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

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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 3.5×10^6 for limonene, 2.2 and 9.0×10^5 for linalool and 39 and 1.0×10^8 for citral. Behavior is bimodal antioxidant/pro-oxidant 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.

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 counteract 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 amounts of thymol and carvacrol, two phenolic components having antioxidant activity similar to that of synthetic phenolic antioxidants, such as butylated hydroxytoluene (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 an H-atom from the phenolic hydroxyl group to a peroxyl radicals ($ROO^{\cdot}$) that is responsible for the propagation of the oxidative radical chain ($PhOH + ROO^{\cdot} \rightarrow PhO^{\cdot} + ROOH$) (Amorati et al., 2013). Unlike peroxyl radicals, the resulting phenoxyl radical ($PhO^{\cdot}$) is

Abbreviations: AIBN, azobisisobutyronitrile; PMHC, 2,2,5,7,8-pentamethyl-6-ch romanol; BHT, butylated hydroxy toluene; EH, oxidizable essential oils components; RH, oxidizable substrate to be protected; $ROO^{\cdot}$, peroxyl radicals formed by the oxidizable substrate; $EOO^{\cdot}$, peroxyl radicals formed by the essential oil component; $HOO^{\cdot}$, hydroperoxyl radical.

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normally unable to propagate the oxidative chain, *i.e.* it is sufficiently unreactive to "wait" in solution until it traps a second peroxyl radical ($PhO^{\cdot} + ROO^{\cdot} \rightarrow$ 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 behavior, 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 et al., 2013).

For instance, Domingues and co-workers studied the antioxidant activity of coriander essential oil, which does not 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 et al., 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 et al., 2005). On the other hand, Ruberto and Baratta, by studying the autoxidation of egg yolk homogenate, found a 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 ($HOO^{\cdot}$) that have very fast self-termination rate constant ($HOO^{\cdot} + HOO^{\cdot} \rightarrow O_2 + HOOH$) (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 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. Azobisisobutyronitrile (AIBN, Fluka, Milan, Italy) was recrystallized from methanol. 2,6-Di-*tert*-butyl-4-methylphenol (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, Zotova, Baschieri, & Valgimigli, 2015; Amorati et al., 2013). 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 et al., 2015; Valgimigli et al., 2009, 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 dodecanal.

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 an 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 (Galano, 2011; La Rocca et al., 2016): the results, summarized in Fig. 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 (*iso*-propylbenzene), which can be described by Eqs. (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 Eq. (1) (initiation), (2) and (4) (propagation), and (8) (termination) (Amorati et al., 2015; Burton et al., 1985).

$$In \rightarrow R^{\cdot} \quad (1)$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \quad (2)$$

$$E^{\cdot} + O_2 \rightarrow EOO^{\cdot} \quad (3)$$