Explaining the antioxidant activity of some common non-phenolic components of essential oils

Highlights

- The antioxidant activity of limonene, linalool and citral was investigated.
- The three EO components act as termination-enhancing antioxidants.
- Activity increases with the concentration up to a limit value, then it decreases
- The three EO components become pro-oxidant above a critical concentration.
- Their potential in food protection versus phenolic antioxidants is discussed.

Explaining the antioxidant activity of some common non-phenolic components of essential oils

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Running Title: Antioxidant activity of non-phenolic EO components

Abstract

Limonene, linalool and citral are common non-phenolic terpenoid components of essential oils, with attributed controversial antioxidant properties. The kinetics of their antioxidant activity was investigated using the inhibited autoxidation of a standard model substrate. Results indicate that antioxidant behavior of limonene, linalool and citral occurs by co-oxidation with the substrate, due to very fast self-termination and cross-termination of the oxidative chain. Rate constants k_p and $2k_t$, ($M^{-1}s^{-1}$) at 30°C were 4.5 and 3.5 $\times 10^6$ for limonene, 2.2 and 9.0 $\times 10^5$ for linalool and 39 and 1.0 $\times 10^8$ for citral. Behavior is bimodal antioxidant/prooxidant depending on the concentration. Calculations at the M05/6-311+g(2df,2p) level indicate that citral reacts selectively at the aldehyde C-H having activation enthalpy and energy respectively lower by 1.3 and 1.8 kcal/mol compared to the most activated allyl position. Their termination-enhancing antioxidant chemistry might be relevant in food preservation and could be exploited under appropriate settings.

Keywords: essential oil, autoxidation, antioxidant, peroxyl radicals, linalool, limonene, citral

Chemical compounds investigated in this study

(*R*)-Limonene (PubChem CID: 440917)
Linalool (PubChem CID: 6549)
Citral (PubChem CID: 8843)
Dodecanal (PubChem CID: 8194)

1. Introduction

Among the various strategies aimed at improving food preservation, antioxidants play an important role because they are able to slow down the oxidation of unsaturated lipids, preventing the development of rancidity in foods (Caleja, Barros, Antonio, Oliveira, & Ferreira, 2017; Guitard, Paul, Nardello-Rataj, & Aubry, 2016). In recent years, essential oils have been actively investigated to replace synthetic antioxidants (Amorati, Foti, & Valgimigli, 2013; Tohidi, Rahimmalek, & Arzani, 2017). Essential oils are complex mixtures of volatile compounds obtained from aromatic and medicinal plants mainly by steam distillation (Amorati & Foti, 2012). For instance, thyme and oregano essential oils have been proposed to contrast oxidative spoilage in various kinds of food (Otoni, Pontes, Medeiros, & Soares, 2014), in particular meat (Fasseas, Mountzouris, Tarantilis, Polissiou, & Zervas, 2008) and fish (Kykkidou, Giatrakou, Papavergou, Kontominas, & Savvaidis, 2009). These two essential oils contain significant amount of thymol and carvacrol, two phenolic components having antioxidant activity similar to that of synthetic phenolic antioxidants, such as butylated hydroxy toluene (BHT) (Perez-Roses, Risco, Vila, Penalver, & Canigueral, 2016). Phenols are in fact prototypical chain-breaking antioxidants. They are able to slow down the peroxidation of unsaturated lipids by formally donating a H-atom from the phenolic hydroxyl group to a peroxyl radicals (ROO•) that is responsible for the propagation of the oxidative radical chain (PhOH + ROO• → PhO• + ROOH)(Amorati, Foti, & Valgimigli, 2013).

Unlike peroxyl radicals, the resulting phenoxyl radical (PhO•) is normally unable to propagate the oxidative chain, *i.e.* it is sufficiently unreactive to "wait" in solution until it traps a second peroxyl radical (PhO• + ROO• → non-radical products), thereby breaking two oxidative chains (Amorati, Baschieri, Morroni, Gambino, & Valgimigli, 2016).

However, in recent years, other essential oils that have no significant content of phenolic components have been claimed to possess relevant antioxidant activity. Unfortunately, such claims have rarely been supported by a clear understanding of the mechanisms at the basis of the purported antioxidant behaviour, and other studies outlining no significant antioxidant activity for the same

essential oils have also appeared in the literature, creating a very confusing picture (Amorati, Foti, & Valgimigli, 2013).

For instance, Domingues and co-workers studied the antioxidant activity of coriander essential oil, which doesn't contain any phenolic component but is rich in linalool, and they reported that "coriander oil and linalool had relevant radical scavenging properties and an exceptional capacity to inhibit the lipid peroxidation" (Duarte, Luis, Oleastro, & Domingues, 2016). Maróstica Junior and co-workers reported that limonene was able to inhibit liver homogenate peroxidation, induced by ferric chloride and ascorbic acid (Marostica, Silva, Franchi, Nowill, Pastore, & Hyslop, 2009). Bruni and co-workers found that lemongrass (*Cymbopogon citratus*) essential oil, rich in citral, had a fairly good antioxidant activity toward the autoxidation of linoleic acid, as assessed by the β-carotene bleaching test (Sacchetti, Maietti, Muzzoli, Scaglianti, Manfredini, Radice, et al., 2005). On the other hand, Ruberto and Baratta, by studying the autoxidation of egg yolk homogenate, found pro-oxidant effect for linalool, and almost negligible antioxidant effect for limonene and citral (Ruberto & Baratta, 2000).

Currently, the only well understood example of a non-phenolic essential oil component endowed with significant antioxidant activity is that of γ-terpinene, a monoterpene component that is able to slow down the autoxidation of methyl linoleate by a co-oxidation mechanism, where the terpene causes a faster oxidative chain-termination due to the generation of hydroperoxyl radicals (HOO•) that have very fast self-termination rate constant ($HOO\bullet + HOO\bullet \rightarrow O_2 + HOOH$) (Foti & Ingold, 2003).

Indeed, the possible contribution of other non-phenolic components to the antioxidant activity of essential oils remains an open question. If confirmed and rationalized their antioxidant activity might be effectively exploited to supplement that of classical phenolic antioxidants, thus contributing to extend the shelf-life of easily oxidizable foods. In this work, we investigate in detail the antioxidant activity of three common non-phenolic essential oil components, limonene, linalool and citral, to rationalize the contrasting or unexplained results about their activity that can be found

in the literature. In order to do so, we studied the kinetics of oxygen uptake in the controlled inhibited autoxidation of a standard substrate (cumene), since this method is the best established and the most reliable to afford accurate mechanistic information on direct antioxidant activity (Amorati, Baschieri & Valgimigli, 2017; Amorati, et al 2016; Amorati, Pedulli, & Valgimigli, 2011; Burton, Doba, Gabe, Hughes, Lee, Prasad, et al., 1985), and we combined the kinetic measurements with quanto-mechanical calculations, to rationalize the results.

2. Materials and Methods.

2.1 Chemicals. (R)-(+)-Limonene, linalool, citral (mixture of *E/Z* isomers) and dodecanal were from Sigma-Aldrich (Milan, Italy) and were stored under argon at -18 °C. Cumene (isopropylbenzene) from Sigma-Aldrich was percolated once on silica and twice on alumina columns. Azobis-isobutyronitrile (AIBN, Fluka, Milan, Italy) was recrystallized from methanol. 2,6-di-*tert*-Butyl-4-metylphenol (BHT) and 2,2,5,7,8-pentamethyl-6-chromanol (PMHC) were both purchased from Sigma-Aldrich at the highest available purity and were recrystallized from hexane.

2.2 Autoxidation experiments. Autoxidation experiments were performed in a two-channel oxygen uptake apparatus, based on a Validyne DP 15 differential pressure transducer, built in our laboratory (Amorati, Lynett, Valgimigli, & Pratt, 2012; Amorati, Valgimigli, Diner, Bakhtiari, Saeedi, & Engman, 2013; Amorati, Zotova, Baschieri, & Valgimigli, 2015). In a typical experiment, an air-saturated solution of the oxidizable substrate (cumene) containing AIBN was equilibrated with an identical reference solution containing excess 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMHC, 25 mM). After equilibration, and when a constant O_2 consumption was reached, a concentrated solution of the essential oil component was injected in the sample flask. The oxygen consumption in the sample was measured after calibration of the apparatus from the differential pressure recorded with time between the two channels. Initiation rates, R_i, were determined for each condition in preliminary experiments by the inhibitor method using PMHC as a reference antioxidant: $R_i = 2[PMHC]/\tau$, where τ is the length of the inhibition period (Matera, Gabbanini, Berretti, Amorati, De

Nicola, Iori, et al., 2015; Valgimigli, Amorati, Petrucci, Pedulli, Hu, Hanthorn, et al., 2009; Valgimigli, Bartolomei, Amorati, Haidasz, Hanthorn, Nara, et al., 2013). The concentration range for the test antioxidants in our experiments was: 28-2800 mM for linalool, 30-1500 mM for limonene, 0.15-90 mM for Citral and 0.11-56 mM for reference dodocanal.

2.3 Calculations.

Geometry optimizations, frequencies, enthalpies and transition states barriers were computed in the gas phase at M05/6-311+g(2df,2p) (Galano, Munoz-Rugeles, Alvarez-Idaboy, Bao, & Truhlar, 2016; Tishchenko & Truhlar, 2012) theory level, by using Gaussian 09, according to previously established protocols. Stationary points were confirmed by checking the absence of imaginary frequencies. Transition states had one imaginary frequency corresponding to the transfer of a H-atom (see Appendix A). Bond dissociations and reaction enthalpies were calculated also by the high accuracy composite method CBS-QB3 (Montgomery, Frisch, Ochterski, & Petersson, 1999; Zielinski, Presseau, Arnorati, Valgimigli, & Pratt, 2014). For the sake of comparison, calculations were also repeated at the M06-2X/6-311++G(d,p) level of theory (La Rocca et al., 2016; Galano, 2011): the results, summarized in Figure 3S (see Appendix), qualitatively confirm those calculated at the M05/6-311+g(2df,2p) level.

3. Results and discussion

3.1 Inhibited autoxidation studies

The antioxidant activity of linalool, limonene and citral was investigated by studying the O_2 consumption during the controlled inhibited autoxidation of cumene (isopropylbenzene), which can be described by eq. 1-10. In the absence of essential oil components (EH) and in the presence of a source of free radicals (In) and atmospheric O_2, cumene (RH) is oxidized to cumene hydroperoxide (ROOH) through a radical-chain mechanism described by equation 1 (initiation), 2 and 4 (propagation), and 8 (termination) (Amorati, et al. 2015; Burton, et al., 1985).

$$In \rightarrow R\bullet \qquad (1)$$

$R\bullet + O_2 \rightarrow ROO\bullet$ (2)

$E\bullet + O_2 \rightarrow EOO\bullet$ (3)

$ROO\bullet + RH \rightarrow ROOH + R\bullet$ (4)

$ROO\bullet + EH \rightarrow ROOH + E\bullet$ (5)

$EOO\bullet + EH \rightarrow EOOH + E\bullet$ (6)

$EOO\bullet + RH \rightarrow EOOH + R\bullet$ (7)

$2\ ROO\bullet \rightarrow$ non radical products (8)

$ROO\bullet + EOO\bullet \rightarrow$ non radical products (9)

$2\ EOO\bullet \rightarrow$ non radical products (10)

The source of radicals is represented by the decomposition of azobis-isobutyronitrile (AIBN), a liposoluble azo-initiator, at 30 °C, which provides a constant initiation rate, indicated as R_i. Upon addition of increasing amounts of reference phenolic antioxidants (PhOH) like BHT, or PMHC a synthetic mimic of natural α-tocopherol having identical antioxidant performance (Burton, et al., 1985) the rate of oxygen consumption is dramatically reduced by the effect of reactions 11 and 12, until the antioxidant is consumed, and it is reversely proportional to the concentration of the antioxidant - i.e. the antioxidant performance increases linearly with the concentration of the antioxidant - as described by Howard-Ingold equation 13 (Burton, et al., 1985) where R_i is the rate of radical initiation (typically 2-9 $\times$ 10^{-9} Ms^{-1} in our system) and k_p is the rate constant of chain propagation for cumene (k_p = 0.34 $M^{-1}s^{-1}$ at 303K; (Amorati, Lynett, Valgimigli, & Pratt, 2012)). The term k_{inh} is usually determined from these kinetic measurements and represents the rate constant of reaction of the antioxidant with chain-propagating peroxyl radicals (ROO•).

$PhOH + ROO\bullet \rightarrow PhO\bullet + ROOH$ (11)

$PhO\bullet + ROO\bullet \rightarrow$ non radical products (12)

184 $$-\frac{d[O_2]_{inh}}{dt} = \frac{k_p[Cumene]R_i}{2k_{inh}[Antiox.]} \qquad (13)$$

185

186 Unlike phenolic antioxidants, when non-phenolic essential oil components (EH) were added to
187 cumene autoxidizing mixture, no distinct inhibition period was observed and the rate of oxygen
188 consumption was decreased or increased as compared to cumene alone, depending on the
189 concentration of EH, but without a monotonic correlation with the concentration. In other words,
190 EH acted as antioxidant or as pro-oxidant depending on the experimental settings, as illustrated in
191 Figure 1A for citral.

192 <Figure 1 about here>

193 When the rate of oxygen consumption (i.e. the rate of autoxidation) was plotted against the
194 concentration of EH added to the system, any of the essential oil components showed a bimodal
195 behavior as illustrated in Figure 1B-D. Below a critical concentration (from 4 % (v/v) for linalool to
196 0.12 % (v/v) for citral) all components EH reduce the oxidation rate, while at higher concentrations
197 they cause an increase of O_2 uptake.

198 These experimental results cannot be ascribed to a classical chain-breaking antioxidant action like
199 that observed in the presence of PMHC or BHT, which strongly inhibits cumene autoxidation at
200 concentrations as low as 5-10 μM (Amorati, et al. 2015). On the contrary, a similar behavior was
201 observed by co-oxidizing cumene and certain non-phenolic substrates such as garlic allylsulfides
202 (Amorati & Pedulli, 2008). We reasoned that the explanation of this phenomenon resides in the
203 complex interplay between peroxyl radicals generated by the two substrates, ROO• for cumene and
204 EOO• for essential oil components, as described by equations 1-10. Essential oils components
205 generate secondary peroxyl radicals that undergo bimolecular self-termination (eq. 10) and cross-
206 termination (eq. 9) much more quickly than tertiary cumylperoxy radicals (eq. 8) (Lucarini, Pedulli,
207 & Valgimigli, 1998). The resulting rate of oxidation for the mixture is lower than for pure cumene
208 because the overall steady-state concentration of peroxyl radicals is reduced (Amorati & Pedulli,

2008). The magnitude of the unusual antioxidant effect increases with the reactivity of the components EH with peroxyl radicals, i.e. with k_5 and k_6 (eq. 5 and 6), because more peroxyl radicals would competitively react with EH rather than with the oxidizable substrate (in this case cumene), and, at the same time, it increases with the rate of termination due to the radicals from EH (sum of eq. 9 and 10) as compared to the self-termination of the substrate (eq. 8). On the other hand, above a critical value of k_5 and k_6 or above a critical concentration of the components EH, autoxidation of EH itself (which acts as co-oxidizable substrate) becomes so significant to carry on the whole autoxidation process, i.e. to increase the rate of O_2 uptake as compared to that measured with neat cumene (that has low rate constant of propagation, k_4), which results in the observed pro-oxidant behavior. It should be stressed that such kinetic behavior sharply differentiates this mechanism of inhibition from the classical chain-breaking activity, as the chain breaking activity does not depend on the rate of chain-termination, but only on the rate of reaction of peroxyl radicals with the antioxidant. Additionally, there is no upper-limit concentration for chain-breaking antioxidants, as their performance grows monotonically with their concentration.

The plots of O_2-consumption rate shown in Figure 1 were analyzed by using the co-oxidation reaction scheme (eq. 1-10). By assuming the usual steady-state approximation, the rate of co-oxidation (R_{ox}) can be derived as in eq. 14 (Amorati & Pedulli, 2008).

$$R_{ox} = \frac{\left\{k_4 k_7 [RH]^2 + 2k_5 k_7 [RH][EH] + k_5 k_6 [EH]^2\right\} R_i^{1/2}}{\left\{k_8 k_7^{\,2} [EH]^2 + k_9 k_5 k_7 [RH][EH] + k_{10} k_5^{\,2} [EH]^2\right\}^{1/2}} \qquad (14)$$

Here the propagation and the termination rate constants of cumylperoxyl radicals, $k_p = k_4$ and $2k_t = k_8$, are known (0.32 M^{-1} s^{-1} and 4.6 × 10^4 M^{-1} s^{-1}, respectively, at 30 °C) (Amorati & Pedulli, 2008). The other rate constants were obtained by fitting the plots of Fig. 1 to eq 14. Results are collected in Table 1.

3.2 Mechanism of reaction of limonene, linalool and citral with peroxyl radicals

Interestingly, in the case of limonene and linalool, very satisfactory fittings of experimental rate of

234 autoxidation (fig 1 C and D) were obtained when eq. 14 was simplified by assuming that the
235 kinetics of H-atom abstraction from cumene (RH) or the essential oil components (EH) is
236 independent from the structure of the peroxyl radical (i.e. $k_4 = k_7$ and $k_5 = k_6$) (Amorati & Pedulli,
237 2008). This finding was not surprising, since it is well known that the reactivity of alkylperoxyl
238 radicals is similar and largely independent of their structure (Amorati, Lynett, Valgimigli, & Pratt,
239 2012; Amorati, et al. 2015). The reactions of limonene and linalool can be exemplified as in Figure
240 2.

241 <Figure 2 about here>

242 Conversely, in the case of citral the same approximation did not afford acceptable matching of
243 experimental results and unrestricted fittings to eq. 14 (Fig. 1B) indicated that reaction of RH or EH
244 with peroxyl radicals derived from citral (eq. 6 and 7) was much faster than the analogous reactions
245 with radicals derived form the substrate cumene (eq. 4 and 5). This implies that the peroxyl radicals
246 derived form citral (EOO•) are much more reactive than those derived from the substrate (ROO•),
247 which suggests that citral autoxidizes by forming acylperoxyl radicals, rather than alkylperoxyl, i.e.
248 in citral H-abstraction by peroxyl radical occurs at the aldehyde group rather than at the available
249 allyl positions, similarly to the oxidation of simpler aldehydes (Li, Hong, Chen, Sun, Yang, Yu, et
250 al., 2016). Ingold and coworkers reported that aliphatic aldehydes are oxidized to peroxycarboxylic
251 acids through a radical chain mechanism sustained by acylperoxyl radicals (see Figure 3). In the
252 same work, acylperoxyl radicals were found to be about 70 and 60 fold more reactive than
253 alkylperoxyl radicals toward H-atom abstraction from hydrocarbons and from aldehydes,
254 respectively (Zaikov, Howard, & Ingold, 1969). Such values are identical, respectively, to the ratios
255 k_7/k_4 and k_5/k_6 determined by us with citral (see table 1). Howard and Ingold reported very high
256 termination constants for acylperoxyl radicals (such as 5.4×10^7 $M^{-1}s^{-1}$ at 0°C for heptanal), these
257 values are in agreement with those reported in Table 1 (Zaikov, et al., 1969).

258 <Figure 3 about here>

To verify this explanation and check the role of unsaturatated hydrocarbon skeleton in the reactivity of citral, we comparatively studied the autoxidation of cumene in the presence of dodecanal, an aliphatic aldehyde. The results are collected in figure 1S (see Appendix) and the corresponding kinetic constants are reported in Table 1 along with those recorded for citral. It can be noted that they are indeed very similar to those of citral, indicating that the aldehyde group is the moiety that characterizes the radical chemistry of citral.

Product studies performed by Karlberg et al. on the spontaneous autoxidation of limonene showed that it yields hydroperoxides in position 6 and epoxides in position 1,2 (Karlberg & DoomsGoossens, 1997). Such products can be explained as arising from secondary peroxyl radicals, which are formed from H-atom abstraction at the allylic CH_2 groups of the cyclohexene ring (see Figure 2), in excellent agreement with our kinetic data, affording $k_5 = k_6 = 4.5$ $M^{-1}s^{-1}$ at 303 K (for comparison the H atom abstraction from the allylic C-H bonds of methyl oleate and cyclohexene occurs with a rate constant of 0.89 and 6.0 $M^{-1}s^{-1}$, respectively; (Valgimigli & Pratt, 2012)).

Similarly, Skold et al. reported that the spontaneous autoxidation of linalool affords products (hydroperoxides and epoxides) derived from both tertiary and secondary peroxyl radicals formed upon H-abstraction in allylic positions (Figure 2) (Skold, Borje, Harambasic, & Karlberg, 2004), again in agreement with our measured average $k_5 = k_6 = 2.2$ $M^{-1}s^{-1}$.

Product studies available on citral are instead at odds with the above two compounds. Indeed, Hagvall et al. showed that the autoxidation products of geranial (one of the two geometric isomers constituting citral) consists mainly of the corresponding carboxylic acid and peracid (Figure 4, path A) along with secondary oxidation products that can only be explained as deriving from the acylperoxyl radicals formed after H-atom abstraction from the aldehydic group; conversely, none of the expected products arising from H-abstraction in allylic position (Figure 4, path B) on the terpenic hydrocarbon backbone could be observed (Hagvall, Backtorp, Norrby, Karlberg, & Borje, 2011). Such findings are in full agreement with our autoxidation experiments: indeed best-fit kinetic

285 constants collected in Table 1 indicate that the peroxyl radicals formed from citral propagate the
286 oxidation of citral itself 62-fold faster that peroxyl radicals derived form cumene with k_6 as large as
287 2.4 x10^3 $M^{-1}s^{-1}$, almost identical to that recorded for saturated dodecanal.

288 <Figure 4 about here>

289 ***3.3 Computational studies***

290 While our current kinetic studies and previous product studies converge indicating that citral reacts
291 with peroxyl radicals at the carbonyl position (Figure 4, path A) rather than at the many available
292 allylic positions (Figure 4, path B), the rationale for such reactivity is not obvious, since C-H bonds
293 at the allylic positions are expected to be weaker than aldehyde C-H groups (Warren, Tronic, &
294 Mayer, 2010), hence their reaction should be more facile. DFT (B3LYP) calculations by Hagvall et
295 al. (2011) indicates that the weakest C-H bond in allylic position in citral is 7.6 kcal/mol weaker
296 than aldehyde C-H bond. The authors suggested that, despite the less favorable thermodynamics,
297 the selective H-abstraction at the aldehyde C-H results from irreversibility of the subsequent
298 reaction of the acyl radical with oxygen to form acylperoxyl radicals (Figure 4, path A), as
299 compared to the reversible addition of oxygen to the stabilized allyl radical (path B) formed upon
300 H-abstraction from allylic positions (Hagvall, et al., 2011). Although this suggestion might be
301 correct, kinetic data reported herein indicate it is unlikely to justify the observed lack of reaction at
302 the allylic position in citral, particularly when the reactivity of citral is compared to that of
303 structurally related linalool, where expectedly similarly reversible formation of allylperoxyl radicals
304 guarantees efficient oxidative-chain propagation. To rationalize the reactivity of citral we turned to
305 quanto-mechanical calculations. The relative reactivities of the allylic and aldehydic C-H bonds
306 were investigated as shown in Figure 5. To economize on computational resources, the unsaturated
307 hydrocarbon portion of citral was simplified to 2-methyl-2-pentene and the aldehydic portion to 3-
308 methyl-2-butenal, i.e. the molecule was "truncated" into its two subunits. This follows the identical
309 approach previously used by Hagvall, et al., (2011) and is fully justified by the fact that the two
310 portions are electronically isolated: detaching the two subunits will not significantly influence the

311 reactivity of either the aldehyde or the allylic C-H group with peroxyl radicals. The enthalpy
312 difference between the products and the reactants, reported in Figure 5, was computed by high-level
313 CBS-QB3 and shows that the reaction at the -CH_2- allylic hydrogens is more exothermic than that at
314 the aldehydic hydrogen by 5.3 kcal/mol, in qualitative agreement with the results reported by
315 Hagvall et al (2011), the difference being attributable to the different level of calculation. Our
316 calculations indicate that bond dissociation enthalpies BDE_{C-H} of allylic and aldehyde moieties are
317 respectively 83.1 and 88.1 kcal/mol at 298K, while the BDE_{O-H} of the methylperoxyl radical was
318 computed as 86.2 kcal/mol. Additionally, transition states were computed at the M05/6-
319 311+g(2df,2p) level of theory, which has been demonstrated to accurately reproduce the barriers of
320 hydrogen atom transfer reactions (Galano et al., 2016;Tishchenko & Truhlar, 2012).
321 <Figure 5 here>
322
323 Most interestingly, calculations showed that, despite the less favorable thermodynamics, the barrier
324 for the abstraction of the aldehyde hydrogen is lower by 1.3 kcal/mol as compared to the abstraction
325 of the hydrogen atom from the most activated allylic position (see Figure 5). For confirmation,
326 calculations were repeated at the M06-2X/6-311++G(d,p) level (Galano, 2011; La Rocca et al.
327 2016), and were in qualitative agreement with the above results (see Appendix A for full
328 comparison among the two methods).
329 When entropy changes are taken into account, the calculated difference in activation energy
330 between the two reaction pathways is 1.8 kcal/mol. This difference indicates that reaction at
331 carbonyl position would be faster than reaction at the allylic position by about twenty-fold at 303 K,
332 fully justifying the experimental observations. Indeed, table 1 indicates that both citral and
333 dodecanal transfer an H-atom to peroxyl radicals at a rate constant (39 and 38 $M^{-1}s^{-1}$ respectively, at
334 303 K) about one order of magnitude higher than linalool or limonene (average value 3.4 $M^{-1}s^{-1}$ at
335 303 K), where reaction clearly occurs at the allylic position.

Natural bond order (NBO) analysis of the two reaction pathways indicated that the bonds being cleaved and being formed have, as expected, an occupancy of about 1. The occupancies of the cleaved CH bonds in the TS are nearly equal (0.98 and 0.95 respectively) for the aldehyde and allylic moieties. The occupancy of the incipient CH_3OO-H bond is 0.84 in the case of hydrogen transfer from the aldehyde, and 0.78 in the case of hydrogen transfer from the allylic position (see Appendix A), supporting the conclusion that reaction at the aldehyde is the preferred reaction path. By analyzing the molecular orbitals in the TS, we found that the orbitals just below the SOMO have a delocalization pattern involving both reacting fragments (see Appendix A), this being coherent with a proton-coupled electron transfer (EPT) mechanism, in analogy with what is found in the case of the reaction of alkylperoxyl radicals with phenols and other compounds (DiLabio & Johnson, 2007).

3.4 Significance of current results for the antioxidant protection of food

The results reported in Table 1 show that limonene, linalool and citral react with the peroxyl radicals of cumene with rather low rate constants (4.5, 2.2 and 39 $M^{-1}s^{-1}$ respectively) as compared to those of phenolic antioxidants (*e.g.* 1.1×10^4 and 3.2×10^6 $M^{-1}s^{-1}$ for BHT and α-tocopherol, respectively, at 30°C in chlorobenzene) (Burton, et al., 1985). However, these constants allow those terpenoids to compete with the oxidizable substrate (bearing relatively unactivated C-H bonds) for reaction with peroxyl radicals, thereby taking part to oxidative chain-propagation. What makes for their antioxidant behavior is the higher rate of self-termination and cross-termination of the terpenoids compared to the oxidizable substrate, thereby increasing the overall chain-termination and cutting the efficiency of autoxidation. This termination-enhancing antioxidant activity differs from the chain-breaking behavior of common phenolic antioxidants by three main aspects of practical relevance: i) their antioxidant performance is less pronounced than that of phenols and requires higher concentrations; ii) it is not linearly related to their concentration, hence it is much less predictable; iii) its effectiveness depends on the rate of chain-termination of the oxidizable

362 substrate, being higher for substrates that have more modest chain-termination (whereas for phenols
363 it depends on substrate's rate of propagation). Despite the above limitations, they could effectively
364 contribute to the antioxidant behavior of natural essential oils: although they are unlikely to afford
365 sufficient protection to foodstuff when used alone, they would sum up to the efficacy of the
366 phenolic components of essential oils and could be effectively exploited in food technology.
367 Among the investigated compounds, citral has the largest reactivity, which arises from the presence
368 of the aldehyde group. In this conjunction it is of interest that simple aliphatic aldehydes like
369 dodecanal had an antioxidant behavior very similar to that of citral (see Appendix A), with might be
370 relevant for their exploitation in food protection.
371
372 **4. Conclusions**
373 Herein, we have provided a rationale and quantitative kinetic data for the antioxidant behaviour of
374 limonene, linalool and citral. Such data are likely to reflect also the behaviour of other non-phenolic
375 terpenoid essential oil components, thereby broadening their relevance. Such compounds act as
376 termination enhancing antioxidants, bearing similitude to the kinetic behaviour previously assessed
377 for γ-terpinene (Foti & Ingold, 2003), although, at variance with γ-terpinene, no involvement of the
378 chemistry of superoxide is found in their antioxidant activity. Although their antioxidant
379 performance will be expressed only in a rather narrow concentration range and only with some
380 oxidizable substrates, they can contribute to the antioxidant activity of essential oils and their
381 chemistry could effectively be exploited in food technology upon testing their performance in the
382 actual system they are called to protect. In this conjunction it should not be overlooked that raw
383 essential oils rather than isolated components are most widely appreciated in food products and the
384 complex interplay among components would deserve deeper understanding, since often synergistic
385 or antagonistic effects might come into action (Amorati et al., 2013). This fascinating chemistry is
386 currently been investigated in our group.
387

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Appendix A

Results of autoxidation studies for dodecanal, details on theoretical calculations and Cartesian Coordinates.

Declaration of interest

The authors declare no competing financial interest.

Abbreviations used

AIBN, azobis-isobutyronitrile; PMHC, 2,2,5,7,8-pentamethyl-6-chromanol; BHT, butylated hydroxy toluene; EH, oxidizable essential oils components; RH, oxidizable substrate to be protected; ROO•, peroxyl radicals formed by the oxidizable substrate; EOO•, peroxyl radicals formed by the essential oil component; HOO•, hydroperoxyl radical.

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Table 1. Rate constants ($M^{-1}s^{-1}$) at 303K obtained from the fitting of co-oxidation plots of cumene and essential oils components (rate constants' numbering refers to eq. 1-10).

EO component	k_5 [a]	k_6 [a]	k_7 [a]	k_9 [a]	k_{10} [a]
limonene	4.5[b]	4.5[b]	0.32[c]	3.4×10^6	3.5×10^6
linalool	2.2[b]	2.2[b]	0.32[c]	9.3×10^5	9.0×10^5
citral [d]	39	2.4×10^3	22.6	1.2×10^8	1.0×10^8
dodecanal [d]	38	2.2×10^3	22.4	0.5×10^8	2.5×10^9

[a] Errors are estimated to be ± 50% of the reported best fit values.

[b] In fittings, k_5 was set equal to k_6 (see text).

[c] In fittings, k_7 was set equal to k_p for cumene (see text).

[d] As EOO• are acylperoxyl radicals, the cross-propagation constant k_7 is ~70-fold larger than k_4, and the k_6/k_5 ratio is ~60.

Figure captions

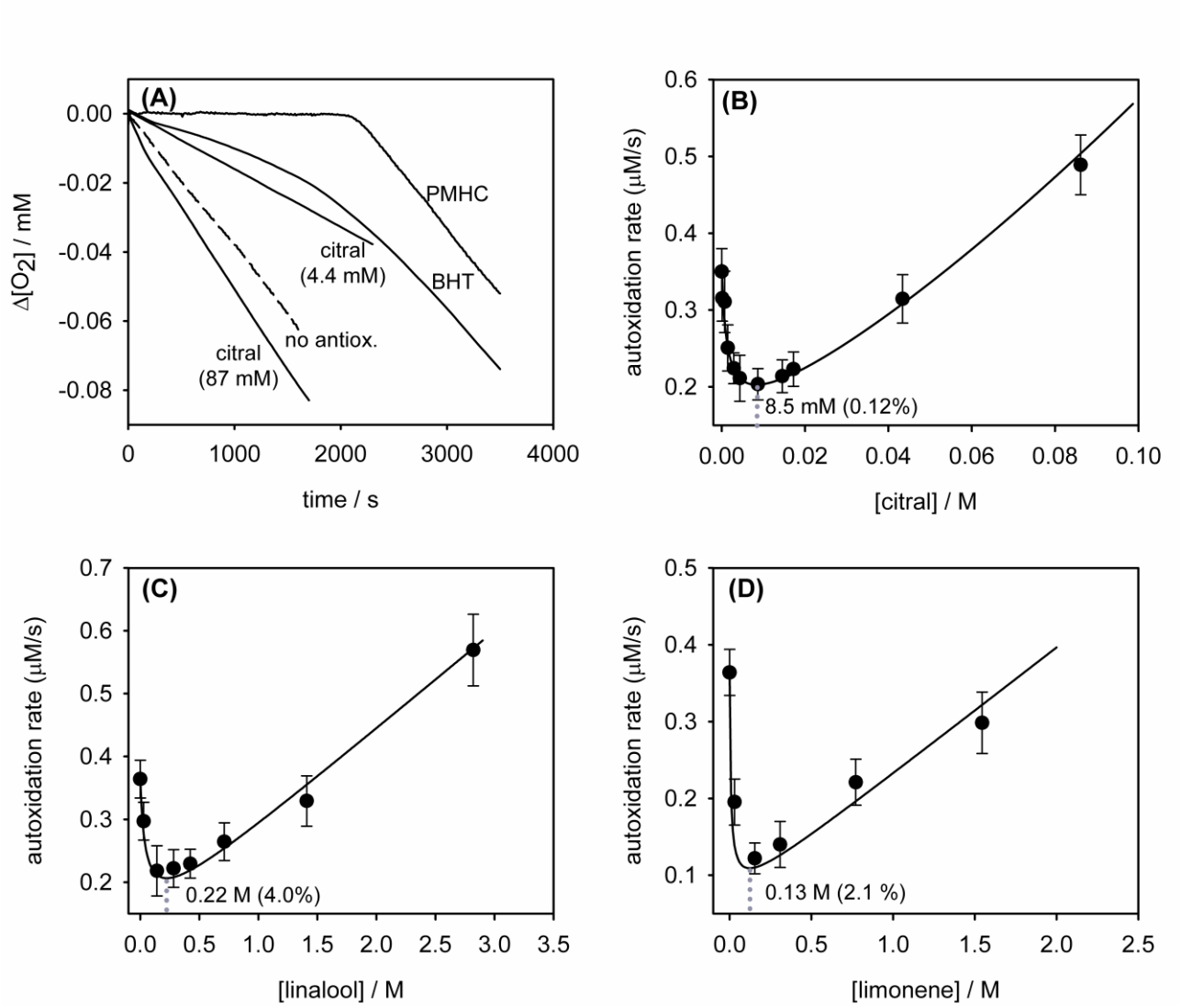
Figure 1 (A-D). Plots of O_2 uptake during the autoxidation of cumene (3.5 M) in chlorobenzene initiated by AIBN (0.05 M) at 30 °C in the absence of antioxidants (dotted line) or in the presence of PMHC 5μM, BHT 5μM, and citral 4.4 or 87 mM (**A**); measured rate of O_2 uptake as function of the concentration of citral (**B**), or of linalool (**C**), or of limonene (**D**) (±SD, n=3).

Figure 2. Peroxyl radicals formed during the autoxidation of limonene and linalool, arising from kinetic measurements in accordance to product studies by Kalberg et al. (1997) and Skold et al. (2004)

Figure 3. Formation of acylperoxyl radicals during the autoxidation of aldehydes.

Figure 4. Suggested reaction pathways for the autoxidation of citral with reference to the products studies on geranial (*E* isomer of citral) by Hagvall et al. (2011).

Figure 5. Calculated enthalpy of hydrogen atom abstraction from the aldehydic (Path A) or the allylic (Path B) portions of of citral (*E*-isomer) by methylperoxyl radicals at the M05/6-311+g(2df,2p) (‡) and CBS-QB3 ($^{\#}$) levels of theory. Values for products or TS are relative to the reactants calculated at the same level of theory. Optimized geometries of the transition states calculated at the M05/6-311+g(2df,2p) level are shown.



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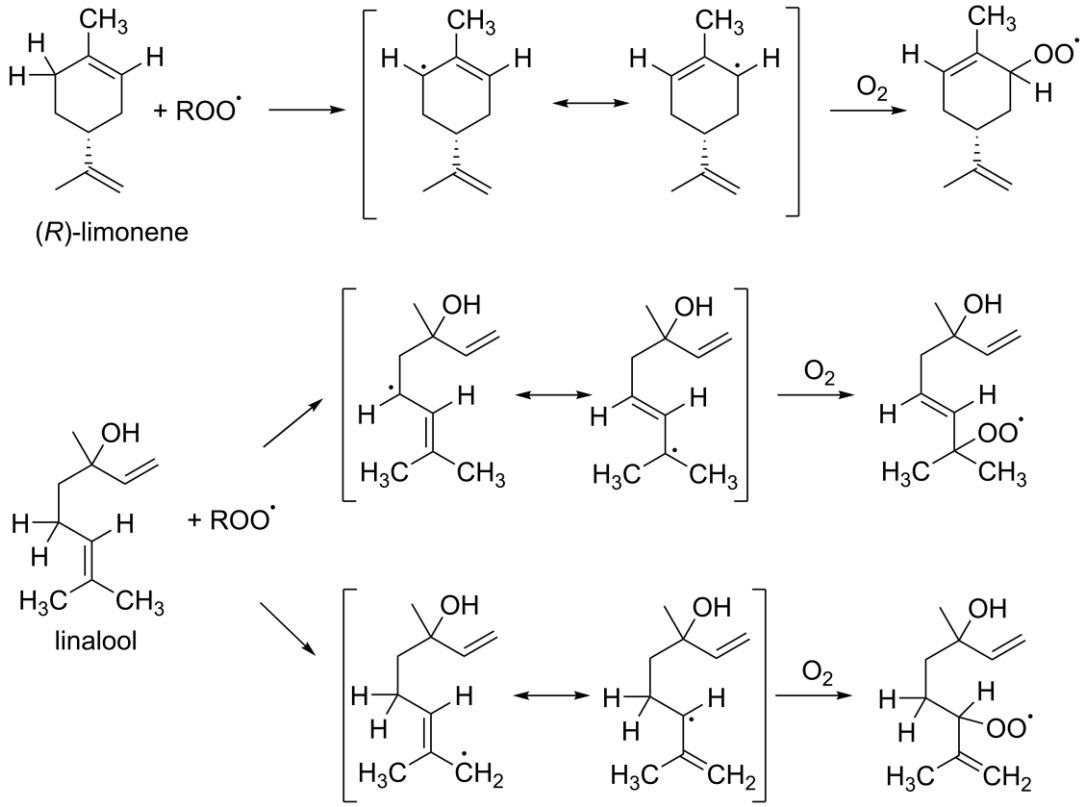

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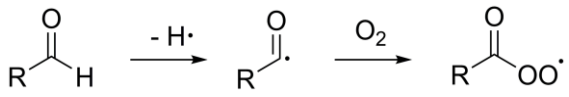

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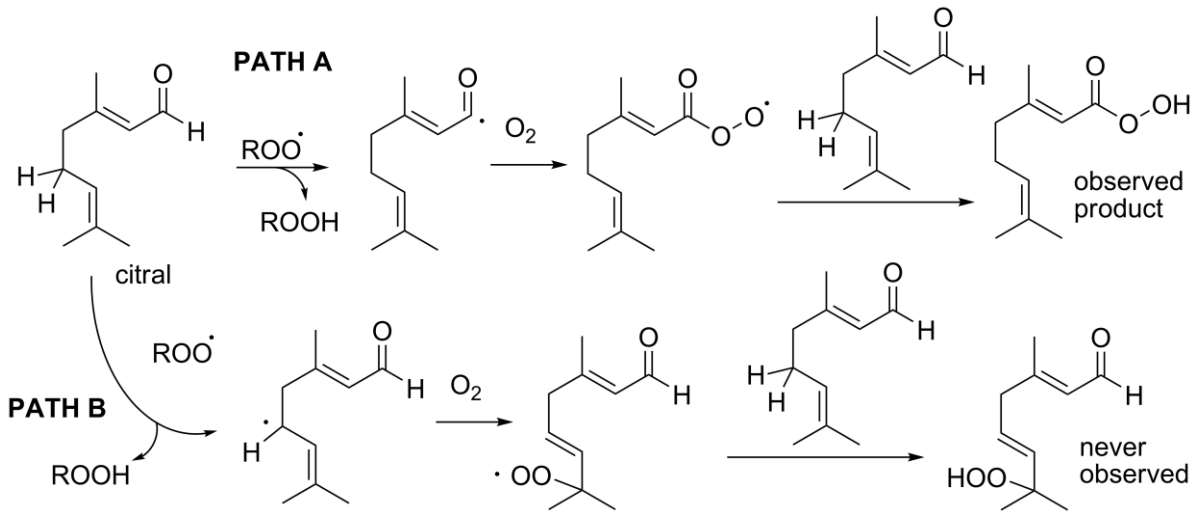

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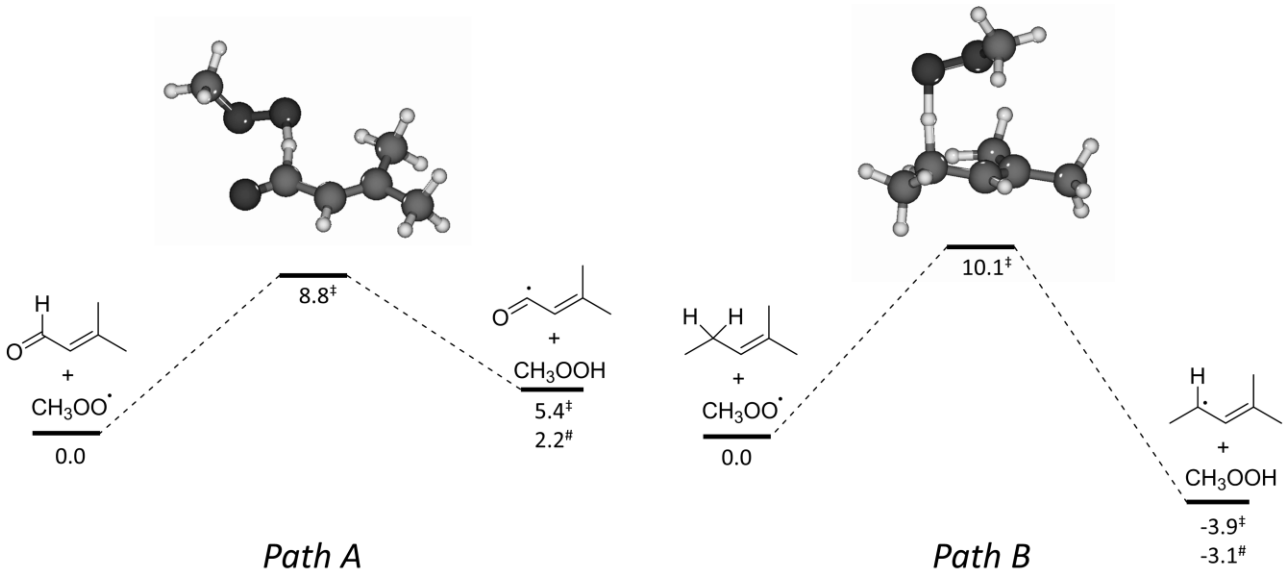

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