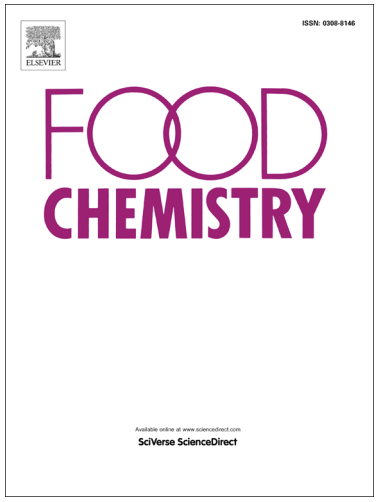

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Contribution of organic acids to α-terpinene antioxidant activity

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ABSTRACT

DPPH• scavenging activity and antioxidant activity in canola oil of α-terpinene, BHT (butylated hydroxytoluene) and acetic, malic and citric acids and their mixtures were determined to examine the synergistic effect on the antioxidant activity. The results demonstrated that α-terpinene and organic acids had low or any activity against DPPH• and BHT showed an IC_{50} of 0.035 mM. When mixed with organic acids, α-terpinene increased its activity showing a synergistic effect. The mix of α-terpinene (1.58 mM) and citric acid (2.91 mM) showed the higher synergistic effect in DPPH• (121.82). BHT scavenging activity was inhibited by organic acids. Although the DPPH• scavenging activity was enhanced for α-terpinene and inhibited for BHT by organic acids, the antioxidant activity in canola oil was not. It indicated that the scavenging DPPH activity and antioxidant activity in canola oil of a compound or a mixture of compounds are not always positively correlated with each other.

Keywords: antioxidant, acid synergy, monoterpenes, non-phenolic compounds.

Chemical compounds studied in this article

α-terpinene (PubChem CID: 7462); acetic acid (PubChem CID: 176); citric acid (PubChem CID: 311); malic acid (PubChem CID: 525)

1. Introduction

Active oxygen and particularly free radicals are well known to be major causes of material degradation and food deterioration. Antioxidants are now known to be prospective protective. In the past few years, addition of synthetic antioxidants has begun to be restricted because of their health risks and toxicity (Furukawa et al., 2001; Lanigan, Yamarik, & Andersen, 2002). Interest in naturally occurring antioxidants for food preservation is emerging due to their technological relevance and positive impact on consumer health. Multiple researches in the field of new antioxidant agents are being conducted in order to replace synthetic antioxidants by natural ones such as essential oils (Asensio, Nepote, & Grosso, 2012; Quiroga, Asensio, & Nepote, 2015; Quiroga, Grosso, & Nepote, 2013) and polyphenols extracts (Larrauri, Zunino, Zygadlo, Grosso, & Nepote, 2016)

Various methods for antioxidant capacity determination have been developed and applied in different systems, but not all of them to concluded results. Should be noticed that there is not a simple and universal method that can be used. It is recommended that for the antioxidant capacity determination, the measurements should be done on the levels of *in vitro* and *in vivo* analysis (Bunaciu, Danet, Fleschin, & Aboul-Enein, 2016)

The antioxidant strength of a compound can be evaluated by investigating its effect on a number of oxidation indicators. Generally *in vitro* antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH• method is furthermore rapid, simple (i.e. not involved with many steps and reagents) and inexpensive in comparison to other test models. This stable free radical has a broad absorption band with a maximum at 517 nm, although if it is quenched by an antiradical compound, it loses this property (Lo Scalzo, 2008). The seeming convenience of these methods is covered by their major limitations, which make them suitable only for

preliminary screening procedures Therefore, the results obtained with these methods indicate a “radical trapping power” or a “reducing power”. They are commonly expressed as IC_{50} and because it depends on the reaction time, this parameter does not provide meaningful information of the actual reactivity of the antioxidant when considered alone; furthermore, data can only be compared when obtained under identical settings.(Amorati & Valgimigli, 2015).

Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids. As oxidation normally proceeds very slowly at the initial stage, the time to reach a sudden increase in oxidation rate is referred to as the induction period (Shahidi & Zhong, 2005). Accelerated stability methods, like the active oxygen method (AOM), the oxygen bomb test, the Rancimat method, and the Schaal oven test, are designed to expedite the oxidation process by manipulating pro-oxidant conditions, such as temperature, oxygen pressure, and light exposure, in order to determine the oxidative stability of fats or fat-containing foods and antioxidant activity of antioxidant compounds, within a short time (Liang & Schwarzer, 1998). The simplicity of the Schaal oven test determination probably explains why it is today still used to measure the effectiveness of an antioxidants in a lipidic matrix. The main difference with an *in vitro* test is that this method tests antioxidants in close-to-real settings, it tests the antioxidant ability to inhibit the oxidation of a substrate (Amorati & Valgimigli, 2015).

Essential oils are complex mixtures of volatile compounds obtained from aromatic and medicinal plants mainly by steam distillation (Amorati & Foti, 2012) that have shown antioxidant activity (Asensio, Grosso, & Juliani, 2015a; Quiroga, Grosso, Lante, et al., 2013). This complexity makes it often difficult to explain the aforesaid activities (Ruberto & Baratta, 2000). In recent years, essential oils and their components have been actively

investigated to replace synthetic antioxidants, being more accepted by consumers because they are perceived as safe and for their functional and sensory properties. However, they showed lower activity than synthetic ones (Asensio, Grosso, & Juliani, 2015b; Asensio et al., 2012; Quiroga et al., 2015; Quiroga, Grosso, & Nepote, 2013).

α-terpinene is a monoterpene found in the essential oils of a large variety of foods and aromatic plants such as *Mentha piperita* (Yadegarinia et al., 2006), *Melaleuca alternifolia* (Baldissera et al., 2014) and oregano (Quiroga, Grosso, Lante, et al., 2013). Its antioxidant activity has been demonstrated (Kim et al., 2004; Ruberto & Baratta, 2000). According to Rudbäck, Bergström, Börje, Nilsson, & Karlberg (2012) α-terpinene is a true antioxidant since it autoxidizes rapidly compared with many other compounds, preventing these from degradation.

Organic acids are recognized for its acidifying properties and have been poor studied as promoter agents of natural antioxidants (Pereira et al., 2010). Some organic acids have shown antioxidant activity preventing lipid peroxidation (Kayashima & Katayama, 2002). To date, few data have been found on the possible synergic action of organic acids when mixed with non-phenolic compounds as DPPH• scavengers molecules (Lo Scalzo, 2008). Previous studies attribute a direct action of some organic acid on the free radicals scavenging activity of plant extracts (Pereira et al., 2010). Knowledge of their contributions to the antioxidant activity would bring new development prospects for these products (Piang-Siong et al., 2017).

The objective of this study was to evaluate the properties of organic acids (acetic, malic and citric acids) as promoter agents for antioxidant activity of α-terpinene.

2. Materials and methods

2.1.Materials

α-terpinene (α-ter), butylated hydroxytoluene (BHT) and 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). Organic acids (OAs): acetic (AA), malic (MA) and citric (CA) acids, were purchased from Cicarelli Laboratorios (Buenos Aires, Argentina). Canola oil (CO) was purchased in a local market (Aceite Krol, Buenos Aires, Argentina).

2.2.Free radical scavenging activity on DPPH

2.2.1. Individual dose–response effects

The DPPH• scavenging method was adapted from Quiroga, Grosso, Lante, et al., (2013). DPPH solution was prepared in methanol (0.1 mM) which provided an absorbance of 1.00 ± 0.2 units at 517 nm. Briefly, 100 µL of tested compounds methanolic solution (α-ter, BHT, AA, CA, MA) were added in 900 µL of DPPH• methanolic solution reaching 1 ml final volume. The absorbance of the samples was measured after 30 min on a spectrophotometer (SP-2100UV, UV-V Spectrophotometer Spectrum, Shanghai, China) at 517 nm. DPPH• inhibition percentage (%I) of the samples was calculated according to the following formula:

$$\%\, I = \left(1 - \frac{A}{A_0}\right) \times 100$$

where A is the absorbance of DPPH solution with the tested compounds (α-ter, BHT, AA, CA, MA), A_0 is the absorbance of DPPH solution. (Quiroga, Grosso, Lante, et al., 2013; Quiroga, Riveros, Zygadlo, Grosso, & Nepote, 2011). The concentration that inhibits the 50% of DPPH• (IC_{50}) was calculated from the curve obtained by plotting the inhibition percentage vs. the final antioxidant concentrations (Larrauri, Zunino, et al., 2016; Quiroga, Grosso, Lante, et al., 2013).

2.2.2. Determination of the DPPH• scavenging activity synergistic effect between organic acids and α-terpinene

Determination of the synergistic effects was adapted from Prieto, Curran, Gowen, & Vázquez (2015). Briefly, 50 μL of each compound methanolic solution were added to 900 μL of DPPH• methanolic solution. All other conditions were the same as previously described. The %I of the mixtures was calculated as was described above

For each α-ter concentration used (1.578 to 25.242 mM), the IC_{50} of each organic acid was calculated.

The synergistic effect in DPPH• assay (SE_{DPPH}) of a mixture was defined by the ratio of the experimental (% I_M) and the theoretical (% I_T) inhibition percentage:

$$SE_{DPPH} = \frac{\%\ I_M}{\%\ I_T}$$

$$\%I_T = \%I_{\alpha-ter} + \%I_{OA} - \frac{\%I_{\alpha-ter} \times \%I_{acid}}{100}$$

where % $I_{α\text{-ter}}$ and % I_{OA} respectively represent the inhibition percentage of DPPH• by the α-ter and each OA used alone. A synergistic effect is found when the SE_{DPPH} is greater than 1 (Piang-Siong et al., 2017).

2.3.Determination of antioxidant activity in canola oil (Schaal oven test)

α-ter and AA were also tested on canola oil by an accelerated oxidation test (Larrauri, Zunino, et al., 2016; Quiroga et al., 2011). Canola oil has the lowest content of saturated fatty acid (SFA) of any oil available in the market. The healthy monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) account for more than 93% of the fats in canola oil (Falahatkar, Asheri, Safarpour Amlashi, & Ershad Langroudi, 2018). The susceptibility of fatty acids to oxidation is directly dependent on the degree of unsaturation of the fatty acids (Quiroga et al., 2015; Riveros et al., 2010). Canola oil was chosen because its high amount of MUFA and PUFA that makes it susceptible to deterioration.

Six canola oil (CO) samples were prepared: CO without additives was used as control sample (CO-C), and CO with BHT (CO-BHT) was used as reference, CO with α-terpinene (CO-α-ter), CO with acetic acid (CO-AA), CO with BHT and AA (CO-BHT+AA), and CO with α-ter and AA (CO-α-ter+AA). All antioxidant compounds were added to canola oil in 0.02% (w⁄w) concentration. This value is the maximum concentration allowed for BHT and other synthetic antioxidants in vegetable oils by the Argentinean Food Code (A.N.M.A.T., 2018).

The accelerated oxidation test was carried out in a laboratory oven at 40 ± 1 °C for 18 days. Samples were analysed at 0, 5, 11 and 18 days. Lipid oxidation of samples was evaluated by determining peroxide value (PV) (AOAC, 1990) and *p*-anisidine value (AV) (IUPAC, 1979). Total oxidation (TOTOX) values were estimated as 2PV + AV (Shahidi & Zhong, 2005; Wang et al., 2018).

2.3.1. Antioxidant activity index (AAI).

The AAI is the ratio between the induction period (IP) of the sample with antioxidant and the sample without antioxidant.

$$AAI = \frac{IP\ of\ canola\ oil\ with\ antioxidant}{IP\ of\ canola\ oil\ without\ antioxidant}$$

AAI > 1 indicates the anti-oxidative potential of additives (Quiroga, Grosso, Lante, et al., 2013; Wang et al., 2018). The induction period was considered as the number of days needed for the PV of the samples to become 20 meq O_2/kg^{-1} of oil. This is in agreement with a general consideration that oils become rancid at PV higher than 20 (Wang et al., 2018).

2.3.2. Determination of the synergistic effects between α-terpinene and acetic acid in canola oil

To evaluate the interaction between α-terpinene and acetic acid in canola oil, it was used a similar approach than DPPH assay. The synergistic effect in canola oil (SE_{CO}) of a mixture was defined by the ratio of the experimental value of the induction period (Wang et al., 2018) of the mixture (IP_M) and the calculated theoretical value (IP_T):

$$SE_{CO} = \frac{IP_M}{IP_T}$$

$$\mathrm{IP}_T = \mathrm{IP}_{\alpha-ter} + \mathrm{IP}_{AA} - \frac{\mathrm{IP}_{\alpha-ter} \times \mathrm{IP}_{AA}}{100}$$

where $IP_{\alpha\text{-ter}}$, IP_{AA} and IP_M represent the induction period of canola oil with α-ter and AA used alone and mixed, respectively. A synergistic effect is found when the SE is greater than 1.

2.4.Statistical analysis

All analyses were carried out in three repetitions. The data were analysed using the InfoStat software, version 2017p (Di Rienzo et al., 2018). Means and standard deviations were calculated. Analysis of variance (ANOVA, α=0.05) and Di Rienzo, Guzmán, & Casanoves (2002) (DGC) test were used to find significant differences among means.

3. Results and discussion

3.1.Free radical scavenging activity on DPPH

3.1.1. Individual dose–response effects

The DPPH• reaction has been widely used for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds (Larrauri, Zunino, et al., 2016; Lo Scalzo, 2008; Prieto et al., 2015; Quiroga, Grosso, Lante, et al., 2013).

The anti-radical activity of α-ter and AA were low, therefore, it was not possible to calculate the IC_{50} with the used concentrations. For the highest concentration used of α-

ter (25.24 mM) and AA (15.52 mM) the %I was 3.02 ± 0.15 and 1.40 ± 0.07, respectively. For the others organic acids antiradical activity was not detected at the used concentrations (0.1 a 1.26 mM for MA and 0.24 to 2.91 mM for CA). Conversely, BHT had a high DPPH• scavenging activity, having an IC_{50} value of 0.035 mM.

Lo Scalzo (2008) and Pereira *et al.* (2010) reported no scavenging activity for AA, MA and CA when used alone. Kim et al. (2004) also found low scavenging activity of DPPH• for α-ter, Larrauri *et al.* (2016) found a similar IC_{50} for BHT against DPPH•.

3.1.2. Determination of the synergistic effects between α-terpinene and each organic acid

When BHT was mixed with OA, at the used concentrations, no activity against DPPH• was observed. This is in agreement with previous reports. AA reduced the rate constant for reaction of DPPH• with different phenols to a limiting value, since AA addition leads to the suppression of the SPLET mechanism in methanol (Litwinienko & Ingold, 2003). Piang-Siong *et al.* (2017) did not found DPPH• synergy scavenging activity for AA and CA when added to phenolic natural antioxidants such as gallic acid and caffeic acid.

When used alone, α-ter or OAs showed low or any activity against DDPH, when those compounds were mixed the activity was much higher. At each α-ter concentration, OAs exhibited a concentration-dependent activity against DPPH•. IC_{50} values of each organic acid at each α-ter concentration are shown in Table 1. Statistically significant differences were found between OAs.

In general, α-ter combined with MA had higher scavenging activity and with AA had the lowest activity. Some concentrations of α-ter with CA had similar activity than α-ter with MA. These results are in agreement with those found by Lo Scalzo (2008) where

OAs had the same behaviour when they are added to a solution of ascorbic acid showing a synergistic effect against DPPH•, with no significant differences between MA and CA.

As the mixture of α-ter and OA showed DPPH• scavenging activity, synergistic effect between them was calculated. Table 2 shows the SE_{DPPH} calculated for the IC_{50} of each OA at different α-ter concentrations. MA and CA had de same SE value since these OAs did not show activity against DPPH•.

The highest SE_{DPPH} (121.8 ± 3.8, %I = 61.5) was found for the combination of α-ter (1.58 mM, %I = 0.5) and CA (2.91 mM, %I = 0), this value had not significant difference with the highest SE_{DPPH} (121.5 ± 5.6 %I = 61.3) found for the combination of α-ter (1.58 mM, %I = 0.5) and MA (1.28 mM, %I = 0). The lower MA concentration suggest that MA had higher synergistic effect than CA when added to α-ter. The combination of α-ter and AA with the highest SE_{DPPH} (48.9 ± 1.6, %I = 70.3) was 6.28 mM (α-ter, %I = 0.7) and 3.88 mM (AA, %I = 0.7). Then for higher OAs concentration SE values were decreased. This trend was observed for all OAs.

On the basis of IC_{50} and SE_{DPPH}, the order of synergistic effect of the OAs tested on the α-ter DPPH• scavenging activity is: MA > CA > AA.

Pereira *et al.* (2010) proposed that *Catharanthus roseus* roots extract, where phenolics compounds were no found, had higher activity against DPPH because of the much higher OAs concentration than other plant parts.

3.2.Determination of oxidative stability of canola oil under an accelerated oxidation test

Results of the chemical indicators: PV, AV and TOTOX values, of different canola oil samples (CO-C, CO-BHT, CO-α-ter, CO-AA, CO-BHT+AA, CO- α-ter+AA) during storage at 40 °C are shown in Figures 1a, 1b and 1c, respectively. In general, these indicators increased significantly ($\alpha = 0.05$) in all treatments with storage time. Initially,

all treatments had the same PVs (3.32 meq O_2 kg^{-1}), AVs (0.50) and TOTOX (7.15) values. Significant differences were detected between samples from the 5^{th} day onwards.

PV is a chemical indicator used to determine primary reaction products of lipid oxidation (Shahidi & Zhong, 2005). From the 11th day onwards, all treatments had significantly lower PV compared with CO-C sample (Fig. 1a). On 18th day of storage, the lowest PVs were found on CO-BHT and CO-BHT+AA samples, followed by CO-AA and CO- α-ter+AA samples, and by CO-α-ter. These results indicate that all compounds (BHT, AA, α-ter) and their combinations (BHT+AA, and α-ter+AA) had antioxidant activity in canola oil. BHT had similar activity when was mixed with AA. The mixture α-ter+AA had higher effect on canola oil than α-ter, and similar to AA, suggesting this combination did not had synergistic effect at these conditions and concentrations.

In advanced stages of lipid oxidation, organic peroxides decompose into secondary products, including alcohols, carboxylic acids, aldehydes and ketones, which can be measured by the AV method (Shahidi & Zhong, 2005). At day 18 of storage, CO-AA, CO-BHT and CO-BHT+AA samples had lower AVs than CO-α-ter, CO-α-ter+AA and CO-C samples, with no significant differences between them (Fig. 1.b). These results suggested that α-ter and the mixture with AA do not prevent canola oil from lipid secondary oxidation.

Since the lipid oxidation is a complex multistep process it is important to study the ability of antioxidants to inhibit the various steps of this process (Marinova, Toneva, & Yanishlieva, 2008).

TOTOX value is calculated as from the PV and AV, indicating overall oxidation of canola oils in the accelerated oxidation test. From day 5 onwards, all treatments had significantly lower TOTOX value compared with the control having CO-BHT and CO-BHT+AA treatments the lowest values (Fig. 1.c). At the end of storage (18 days), CO-C

had the higher TOTOX value (135.05). CO-BHT (101.75) and CO-BHT+AA (100.75) had the lower values, without significant differences between them. CO-AA (107.66), CO-α-ter (117.96) and CO-α-ter+AA (111.48) had higher values than BHT samples but lower than control, without significant differences between them. These results indicate AA, α-ter, BHT and the mixtures of α-ter+AA and BHT+AA showed antioxidant activity on canola oil.

3.2.1. Antioxidant activity index (AAI) and synergistic effect between α-terpinene and acetic acid in canola oil

IP, AAI and SE for all tested antioxidants and their mixtures on canola oil are presented in Table 3. The highest AAI was observed in the sample with BHT+AA and the lowest in the sample with α-ter. These results indicate AA, α-ter and the mixtures α-ter+AA and BHT+AA showed antioxidant activity in canola oil.

Previous studies report the antioxidant effects of BHT added to sunflower (Quiroga et al., 2015; Quiroga, Grosso, & Nepote, 2013) and almonds products (Larrauri, Demaría, et al., 2016). OA and MA have an effective antioxidant activity inhibiting TBARS (Kayashima & Katayama, 2002).

On the basis of PV, AV, TOTOX values measurements and AAI, the order of anti-oxidative activity is: BHT+AA = BHT > AA = α-ter+AA > α-ter.

The SE_{CO} calculated for the mixture of α-ter with AA and BHT with AA are showed in Table 3. This value (SE_{CO}< 1) indicate any synergic effect between the compounds in preventing the lipid oxidation of canola oil sample.

As DPPH• scavenging free radical activity is based on an environment different from a real lipid oxidation condition, it is hardly associated with the potential of an antioxidant

in preventing the lipid oxidation of food (Van Ha, Pokorný, & Sakurai, 2007; Zhang, Shen, Zhu, & Xu, 2015).

4. Conclusions

The DPPH• scavenging activity of α-ter is influenced by other components like OAs that are not directly involved in the free radical scavenging activity. It is evident that an interaction exists between α-ter and some widely diffused OAs. MA had a greater effect enhancing the α-ter DPPH• scavenging activity. The DPPH• assay was not able to predict which compounds o mixture of compounds were the most effective antioxidants in canola oil. While α-ter and AA had very low activity when used alone, the mixture of them at different concentrations was more effective as DPPH free radical scavenger. Conversely, in the canola oil accelerated oxidation test, AA was more effective than the mixture α-ter+AA and α-ter had the lowest activity. In another hand, BHT used alone had high DPPH• scavenging activity and when is mixed with an OA had any activity against DPPH•. Contrariwise, when BHT was tested in the canola oil accelerated oxidation test had the same activity alone and mixed with AA. Because DPPH• assay was made in a polar solvent, it is unable to determine the antioxidant activity of the different compounds in canola oil. This could explain why the free radical scavenging activity of a compound did not relate to its ability to inhibit canola oil lipid oxidation.

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Table 1. IC_{50} (average and standard deviation) of each organic acid: malic (MA), citric (CA) and acetic (AA), at different α-terpinene (α-ter) concentrations

[α-ter] mM	[MA] mM				[CA] mM				[AA] mM			
1.578	0.650	±	0.020	a	1.117	±	0.022	b	4.791	±	0.191	c
3.155	0.246	±	0.004	a	0.305	±	0.008	b	2.063	±	0.037	c
6.311	0.161	±	0.002	a	0.153	±	0.005	a	1.274	±	0.041	b
9.466	0.116	±	0.002	a	0.131	±	0.010	a	0.945	±	0.002	b
12.621	0.093	±	0.004	a	0.123	±	0.006	a	0.709	±	0.037	b
18.932	0.072	±	0.004	a	0.109	±	0.008	b	0.561	±	0.010	c
25.242	0.058	±	0.002	a	0.092	±	0.004	b	0.546	±	0.017	c

Values with different letters within each raw are significantly different (DGC test. α = 0.05. n = 3).

Table 2. SE_{DPPH} of IC_{50} of each organic acid: malic (MA), citric (CA) and acetic (AA), at different α-ter concentrations.

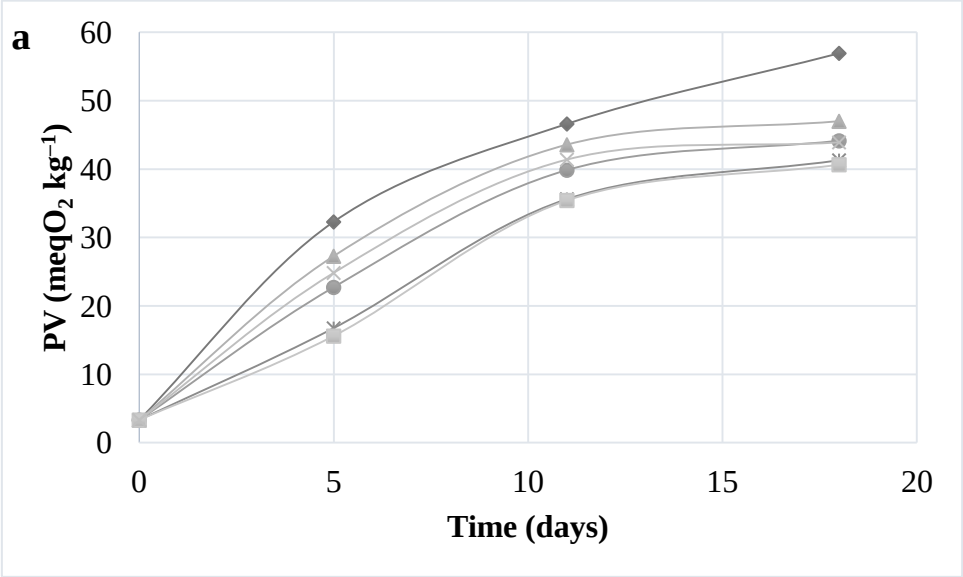
[α-ter] mM	SE MA	SE CA	SE AA
1,578	99,1 ± 4,0 b	99,1 ± 4,0 b	36,2 ± 0,8 a
3,155	73,8 ± 2,4 b	73,8 ± 2,4 b	40,0 ± 2,7 a
6,311	67,9 ± 2,4 b	67,9 ± 2,4 b	44,1 ± 3,9 a
9,466	46,1 ± 2,4 b	46,1 ± 2,4 b	36,3 ± 2,9 a
12,621	42,8 ± 1,0 b	42,8 ± 1,0 b	36,9 ± 3,5 a
18,932	29,3 ± 1,6 a	29,3 ± 1,6 a	26,5 ± 2,1 a
25,242	16,6 ± 0,8 a	16,6 ± 0,8 a	15,7 ± 0,3 a

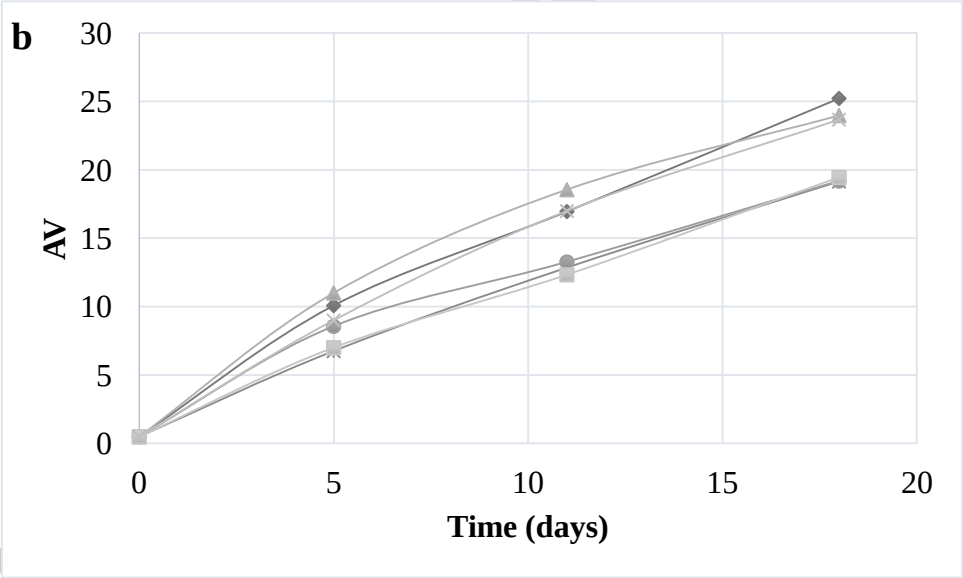
Values with different letters within each raw are significantly different (DGC test. α = 0.05. n = 3).

Table 3. Induction period (IP), Antioxidant activity index (AAI) and synergistic effect in canola oil (SE_{CO}) (averages and standard deviations) for samples: canola oil control (CO-C), canola oil with BHT (CO-BHT), with α-terpinene (CO-α-ter), with acetic acid (CO-AA), with BHT and acetic acid (CO-BHT+AA), and with α-terpinene and acetic acid (CO-α-ter+AA), stored at 40 ºC.

Sample	IP	AAI	SE_{CO}
CO-C	2,99 ± 0,10 a	1,00 ± 0,03 a	
CO-α-ter	3,53 ± 0,08 b	1,18 ± 0,03 b	
CO-α-ter+AA	4,05 ± 0,19 c	1,36 ± 0,06 c	0,54 ± 0,02
CO-AA	4,17 ± 0,06 c	1,40 ± 0,02 c	
CO-BHT	5,45 ± 0,08 d	1,82 ± 0,03 d	
CO-BHT+AA	5,51 ± 0,06 d	1,84 ± 0,02 d	0,58 ± 0,01

Values with different letters within each column are significantly different (DGC test. α = 0.05. n = 3).





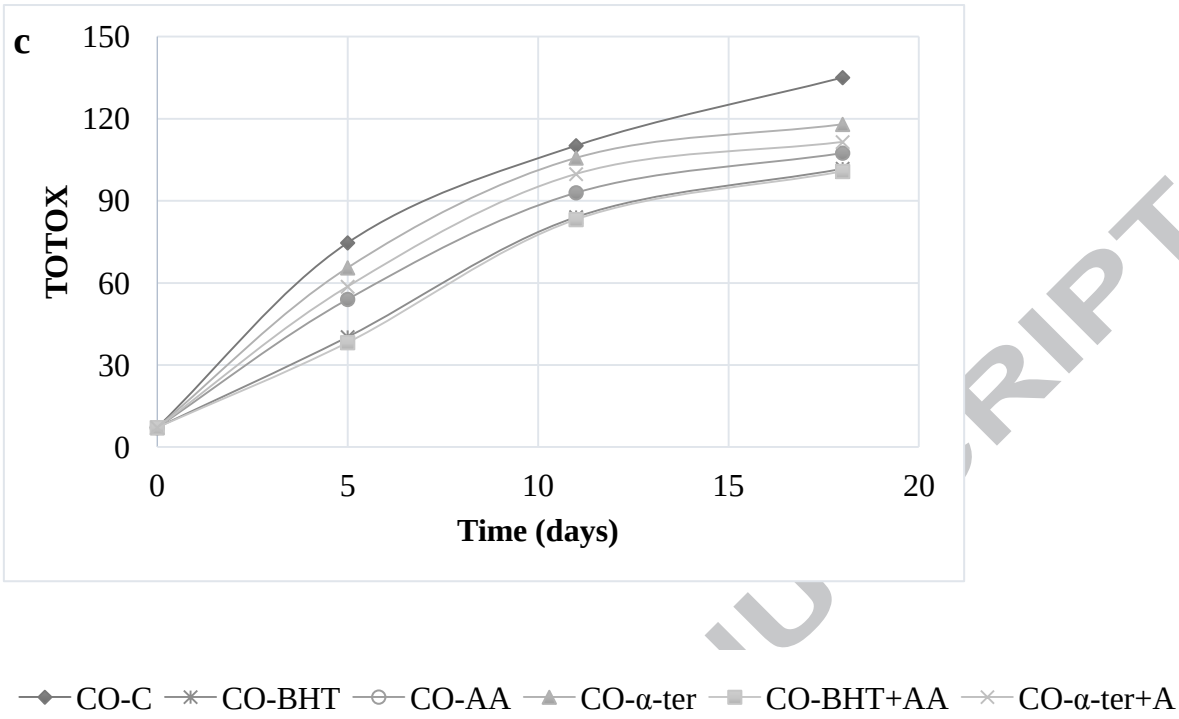


Figure 1. (a) Peroxide value (PV), (b) p-anisidine value (AV) and (c) TOTOX value evaluated in samples of canola oil control (CO-C), with BHT (CO-BHT), with α-ter (CO-α-ter) with AA (CO-AA) and α-ter + AA (CO-α-ter+AA) during storage.

Highlights

- α-terpinene DPPH• radical scavenging activity is enhanced by organic acids
- Acetic acid does not interact with α-terpinene in preventing oil lipid oxidation
- DPPH• radical scavenging activity does not associate with lipid oxidation test