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Antioxidant activity of selected essential oil components in two lipid model systems

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Dedicated to the memory of Dr. Angelo Starrantino

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Abstract

About 100 pure components of essential oils have been tested for their antioxidant effectiveness. The main classes of compounds, namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, benzene derivatives, and non isoprenoid components comprising alcohols, aldehydes, ketones, which are the most common constituents of essential oils, have been analysed. Two model systems for the antioxidant efficacy have been used; the first exploiting the thiobarbituric acid reactive species (TBARS) method using egg yolk as oxydable substrate, the second measuring the formation of hydroperoxydienes from linoleic acid in a micellar system, using in both cases 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) as a radical initiator, and α-tocopherol as a reference compound. From a general point of view phenols were confirmed to possess the highest antioxidant activity. In particular some monoterpene hydrocarbons, namely, terpinolene, α- and γ-terpinene showed a significant protective action, whereas among the oxygenated components, beside the aforesaid phenols, allylic alcohols manifested an appreciable activity. Sesquiterpene hydrocarbons and non isoprenoid components subjected to this study showed a low, if any, antioxidant effect. The role of the different model systems and the relationship between structure and antioxidant effectiveness are discussed.

1. Introduction

Essential oils are known to possess potential as natural agents for food preservation; in fact their effectiveness against a wide range of microorganisms has been repeatedly demonstrated (Baratta, Dorman, Deans, Biondi & Ruberto, 1998; Barratta, Dorman, Deans, Figueiredo, Barroso & Ruberto, 1998; Deans, 1991; Deans & Ritchie, 1987; Helander et al, 1998). Besides this activity many essential oils, more recently, have been qualified as natural antioxidants (Aeschbach et al., 1994; Baratta, Deans et al.; Baratta, Dorman et al.; Yanishlieva, Marinova, Gordon & Raneva, 1999), and proposed as potential substitutes of synthetic antioxidants in specific sectors of food preservation where their use is not in contrast with their aroma. Essential oils are, from the chemical point of view, quite complex mixtures constituted by several tens of components, and this complexity makes it often difficult to explain the aforesaid activities. If we exclude the case of some phenolic components, whose antimicrobial and antioxidant activity is well known and widely documented (Helander et al.; Yanishlieva et al.), and some other examples of pure compounds (Aeschbach et al.; Madsen & Bertelsen, 1995), nothing is known about the effectiveness of most components. Many reports on the essential oil activities, often refer to concepts, such as synergism, antagonism, additivity, but they are rarely experimentally supported, being for the most part purely speculative (Janssen, Tsai Ribe, Scheffer & Baerheim Svendsen, 1988). However, when a study on the antimicrobial activity of an essential oil comprises analysis on the activity of its individual components, antagonistic and/or synergistic effects assume a more complete significance, and become useful to explain the observed

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biological activity (Cuong et al., 1994; Griffin, Leach, Markham & Johnstone, 1998; Helander et al; Knobloch, Pauli, Iberl, Weigand & Weis, 1986).

Pursuing our activity on the studies of natural antioxidants, exploitable in strategic fields such as the food, pharmacological and cosmetic sectors, we have analysed the antioxidant effectiveness of about one hundred pure components of essential oils, and here we wish to report the results of this research. The protective action of these compounds has been evaluated in two model systems, by measuring the formation of primary (hydroperoxydienes) and secondary (malonaldehyde) components of the oxidative process of a lipid matrix.

2. Materials and methods

2.1. Materials

Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA), Fluka AG (Buchs, Switzerland), Aldrich Chemical Co. (Milwaukee, WI, USA), 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) was from Wako Chemical GmbH (Neuss, Germany). Purity of tested compounds was performed, when necessary, by gas chromatographic analysis on a Hewlett-Packard gas-chromatograph, model 5890 equipped with a flame ionisation detector (FID) and coupled to an electronic integrator. Analytical conditions: DB-5 capillary column (30 m×0.25 mm×0.25 μ film thickness), helium as carrier gas. The oven temperature was programmed according to the retention time of each component.

2.2. Methods

2.2.1. Method A

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the potential antioxidant capacity of the compounds (Wong, Hashimoto & Shibamoto, 1995), using egg yolk homogenates as lipid rich media (Noble & Cocchi, 1990). Briefly, 0.5 ml of 10 % (w/v) tissue homogenate and 0.1 ml of solutions of compounds to be tested in methanol, prepared immediately before use, were added to a test tube and made up to 1.0 ml with distilled water. 0.05 ml of ABAP solution (0.07 M) in water was added to induce lipid peroxidation. 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml 0.8% (w/v) thiobarbituric acid (TBA) in 1.1% (w/v) sodium dodecyl sulphate (SDS) solution was added and the resulting mixture was vortexed, and then heated at 95°C for 60 min. After cooling, 5.0 ml of butan-1-ol was added to each tube and centrifuged at 1200 *g* for 10 min. The absorbance of the organic upper layer was measured using a Beckman DU-65 spectrophotmeter, set at 532 nm (band width 0.5 nm).

All the values were based on the percentage antioxidant index (AI%) calculated using the formula:

$$AI\% = (1 - T/C) \times 100 \quad (1)$$

where C is the absorbance value of the fully oxidised control and T is the absorbance of the test sample.

2.2.2. Method B

The method is based on the spectrophotometric determination of the rate of conjugated diene formation from linoleic acid, in the absence and in the presence of a potential antioxidant (Foti, Piatteli, Baratta & Ruberto, 1996; Pryor et al., 1993). Conjugated diene hydroperoxides are end products of linoleic acid peroxidation induced into the micellar phase by a radical initiator (ABAP), which, on thermolysis, provides radicals at constant rate. These hydroperoxides have strong UV absorption, in micelles of SDS in buffer solution at pH 7.4, with a maximum at 232 nm and a molar coefficient of 26,100 M^{-1} cm^{-1}.

A 0.1 M solution of SDS was prepared in aqueous 0.01 M Na_2PO_4 and adjusted to pH 7.4 with concentrated aqueous NaOH. Linoleic acid was added, immediately before each experiment, to a concentration 0.0026 M. A stock solution (0.07 M) of ABAP in water, stored at 5–10°C was used within a week. Solutions of compounds to be tested (10^{-2}, 10^{-3}, 10^{-4} M) in methanol were prepared immediately before use. The procedure was as follows: an aliquot (2 ml) of the micellar suspension of linoleic acid was stirred in the sample compartment of the spectrophotometer at 50°C. The buffered SDS solution was used as blank. After equilibration (20 min), 10 μl of the radical initiator solution and 50 μl of the antioxidant solution were added and the progress of the peroxidation monitored by recording the absorbance at 232 nm for 15 min. The absorbance value of the linoleic acid subjected to full peroxidation (without antioxidant) measured at the 15th minute was used as control. The antioxidant efficiency of the oils, expressed in terms of their capacity to protect linoleic acid from peroxidation, was evaluated by using formula (1), where C is the absorbance value at 232 nm of the oxidised linoleic acid after 15 min and T is the absorbance of the oxidised linoleic acid in the presence of the test sample at the same conditions.

Each determination was performed in quadruplicate and results are reported as mean ± standard deviation.

3. Results and discussion

Ninety-eight pure essential oil components have been subjected to a screening for their antioxidant activity. The components, listed in Table 1, have been chosen to represent the main classes of compounds typical of the

Table 1
Antioxidant effectiveness of essential oil components[a]

Compound	Method A			Method B		
	1000 ppm	500 ppm	100 ppm	10^{-2} M	10^{-3} M	10^{-4} M
Monoterpene hydrocarbons						
Mycene	32.1 (2.7)	29.4 (2.5)	24.0 (3.4)			
β-Ocimene[b]	30.6 (1.3)	26.8 (2.1)	16.3 (1.5)			
Terpinolene	64.6 (2.4)	56.3 (4.2)	40.3 (1.2)	78.3 (3.8)	22.0 (1.9)	12.2 (2.6)
α-Terpinene	71.5 (1.3)	57.4 (2.1)	31.8 (2.0)	92.2 (0.9)	73.4 (3.6)	14.8 (1.3)
γ-Terpinene	79.5 (2.2)	76.1 (3.7)	61.6 (1.6)	95.1 (2.6)	78.5 (1.9)	15.3 (3.2)
(S)-(−)-Limonene	27.4 (2.0)	26.3 (1.3)	2.9 (1.6)	21.0 (3.4)	15.7 (2.1)	n.a.
p-Cymene	42.6 (0.3)	25.5 (1.2)	14.9 (0.8)			
α-Thujene	38.6 (2.7)	30.5 (3.1)	19.0 (1.9)			
(+)-Sabinene	59.9 (4.1)	56.5 (1.8)	43.0 (1.2)	8.7 (2.4)	7.6 (1.5)	4.9 (3.1)
(+)-3-Carene	17.7 (3.1)	15.3 (4.8)	3.7 (1.9)			
(−)-α-Pinene	12.6 (2.2)	6.4 (3.8)	n.a.			
(−)-β-Pinene	27.6 (3.1)	18.5 (0.7)	1.0 (1.2)			
(−)-Camphene	9.8 (3.2)	7.5 (3.3)	3.2 (1.2)			
Oxygenated monoterpenes						
Nerol	42.7 (4.0)	28.7 (1.7)	19.4 (3.3)	30.8 (1.7)	20.5 (1.4)	16.1 (5.5)
Geraniol	34.9 (2.4)	23.1 (2.5)	22.3 (1.0)	26.5 (0.5)	20.7 (1.4)	15.8 (3.9)
Linalol	−32.9 (2.8)	−19.6 (2.6)	−16.1 (0.9)	n.a.	n.a.	n.a.
(±)-Citronellol	27.5 (3.3)	21.0 (3.6)	6.2 (3.0)	13.3 (2.1)	0.9 (0.2)	n.a.
(±)-Lavandulol	34.9 (1.8)	21.9 (2.2)	10.7 (1.4)	9.9 (2.0)	9.8 (0.5)	5.9 (0.6)
(S)-(−)-Peryllyl alcohol	53.6 (1.8)	50.3 (2.1)	39.1 (3.3)	18.9 (3.5)	9.7 (0.3)	6.5 (1.0)
Carveol[b]	31.0 (3.3)	24.6 (2.3)	17.5 (2.2)	15.7 (2.3)	5.6 (1.0)	6.0 (0.9)
(−)-Menthol	22.2 (2.4)	11.0 (2.7)	6.5 (2.5)			
(±)-Terpinen-4-ol	31.0 (2.7)	21.6 (3.5)	8.1 (2.4)			
α-Terpineol	28.1 (3.6)	13.0 (3.0)	0.3 (0.1)			
(±)-Chrysantemyl alcohol	25.9 (4.0)	14.6 (1.3)	n.a.			
(−)-*cis*-Myrtanol	49.7 (2.4)	43.3 (6.5)	17.6 (3.6)	8.1 (0.4)	3.2 (1.8)	n.a.
(S)-*cis*-Verbenol	39.4 (1.5)	28.0 (0.4)	11.4 (0.7)	28.5 (1.3)	8.2 (1.3)	3.1 (0.6)
(+)-Fenchol	3.0 (1.3)	n.a.	n.a.			
(−)-Borneol	6.6 (2.0)	1.8 (2.2)	n.a.			
(±)-Linalyl acetate	33.2 (3.3)	23.7 (0.7)	16.4 (2.1)			
(−)-Bornyl acetate	18.7 (2.5)	17.1 (2.0)	10.5 (2.1)			
(+)-*cis*-Limonene-1,2-epoxide	21.6 (1.4)	9.0 (0.5)	3.3 (1.8)			
(+)-*trans*-Limonene-1,2-epoxide	24.6 (3.4)	12.5 (0.7)	5.2 (2.1)			
1,8-Cineole	20.3 (1.0)	3.3 (0.8)	n.a.			
Linalol oxide	3.2 (1.0)	4.1 (0.4)	n.a.			
(±)-Citronellal	n.d.	n.d.	n.d.	21.9 (4.1)	21.1 (3.6)	16.9 (2.4)
Citral	n.d.	n.d.	n.d.	n.d.	18.3 (0.9)	8.4 (2.3)
(−)-Peryllaldehyde	n.d.	n.d.	n.d.	n.d.	4.5 (0.7)	0.7 (0.6)
(−)-Menthone	3.1 (1.3)	2.1 (1.9)	n.a.			
(S)-(+)-Carvone	20.5 (4.0)	12.6 (2.1)	7.9 (1.5)			
(R)-(−)-Carvone	23.6 (4.2)	18.2 (2.6)	16.3 (3.7)			
Pulegone	31.1 (3.1)	17.5 (2.4)	5.0 (2.0)			
(−)-Thujone	15.9 (3.5)	7.5 (1.5)	3.5 (2.8)			
(1S)-(−)-Verbenone	14.7 (1.0)	9.4 (2.0)	n.a.			
(±)-Camphor	6.6 (2.4)	2.3 (0.9)	n.a.			
(−)-Fenchone	25.6 (2.8)	10.6 (3.3)	7.2 (2.2)			
Thymol	55.7 (0.4)	43.3 (2.1)	25.5 (1.9)	91.7 (1.9)	60.9 (2.1)	23.0 (3.9)
Carvacrol	69.9 (1.6)	67.3 (0.8)	59.1 (3.4)	89.9 (2.8)	61.1 (3.8)	30.7 (1.5)
Sesquiterpene hydrocarbons						
α-Humulene	15.7 (4.7)	9.7 (2.6)	n.a.			
(−)-*trans*-Caryophyllene	8.9 (1.5)	3.0 (3.5)	n.a.			
(+)-Valencene	10.0 (1.4)	8.4 (1.5)	7.7 (1.1)			
(+)-Calarene	34.6 (1.0)	25.7 (2.1)	16.8 (3.0)			
(+)-Aromadendrene	17.5 (4.0)	3.4 (2.6)	n.a.			
α-Cedrene	11.7 (1.3)	25.2 (3.8)	6.6 (3.6)			
(+)-Longiciclene	n.a.	n.a.	n.a.			
(+)-Longifolene	n.a.	n.a.	n.a.			

(*continued on next page*)

Table 1 (*continued*)

Compound	Method A			Method B		
	1000 ppm	500 ppm	100 ppm	10^{-2} M	10^{-3} M	10^{-4} M
(+)-α-Longipinene	7.8 (2.3)	4.2 (0.7)	2.1 (0.7)			
α-Panasinsene	12.8 (0.7)	11.6 (4.1)	n.a.			
β-Panasinsene	3.8 (1.7)	3.8 (3.3)	1.4 (2.9)			
Oxygenated sesquiterpenes						
trans-trans-Farnesol	35.5 (1.8)	21.8 (2.7)	22.2 (3.7)			
Farnesol[b]	46.7 (0.3)	46.6 (2.3)	38.9 (0.5)	16.6 (1.2)	5.5 (3.2)	0.3 (0.3)
Farnesyl acetate	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
(±)-*cis*-Nerolidol	−44.0 (4.6)	−23.3 (2.6)	−11.7 (1.7)	n.a.	n.a.	n.a.
(±)-α-Bisabolol	25.5 (2.9)	20.7 (2.3)	10.0 (3.1)			
Guaiol	35.9 (2.4)	33.1 (3.8)	12.8 (1.6)			
(−)-Globulol	7.7 (3.0)	7.2 (0.2)	n.a.			
(+)-8-(15)-Cedren-9-ol	36.5 (2.4)	32.7 (2.7)	19.0 (3.1)	n.a.	n.a.	n.a.
Germacrone	32.0 (3.1)	25.3 (2.1)	19.0 (2.6)	44.5 (2.1)	20.4 (0.8)	15.4 (0.5)
(−)-Caryophyllene oxide	n.a.	n.a.	n.a.			
Diterpene						
Phytol[b]	36.7 (3.6)	36.7 (1.6)	30.9 (1.4)			
Benzene derivatives						
Eugenol	81.2 (1.7)	67.2 (1.3)	64.7 (3.0)	93.6 (3.1)	73.0 (2.8)	39.1 (2.1)
Eugenol methyl ether	40.7 (1.6)	31.2 (2.2)	10.4 (1.3)			
Estragol	32.3 (2.3)	9.3 (0.9)	4.5 (0.5)			
trans-Anethole	41.9 (2.1)	35.5 (1.2)	30.1 (2.2)			
Guaiacol	49.7 (2.4)	46.9 (1.1)	19.5 (1.5)	86.3 (2.3)	49.0 (1.6)	23.1 (3.1)
Mesitaldehyde	n.d.	n.d.	n.d.			
p-Anisaldehyde	n.d.	n.d.	n.d.			
Non isoprenoid components						
1-Penten-3-ol	6.1 (3.3)	3.6 (1.8)	n.a.			
1-Hepten-3-ol	22.3 (2.4)	11.9 (3.4)	7.7 (2.7)	n.a.	n.a.	n.a.
Heptanol	8.4 (1.1)	3.5 (2.6)	0.8 (1.2)			
2-Octanol	18.4 (2.6)	9.7 (1.8)	7.7 (2.7)			
1-Octen-3-ol	17.3 (2.3)	11.4 (2.5)	n.a.	n.a.	n.a.	n.a.
Octyl acetate	12.6 (2.9)	3.8 (2.3)	n.a.			
Nonanol	10.8 (1.6)	n.a.	n.a.			
Decanol	13.6 (2.0)	10.7 (3.0)	4.0 (1.6)			
trans-2-Pentenal	n.d.	n.d.	n.d.			
Heptanal	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
Octanal	n.d.	n.d.	n.d.	7.7 (0.5)	6.8 (0.9)	n.a.
Nonanal	n.d.	n.d.	n.d.	7.9 (1.5)	7.3 (2.2)	5.3 (1.2)
Decanal	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
cis-4-Decenal	n.d.	n.d.	n.d.			
Undecanal	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
3-Decanone	n.a.	n.a.	n.a.			
2-Undecanone	11.0 (2.8)	n.a.	n.a.			
1-Adamantanol	18.4 (3.9)	15.2 (4.4)	n.a.			
2-Adamantanol	14.0 (0.8)	3.2 (3.7)	4.9 (2.2)			
Maltol	19.6 (2.7)	14.2 (2.3)	14.1 (1.3)			
cis-Jasmone	34.4 (2.7)	23.9 (3.0)	10.1 (0.6)			
α-Tocopherol	93.5 (0.1)	89.3 (0.9)	82.6 (0.1)	94.8 (1.7)	91.6 (2.1)	88.4 (0.9)

[a] Antioxidant effectiveness expressed as antioxidant index (AI%), and values represents average of four determinations with ±Standard Deviation (S.D.) given in parentheses; n.a. = not active; n.d. = not detectable.

[b] Mixture of isomers.

chemical composition of essential oils: 13 monoterpene hydrocarbons, 34 oxygenated monoterpenes, 11 sesquiterpenene hydrocarbons, 10 oxygenated sesquiterpenes, one diterpene, seven benzene derivatives, 22 non isoprenoid components, comprising alcohols, aldehydes, ketones. The antioxidant effectiveness has been evaluated by two different methods, since, given that the lipid oxidation is a multistep process (Abdalla & Roozen, 1999; Hamilton, Kalv, Prisk, Padley & Pierce, 1997; Huang, Frankel & German, 1994), it is opportune to analyse the action of a potential antioxidant on the different steps of the oxidation process (Frankel, 1993).

The first method, known as thiobarbituric acid (TBA) assay, by which all compounds were tested, concerns the spectrophotometric detection of thiobarbituric acid reactive substances (TBARS), namely being malonaldehhyde (MDA), one of the secondary lipid peroxidation products, whose quantification gives a measure of the extent of lipid degradation (Belitz & Grosch, 1987; Frankel, 1985). TBA test has been criticised for its scarce specificity and because it appeared to give overestimated results (Frankel, 1985; Janero, 1990). However, more recent studies show that despite elevated levels, TBARS formation is correlated and similar to the actual MDA formation (Kikugawa, Kojima, Yamaki & Kosugi, 1992; Kishida, Kamura, Tokumaru, Oribe, Iguchi & Kojo, 1993, Wong et al., 1995). The second method deals with the first step of the degradation process of a lipid matrix, whose final products are hydroperoxydienes. These compounds, coming from the oxidation of linoleic acid subjected to radical attack of an initiator, were spectrophotometrically quantified by a measurement at 232 nm (Foti et al., 1996; Pryor et al, 1993).

Table 1 lists all compounds subjected to this study and their activity evaluated with both model systems.

3.1. Monoterpene hydrocarbons

Thirteen monoterpenes (Fig. 1), representing the common acyclic, mono- and byciclic components of this class, have been analysed. Normally a scarce, if any, antioxidant activity is accredited to these compounds. This is substantially confirmed in this study, but with a remarkable exception, three monocyclic components, namely terpinolene, α-terpinene and γ-terpinene, and to a less extent, sabinene, a bicyclic one, show a very high activity. In particular α- and γ-terpinene have a comparable activity to that of α-tocopherol (Kamal-Eldin & Appelqvist, 1996) and other phenols at the highest concentration, clearly decreasing with reduced concentrations. The presence in these molecules of strongly activated methylene groups is probably the reason for this behaviour, which is more clear in Method B where a competition with the activated methylene in C-11 of linoleic acid may be hypothesised. However the high activity of the aforesaid monoterpenes is also confirmed by the TBARS assay (Method A), in which also the bicyclic sabinene shows an appreciable activity.

The formation of secondary products, including malonaldehyde, during the oxidation processes, by which monohydroperoxides are transformed into smaller oxygenated products, is a quite complex series of reactions, if compared to the formation of primary oxidation products, such as hydroperoxides (Belitz & Grosch, 1987; Frankel, 1985; Janero, 1990). Then, considering that the capacity to give a hydrogen atom to stop the radical chain is probably one of the most important processes, we cannot exclude that different processes can occur depending on the particular molecular features of a potential antioxidant.

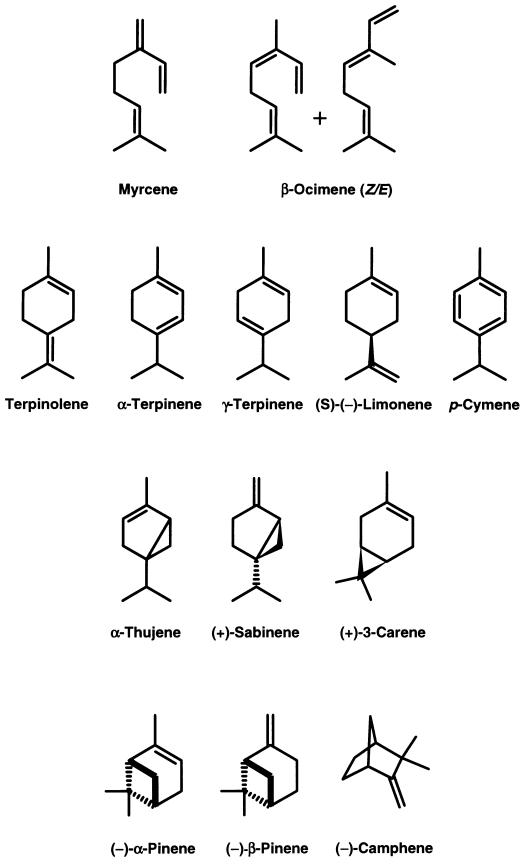

Fig.1. Monoterpene hydrocarbons.

3.2. Oxygenated monoterpenes

This is the most significant class of compounds in this study. Fig. 2 shows the 34 analysed components. The choice to focus on this chemical class is not only due to the fact that it normally strongly characterises an essential oil, but mainly because many examples of different functional groups (alcohols, aldehydes, ketones, ethers, etc.) with several variants are present within it.

The most active components of this class are the two phenols thymol and carvacrol as is reported in Table 1. The activity is confirmed by the two methods and is close to that of α-tocopherol, at least, at higher concentrations. That phenols are efficient antioxidants is well known (Deighton, Glidewell, Deans & Goodman, 1993; Madsen & Bertelsen, 1995; Yanishlieva et al., 1999), thymol and carvacrol are in fact responsible for the antioxidant activity of many essential oils which contain them (Baratta, Dorman, Deans, Biondi, et al., 1998b; Lagouri, Blekas, Tsimidov, Kokkini & Boskov, 1993; Aeschbach et al., 1994). Other components of this class show a generally lower activity (Table 1), but with

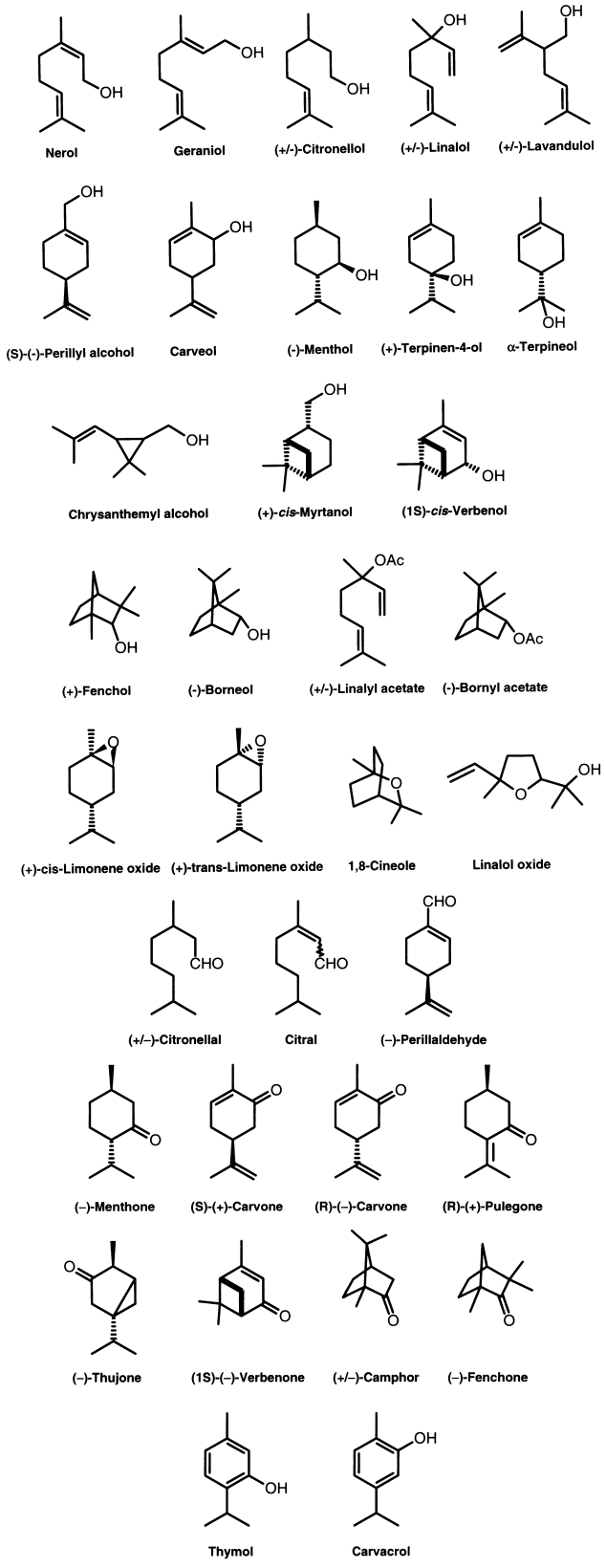

Fig.2. Oxygenated monoterpenes.

interesting distinctions depending on the presence of particular molecular moieties. Alcohols are the most active components, with a slight predominance of allylic alcohols (perillyl alcohol, nerol, *cis*-verbenol, geraniol); this is confirmed by both methods, even when with Method B the activities are lower. An interesting exception is represented by linalool, a tertiary allylic alcohol, which shows a pro-oxidant effect with Method A and a total absence of activity with Method B. A satisfactory explanation of this anomalous behaviour is still not available. Prosecuting the analysis of results the four ethers (*cis*- and *trans*-limonene oxide, 1,8-cineole and linalool-oxide) do not show significant activity with Method A, whereas the activity of aldehydes is determinable only with Method B, since they interact with thiobarbituric acid (TBA) of Method A avoiding any possibility of measurement. However, only citronellal, a saturated aldehydes, shows a slight activity. Finally, the antioxidant index (%) of ketones is not particularly high, the α,β-insaturated ketones being more active.

3.3. Sesquiterpenes — hydrocarbons and oxygenated derivatives

Concerning the sesquiterpene hydrocarbons (Fig. 3), unlike the homologous monoterpenes, their activities are very low. Instead the oxygenated sesquiterpenes analysed here (Fig. 4) show a result comparable to that of the oxygenated monoterpenes. The allylic alcohols are the most active components, but their activity is

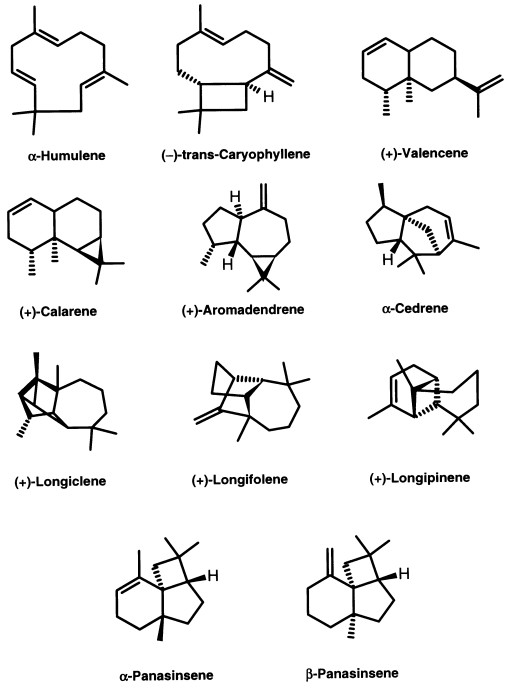

Fig.3. Sesquiterpene hydrocarbons.

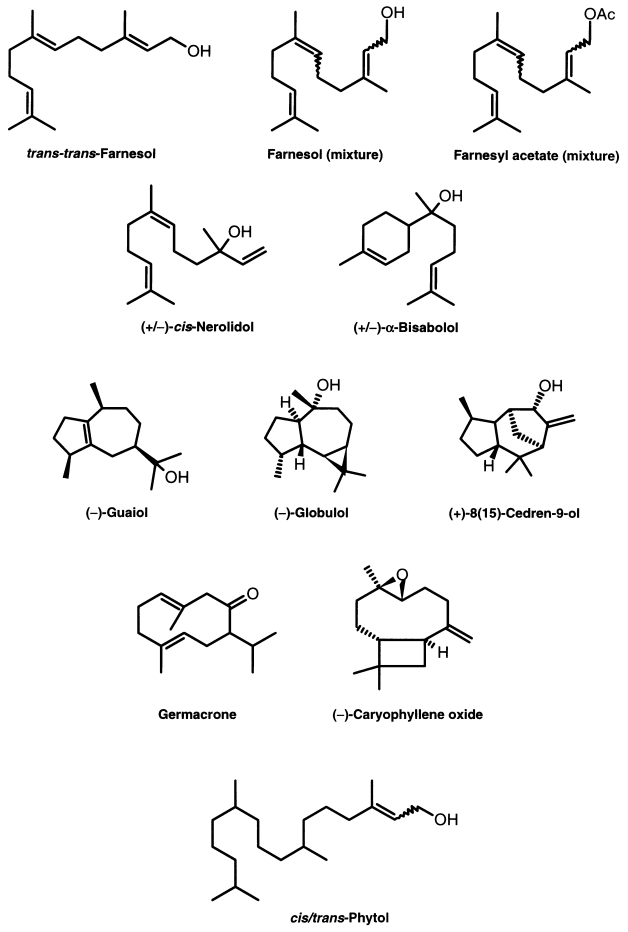

Fig.4. Oxygenated sesquiterpenes and phytol.

totally annulled by acetylation of the alcoholic function, which, as was foreseeable, is essential for the effectiveness. Nerolidol, like linalol, shows a pro-oxidant activity in Method A and no activity in Method B, confirming that the particular molecular characteristics of these compounds constitute the reason for this behaviour. Endowed with a certain degree of activity, confirmed in both methods, is the cyclic ketone germacrone.

The activity of phytol, the sole diterpene subjected to this study, is comparable to that of the other allylic alcohols previously discussed.

3.4. Benzene derivatives

Among the benzene derivatives (Fig. 5) phenols show the best results. As previously mentioned for thymol and carvacrol, these results are in accordance with the numerous literature data (Aeschbach et al., 1994; Yanishlieva et al., 1999). In particular these compounds seem to be more efficient in preventing the formation of primary oxidation products (Method B), than their effect on the formation of secondary oxidation products (Method A).

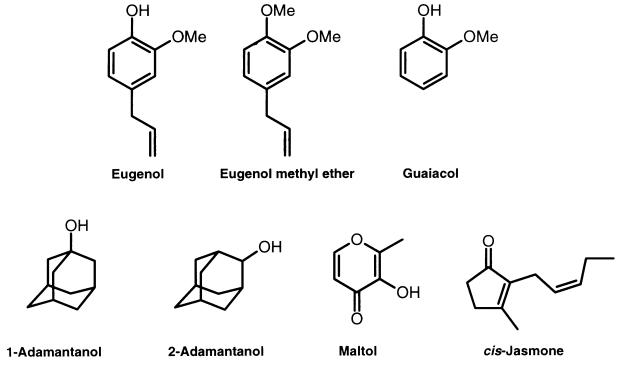

Fig.5. Selected benzene and non isoprenoid components.

3.5. Non isoprenoid components

The last class of components is represented by hydrocarbons (Fig. 5), alcohols, aldehydes, ketones, widely present in the most of essential oils, even if at hardly ever elevated levels.

In absolute the highest activity is shown by *cis*-jasmone which contain both an α,β-unsaturated ketone function and two allylic methylenes. Also in this case the allylic alcohols show some slight prevalence, whereas the aldehydes, not detectable with Method A, show a very low value with Method B. Therefore, this class of components, together with the sesquiterpene hydrocarbons, possesses the least antioxidant effectiveness.

4. Conclusion

It is well known that many parameters must be carefully taken into consideration when oxidative processes and attempts at their elimination and/or control are studied. The model system used is perhaps one of the most important, since it allows us to follow a defined step (primary or secondary) of the oxidative process. This is important because if, on one hand, the primary oxidation products (monohydroperoxides) are odourless and tasteless and do not affect food quality, even though their toxicity is in discussion (Sun, Stahr & Love, 1998), on the other hand they are the precursors of the secondary products (aldehydes, ketones, acids), which instead strongly affect odour and flavour of food, decidedly compromising its quality (Belitz & Grosch, 1987). Therefore, it is necessary, as emphasised throughout, to assess the antioxidant effectiveness in model systems dealing with both steps of lipid oxidation.

The results of this study show that the primary oxidation is a quite "easily" controllable process, and the presence of available hydrogen atoms from phenol and/or allylic groups represents a good barrier against the primary oxidative process. In effect the most widespread synthetic antioxidants act in such way (Haumann, 1990). It is very difficult to explain the molecular capacities of

compounds in the secondary oxidative process, this being much more complex than primary one. Considering that the possibility to yield a hydrogen atom, as in the primary process, still has a good chance of success, different mechanisms cannot be ruled out.

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