myrcene, β-pinene, sabine or γ-terpinene, and an increase in *p*-cymene. In addition, other authors in the same review detected several chemical alterations in lemon oil stored at 50 °C for 2 weeks; mainly decreased geranial, neral, and terpenic hydrocarbons, together with an increase in *p*-cymene. The results of the current work did not show a strong association between the changes in the volatiles profile and the physical and chemical characteristics of the compositional molecules in the EOs.

3.4. Chemical oxidation indicators from the accelerated oxidation test

Chemical oxidation. All chemical oxidation indicators increased during storage at 60 ºC for 14 days, as shown in Fig. 1 and 2, for AT and MM EOs, respectively. Three levels (0.02, 0.10, and 0.20% w/w) of the EOs were used. The PVs (Fig. 1A and 2B) increased in all samples but to varying extents. Among the samples, S (vegetal oil control) showed the highest PV while the lowest PV was presented by SBHT. Only SAT 0.02, SMM 0.02, and SMM 0.10 were not significantly different to SBHT. The high PVs obtained in samples with the addition of the respective EOs illustrates that the proportion of these EOs does not necessarily parallel the efficacy of protection against oxidation. The highest degree of oil protection with AT and MM EOs, separately, was observed at 0.02% p/p. There is not a linear association between the EOs concentration and antioxidant effect (monotonic correlation) [31]. A major explanation about this point will be addressed in section 3.5.

The highest and lowest AVs at day 14 were associated with S (11.8 AnV) and SBHT (2.4 AnV), respectively. No significant differences were observed in the AT and MM samples. However, a significant difference was noted between the EOs and SBHT, but only at day 14. Otherwise, all samples with AT and MM had similar AVs to SBHT (Fig. 1B and 2B). The AV determines secondary oxidation compounds and the values were low because the peroxides did not undergo further degradation, due to the antioxidant action.

The CD values showed a similar trend to the PVs (Fig. 1C and 2C). The highest and lowest values were detected in S and SBHT, respectively. Only SA 0.10 had similar CD contents (no significant difference) to SBHT. In MM EO, all samples had comparable CD levels, irrespective of the concentration.

The total oxidation (totox) value combines the primary and secondary oxidation indicators, by summing the PVs and AVs. The totox values had a similar behavior to the PVs (Fig. 1D and 2D) because low AVs were detected during storage. AT and MM EOs showed a higher antioxidant efficacy than sample S. Nonetheless, when the concentrations increased, the antioxidant effect did not improve. Based on the oxidation indicators, the samples with 0.10% EO showed the best antioxidant outcome, being comparable to SBHT.

Volatile oxidation compounds. The volatile oxidation compounds from sunflower oil exposed to the different treatments are presented in Fig. 3 and 4. With 63% linolenic acid (18:2) and 18% oleic acid (18:1), sunflower oil has a fatty acid profile susceptible to oxidation. When fatty acid oxidation occurs, odor-activated compounds are generated, which are responsible for off-flavors in food. In this study, hexanal (Fig. 3A and 4A), 2-heptanal (Fig. 3B and 4B), (*E*,*E*)-2,4-decadienal (Fig. 3C and 4C), and (*E*,*Z*)-2,4-decadienal (Fig. 3D and 4D) were detected. All volatile oxidation compounds increased during the 14-day storage. Significant differences were observed among the samples. Sample S had the highest concentrations of all volatile oxidation compounds while SBHT had the lowest, except for (*E*,*Z*)-2,4-decadienal, which was least abundant in both EOs. For sunflower oil with added EO (AT and MM), low concentrations of the volatile oxidation compounds were generated during storage. Furthermore, in some treatments, there were no significant differences in the concentrations of the volatile compounds analyzed, for example, (*E*,*Z*)-2,4-decadienal in MM EO-treated samples. Overall, the volatile oxidation compound concentrations ranged between 13–60 ppm. Similar levels (10–80 ppm) for the same volatile oxidation compounds were found by Olmedo et al. [17], in sunflower oil with laurel, oregano, and rosemary EOs. Elsewhere, it was reported that 1 g oil could produce 5100 ppm hexanal, 450 ppm 2-heptenal, 150 ppm (*E*,*E*)-2,4-decadienal, and 250 ppm (*E*,*Z*)-2,4-decadienal, but the conditions necessary to obtain these values are considerably drastic for food under normal conditions [36].

3.5. Linear regression slope and principal component multivariate analysis

The values obtained for the chemical and volatile oxidation compounds were used to calculate the linear regression slopes, which were analyzed by ANOVA and Fisher's LSD test. The dependent variables were good descriptors of the oxidation during storage because the R^2 values were all above 0.70. Analysis of the slopes revealed that sample S had the highest slope among the treatments and thereby the least chemical stability. Significant differences were found between the samples. Olmedo et al. [17] found a similar behavior between the slope values from the regression analysis for laurel, oregano, and rosemary EOs. In other research, Olmedo et al. [13] showed that oregano EO and fractions obtained from oregano EO separated by molecular distillation presented a lower slope value than the control sample of vegetable oil. The outcome was related to the less extensive oxidation in vegetable oil with EOs than without [13].

A principal component multivariate analysis was performed, to visually observe which samples exhibited the highest antioxidant property. Fig. 5 shows that the sum of two principal components explained 91.1% data variability. Therefore, the chemical and volatile compound values were mainly responsible for the variability. The most important component is PC1, which has 82.2% total variability. The oxidation indicators were on the positive side of PC1 and were accompanied by the samples with the highest values for the chemical (oxidation) indicators. Samples located on the opposite side (negative values of PC1), and far from the lipid oxidation indicators, had the best antioxidant properties. Only SM 0.20 and SA 0.20 were located on the positive side and linked to the chemical indicators. Therefore, these samples were considered poor antioxidants. In contrast, S-BHT 0.20, SM 0.02, and SA 0.02 had the highest antioxidant properties while SA 0.10 and SM 0.10 displayed good (intermediate) antioxidant properties. In this analysis, it was observed that the maximal EO content (0.20%) did not correlate with the maximum antioxidant activity, suggesting a pro-oxidant behavior at high EO levels.

Amorati et al. [31] explained that terpenoids do not display the same behavior as phenols but do show antioxidant activity. Also, terpenoids co-oxidize and combine with the oxidizable lipids because they are lipid oxidation initiators. Furthermore, the antioxidant mechanisms differ between phenols and terpenoids. Phenols act in the initial step and provide a chain-breaking effect on radicals, whereas terpenoids contribute to the termination step, called "termination-enhancing", when two radicals meet resulting in cessation of their auto-oxidation activities [31]. A study of non-phenolic terpenoids in EO from *Cleistocalyx operculatus* showed that the antioxidant effect was related to the "termination-enhancing" mechanism [37]. Baschieri et al. [38] confirmed "termination-enhancing" as the antioxidant mechanism in citral, linalool, and limonene components.

Also, these components exhibited two different behaviors depending on their concentration, which was tested at 0 to 0.10, 3.5, and 2.5 M for citral, linalool, and limonene, respectively. At a very low concentration of these EO components, the antioxidant effect was minimal, but it rapidly improved when the concentration increased [38]. Nevertheless, at a determinate concentration (0.0085 M citral or 0.12% v/v, 0.22 M linalool or 40% v/v, and 0.13 M limonene or 2.1% v/v), a pro-oxidant effect was observed. This pro-oxidant effect can be explained because non-phenolic compounds are oxidized, then, at a high concentration, they become significantly dominant to carry on the autoxidation process, increasing propagation at a constant rate until reaching values greater than those coming from lipid oxidation [38]. Work by Frutos and Hernandez-Herrero [39] showed similar behavior in the oxidative stability of sunflower oil containing various concentrations of rosemary extract (0, 2, 4, and 6 g/L). Based on the thiobarbituric acid test and PVs, 4 g/L extract exhibited better antioxidant activity than 6 g/L extract. Simic et al. [40] examined the effect of crude laurel extract on Fe^{2+}/ascorbate-induced lipid peroxidation in liposomes. In that study, the most effective inhibition of lipid peroxidation was obtained with 1 mg of extract (70.6% inhibition), when compared with 2, 3, and 5 mg (60.5, 64.6, and 59.4% inhibition, respectively). Therefore, the effectiveness of a natural antioxidant must be tested in each product to determine the optimal concentration while also considering the storage conditions [32, 41].

4. Conclusions

At all concentrations of the AT and MM EOs tested, the oxidative processes were inhibited. The accelerated stability test results confirm that at 0.02% w/w, the EOs show enhanced antioxidant capacity. These EOs are potential natural antioxidants and could be used as an antioxidant additive in food lipid matrices.

Acknowledgement

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Secretaria de Ciencia y Tecnología - Universidad Nacional de Córdoba (SECYT-UNC).

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Table 1. Chemical composition; volatile composition (VC) from volatile profile at day 0, 7, and 14; free radical scavenging activity (FRSA); and total phenol content from *Aloysia triphylla* essential oil.

Retentionindex	Components	EO	VCday 0	VC day 7	VC day 14	Methods of identification[B]
		g/100g ± SD[A]	g/100g ± SD[A]	g/100g ± SD[A]	g/100g ± SD[A]	
933	alpha pinene	1.4 ± 0.1	0.7 ± 0.1 b	0.3 ± 0.1 a	0.3 ± 0.1 a	GCMS-Co
985	Sulcatone	0.6 ± 0.1	0.5 ± 0.1 a	0.5 ± 0.1 a	0.3 ± 0.1 a	GCMS
992	myrcene	6.6 ± 0.2	4.3 ± 0.1 b	4.1 ± 0.1 b	3.8 ± 0.1 a	GCMS-Co
1026	p-cymene	1.1 ± 0.1	1.3 ± 0.1 b	1.1 ± 0.1 b	0.7 ± 0.1 a	GCMS
1031	Limonene	25.6 ± 0.2	27.8 ± 0.2 a	28.9 ± 0.2 b	28.8 ± 0.3 b	GCMS-Co
1132	limonene oxide cis	0.5 ± 0.2	0.3 ± 0.1 a	Trace	Trace	GCMS
1139	trans sabinol	0.4 ± 0.1	0.5 ± 0.1 a	0.6 ± 0.1 a	0.5 ± 0.1 a	GCMS
1192	dihydrocarvone trans	0.5 ± 0.2	0.7 ± 0.2 a	0.7 ± 0.1 a	0.8 ± 0.1 a	GCMS
1196	dihydrocarvonecis	0.9 ± 0.2	0.6 ± 0.1 a	0.6 ± 0.1 a	0.7 ± 0.1 a	GCMS
1247	neral	27.3 ± 0.2	28.6 ± 0.3 a	29.3 ± 0.2 b	30.4 ± 0.2 c	GCMS-Co
1277	geranial	24.4 ± 0.3	25.4 ± 0.2 a	25.7 ± 0.2 a	26.2 ± 0.2 b	GCMS-Co
1299	cispinocarvyl acetate	0.4 ± 0.1	0.5 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a	GCMS
1418	beta caryophyllene	1.6 ± 0.1	1.2 ± 0.1 a	1.4 ± 0.1 a	1.2 ± 0.1 a	GCMS
1477	gamma muurolene	2.6 ± 0.2	2.2 ± 0.1 a	2.3 ± 0.1 a	2.1 ± 0.1 a	GCMS
1480	germacrene D	0.2 ± 0.1	Trace	0.5 ± 0.1 a	0.4 ± 0.1 a	GCMS
1509	beta bisabolene	0.5 ± 0.2	0.4 ± 0.1 a	0.7 ± 0.1 b	0.7 ± 0.1 b	GCMS-Co
1553	Alfa curcumene	0.7 ± 0.2	0.9 ± 0.1 a	1.2 ± 0.1 b	1.1 ± 0.1 ab	GCMS
1652	alpha-cadino	0.2 ± 0.1	Trace	0.3 ± 0.1 a	Trace	GCMS
1818	farnesyl acetate ZE	2.1 ± 0.2	2.2 ± 0.1 a	Trace	Trace	GCMS
1843	farnesyl acetate EE	1.6 ± 0.2	1.7 ± 0.1 b	1.1 ± 0.1 a	0.9 ± 0.1 a	GCMS
	Total	99.2	99.8	99.9	99.5	
	FRSA[C] percentage	48.2 ± 1.1				
	Phenol[D]content (mg/g)	8.3 ± 0.2				

[A] values with different letter in the same raw are significantly different (n=3, LSD Fisher, α=0.05).

[B] GCMS: Peak identifications are based on MS comparation 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.

Table 2. Chemical composition; volatile composition (VC) from volatile profile at day 0, 14, and 28; free radical scavenging activity (FRSA); and total phenol content from *Minthostachys mollis* essential oil.

Retentionindex	Components	EO	VCday 0	VC day 7	VC day 14	Methods of identification[B]
		g/100g ± SD[A]	g/100g ± SD[A]	g/100g ± SD[A]	g/100g ± SD[A]	
933	alpha pinene	0.3 ± 0.1	0.6 ± 0.1 a	0.5 ± 0.1 a	0.5 ± 0.1 a	GCMS-Co
973	sabinene	Trace	0.4 ± 0.1 b	0.2 ± 0.1 a	Trace	GCMS-Co
980	beta pinene	0.3 ± 0.1	0.7 ± 0.1 a	0.7 ± 0.1 a	0.8 ± 0.1 a	GCMS-Co
1098	linalool	0.6 ± 0.2	0.9 ± 0.1 b	0.8 ± 0.1 b	0.5 ± 0.1 a	GCMS
1154	menthone	29.5 ± 0.3	28.4 ± 0.3c	27.6 ± 0.2 b	26.8 ± 0.2 a	GCMS
1164	isomenthone	2.9 ± 0.2	2.7 ± 0.1 a	2.8 ± 0.1 a	2.7 ± 0.1 a	GCMS
1196	dihydrocarvonecis	4.7 ± 0.2	4.2 ± 0.2 a	4.1 ± 0.1 a	4.3 ± 0.1 a	GCMS
1237	pulegone	53.1 ± 0.3	54.2 ± 0.2 a	55.3 ± 0.3 b	55.4 ± 0.2 b	GCMS-Co
1242	carvone	3.1 ± 0.2	2.7 ± 0.1 a	2.9 ± 0.1ab	3.1 ± 0.1b	GCMS-Co
1282	piperitone	2.3 ± 0.1	1.9 ± 0.1 a	1.9 ± 0.1 a	2.2 ± 0.1 b	GCMS
1573	caryophyllene oxide	0.7 ± 0.1	0.6 ± 0.1 a	0.7 ± 0.1 ab	0.9 ± 0.1 b	GCMS
1619	spathulenol	1.9 ± 0.2	1.8 ± 0.1 a	1.8 ± 0.1 a	2.2 ± 0.1 b	GCMS
	Total	99.2	99.1	99.2	99.4	
	FRSA[C] percentage	24.6 ± 0.9				
	Phenol[D]content (mg/g)	5.7 ± 0.3				

[A] values with different letter in the same raw are significantly different (n=3, LSD Fisher, α=0.05).

[B] GCMS: Peak identifications are based on MS comparation 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.

Figure 1. Peroxide value (PV), anisidinine value (AV), conjugated dienes (CD), and Totox value (chemical indicators) of sunflower oil added with *Aloyzia triphylla* essential oil during storage at 60 ºC.

Figure 2. Peroxide value (PV), anisidinine value (AV), conjugated dienes (CD) and Totox value (chemical indicators) of sunflower oil added with *Minthostachys mollis* essential oil during storage at 60 ºC.

Figure 3. Volatile compounds oxidation indicators evaluated in sunflower oil added with *Aloyzia triphylla* essential during storage at 60ºC.

Figure 4. Volatile compounds oxidation indicators evaluated in sunflower oil added with *Minthostachys mollis* essential during storage at 60ºC.

Figure 5. Biplots of the 1st and 2nd principal components of PCA. Variables: chemical and volatiles oxidation indicators and all treatments.

Figure 1.

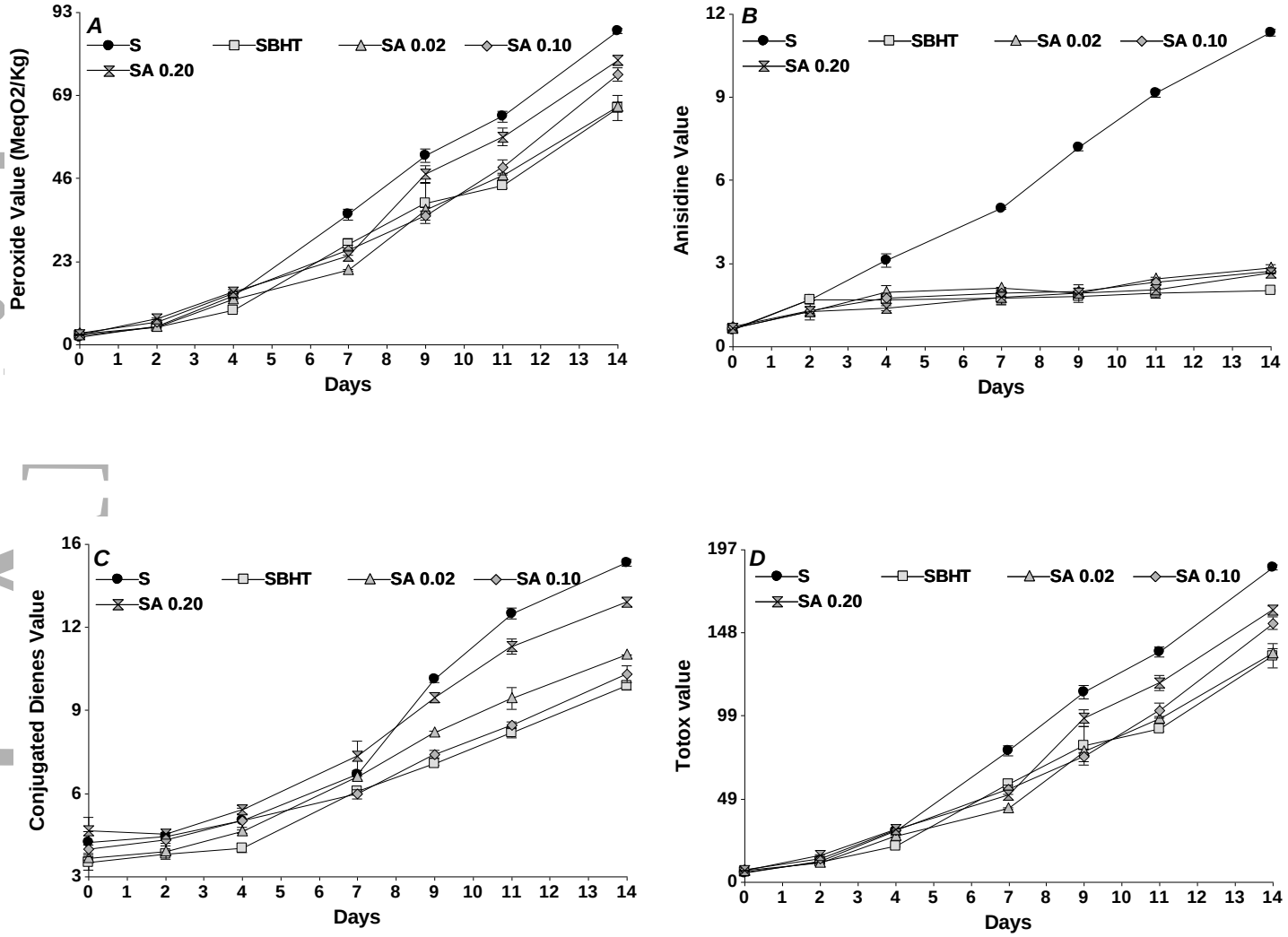

Figure 2.

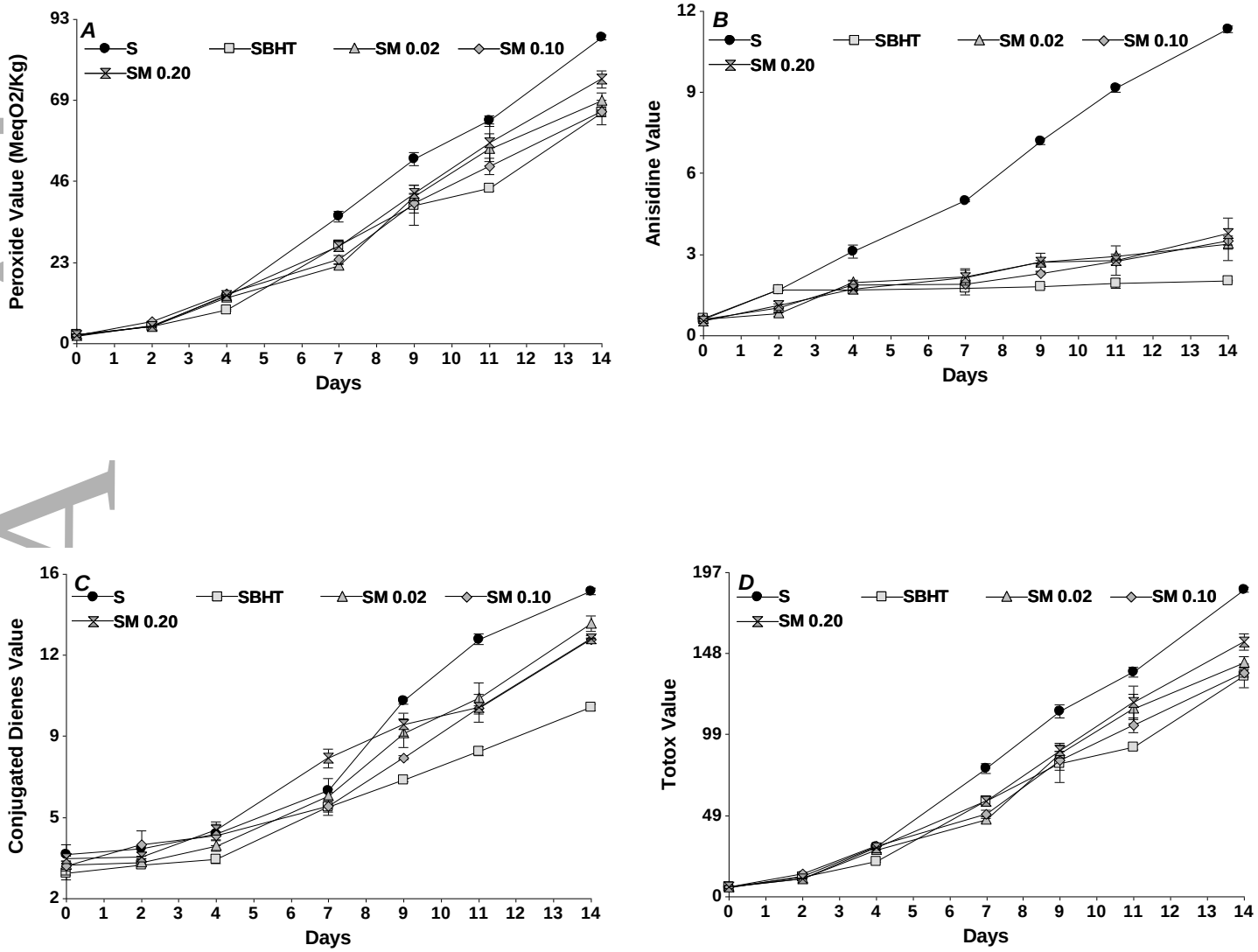

Figure 3.

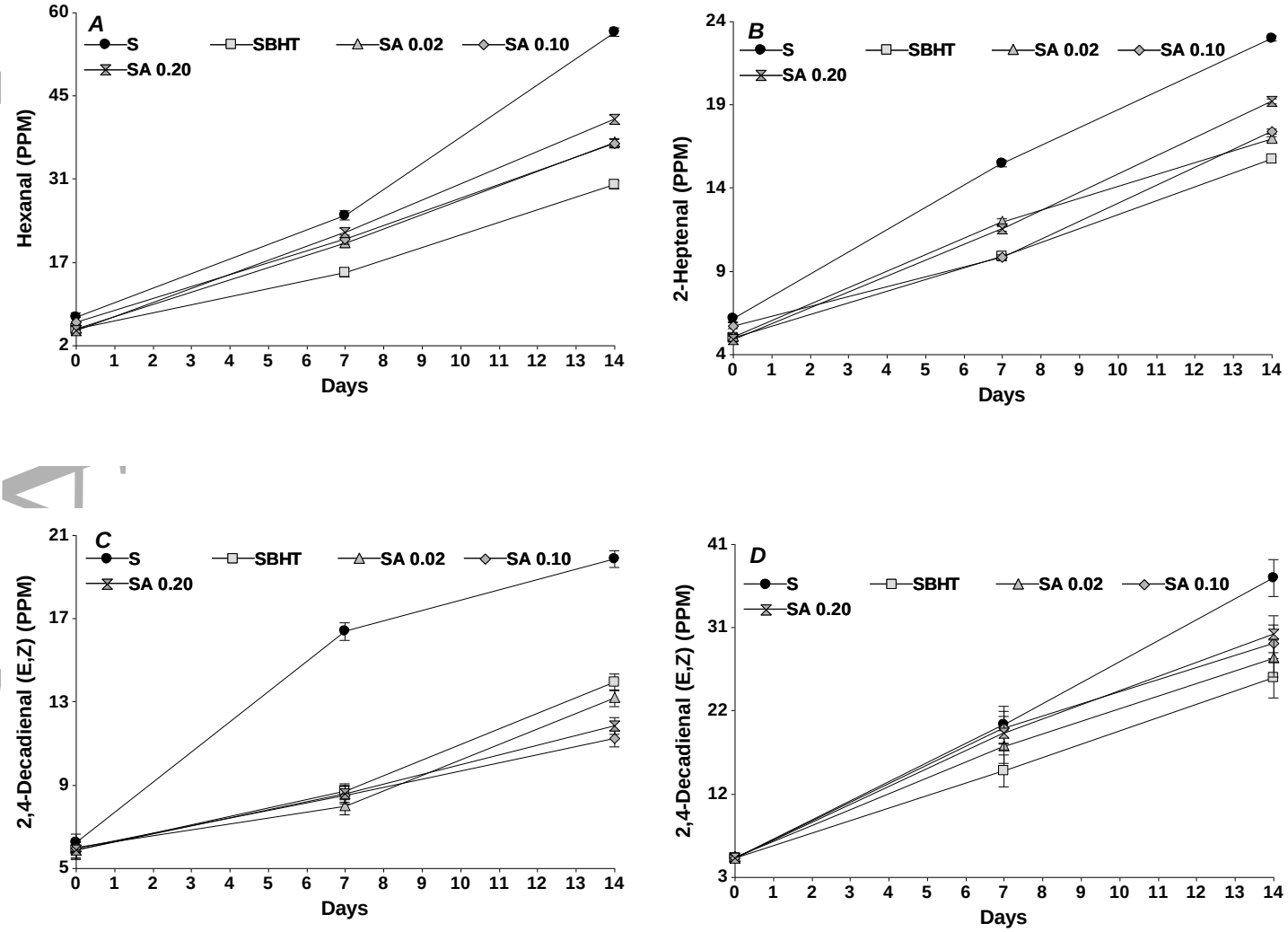

Figure 4.

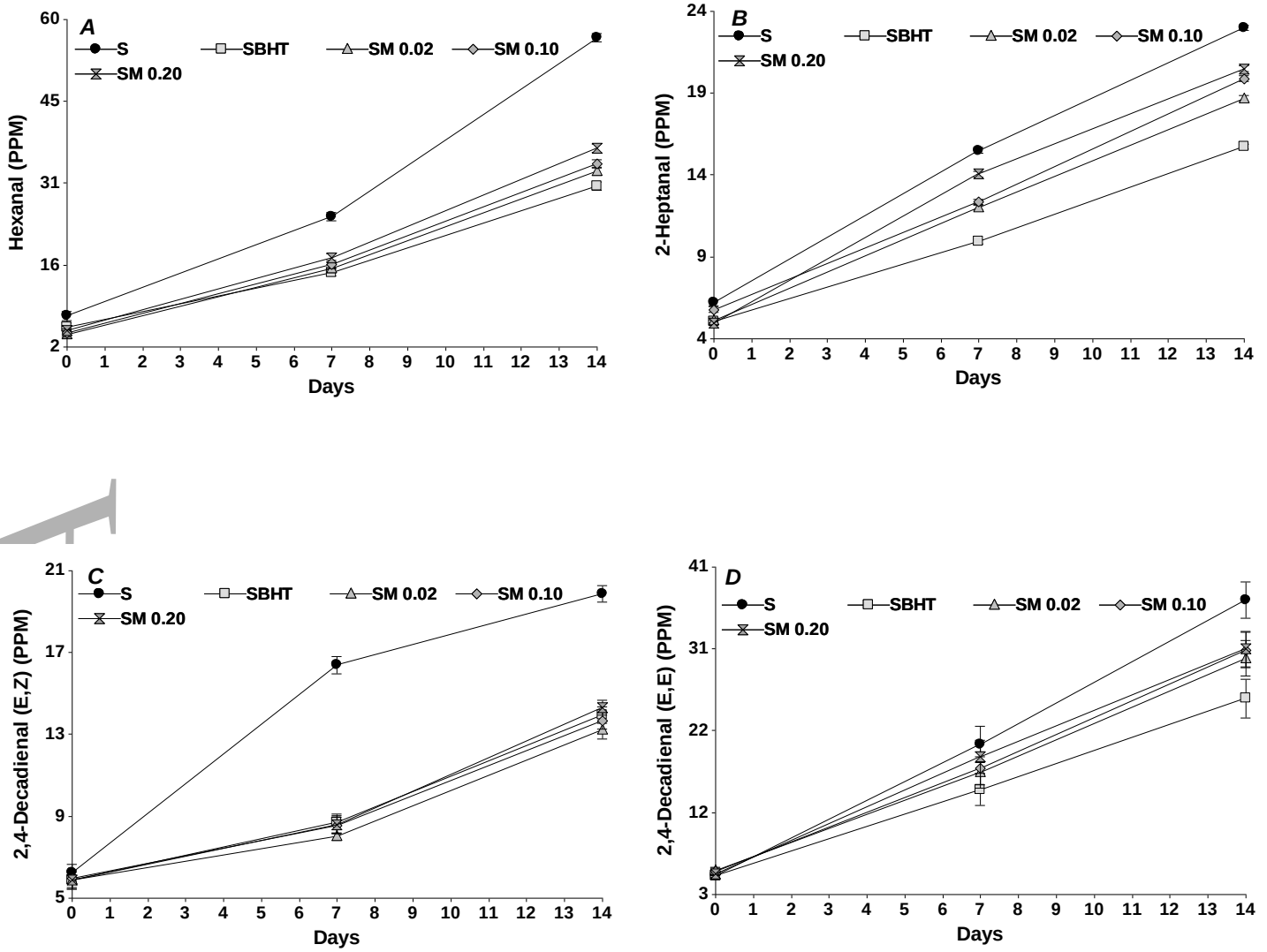

Figure 5.