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Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems

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Abstract

The autoxidation of purified triacylglycerols of lard and sunflower oil (TGL and TGSO) containing 0.02, 0.05, 0.10 and 0.20% thymol and carvacrol was studied at ambient temperature. The results obtained with the inhibited lipid systems (thymol - TGL; carvacrol - TGL; thymol - TGSO and carvacrol - TGSO) showed that thymol had the highest antioxidant effectiveness and activity during TGSO oxidation. Thymol and carvacrol participated in one side reaction during inhibited TGL oxidation, and thymol took part in two side reactions during TGSO oxidation. Carvacrol molecules did not participate in side reactions during TGSO oxidation. Thymol and carvacrol contributed to chain initiation to a higher degree during TGSO oxidation than during TGL oxidation. Thymol radicals did not participate in chain propagation during TGL and TGSO oxidation. Carvacrol radicals took part in one reaction of chain propagation in both lipid systems. In general, during autoxidation of lipids at ambient temperature, thymol is a more effective and more active antioxidant than carvacrol. Both antioxidants differ in the mechanism of their inhibiting action which depends on the character of the lipid medium. Thymol is a better antioxidant in TGSO than in TGL, whereas the activity of carvacrol in the two lipid systems does not differ significantly.

1. Introduction

Autoxidation of lipids has long been recognised as a major deterioration process affecting both the sensory and nutritional quality of foods. The high oxidation stability of lipids, which can be ensured by addition of antioxidants, is important for health protection and for economic reasons.

Some toxicological studies (Lindenschmidt et al., 1986; Kahl and Kappus, 1993) have implicated the widely-used synthetic inhibitors butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in promoting the development of cancerous cells in rats.

These findings, together with consumer interest in natural food additives, have reinforced the interest in natural antioxidants (Pokorny, 1991; Evans and Reyhout, 1992; Gordon, 1996).

Natural antioxidants are extensively studied also for their capacity to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases and cancer (Cozzi et al., 1997).

Herbs and spices are harmless sources for obtaining natural antioxidants (Gordon and Weng, 1992; Kim et al., 1994). Our investigations on the antioxidant activity of spices from the family *Lamiaceae* grown in Bulgaria (Yanishlieva and Marinova, 1995a; Marinova and Yanishlieva, 1997) have shown that the ethanolic extract from *Saturejae hortensis* L. (summer savory) stabilised lipids against oxidative changes in air. This extract is also promising for stabilisation of sunflower oil at frying temperature (Yanishlieva et al., 1997).

Main components of the ethanolic extract of *Saturejae hortensis* L., as well as of the essential oils of *Saturejae montana* L. (Kustrak et al., 1996), *Saturejae spicigera* (C. Koch) (Tumen and Baser, 1996), *Saturejae odora* L. and *Saturejae parvifolia* L. (Muschietti et al., 1996), are the isomers thymol (5-methyl-2-isopropylphenol) and carvacrol (5-isopropyl-2-methylphenol) (Fig. 1). It is emphasised (Tsimidou and Boskou,

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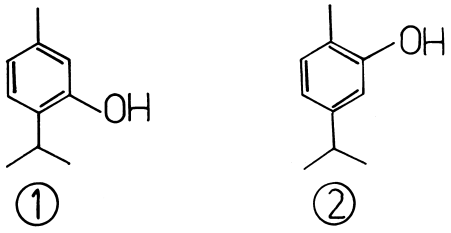

Fig. 1. Structure of the investigated antioxidants 1 – thymol; 2 – carvacrol.

1994) that, in *Lamiaceae* plants, thymol is always accompanied by its isomer carvacrol. Both compounds are biologically active – thymol has antiseptic, and carvacrol possesses antifungal properties (Menphini et al., 1993).

It has been established (Aeschbach et al., 1994) that thymol and carvacrol inhibit the peroxidation of liposome phospholipids in a concentration dependent manner. It appeared (Lagouri et al., 1993) that both isomers were equally effective in the autoxidation of lard at 35°C at a concentration of 0.1%. The inhibition of oxidation by the essential oils from plants of oregano species was highly dependent on the content of carvacrol + thymol (Lagouri and Boskou, 1995).

Our previous investigations showed that the positional isomers of some phenolic antioxidants, e.g. quercetin and morin, may exert different antioxidant behaviour depending on lipid substrate and temperature (Yanishlieva and Marinova, 1996; Marinova and Yanishlieva, 1998). This fact motivated us to carry out an investigation on the antioxidant activity and mechanism of action of thymol and carvacrol. There are no data in the literature concerning the mechanism of antioxidant action of these phenolic antioxidants in lipids.

We have performed a preliminary study on the antioxidant action of thymol and carvacrol at different concentrations (0.02–0.20%) during autoxidation of pure triacylglycerols of lard and sunflower oil at 100°C. The results obtained showed that at this temperature, commonly used for accelerated stability determination, the effectiveness of either substance is not high. The highest effectiveness was achieved at a concentration 0.2% (*ca* 10^{-2}M) when the increase of the oxidation stability was 2.5–3 times. The stabilisation factor for some other natural antioxidants under the same experimental conditions was much higher, e.g. for 3,4-dihydroxybenzoic, ferulic, sinapic and caffeic acids (Marinova and Yanishlieva, 1992b; Yanishlieva and Marinova, 1995b), esculetin and fraxetin (Marinova et al., 1994); it ranged from 6 to 340. It is most likely that the volatilities of thymol and carvacrol were responsible for their relatively low stabilising effect observed at 100°C.

Here we present the kinetic results obtained by following the autoxidation process at room temperature. Since the type and unsaturation degree of the lipid medium may strongly influence the inhibiting effect of the antioxidants (Popov and Yanishlieva, 1970; Yanishlieva and Popov, 1971b; Marinova and Yanishlieva, 1994; Marinova et al., 1994) we report the stabilizing eflect of thymol and carvacrol in two lipid systems – purified triacylglycerols of lard (TGL) and of sunflower oil (TGSO). As established (Yanishlieva and Popov, 1973), during the initial stage of the autoxidation of TGSO the linoleate moieties alone are attacked by oxygen and peroxide radicals, whereas both the linoleate and oleate in TGL participate in chain generation and chain propagation.

2. Materials and methods

2.1. Materials

Purified triacylglycerols of lard (TGL) and sunflower oil (TGSO) were obtained by removing pro- and antioxidants and trace metals from commercially available lard and sunflower oil by adsorption chromatography (Popov et al., 1968): 50 g lipid substrate in 500 ml distilled hexane were passed through a column (i.d. 2 cm) filled with 35 g alumina (type 507C, neutral, Fluka AG, Buchs, Switzerland) activated for 4 h at 180°C, and collected in nitrogen in the dark. The products obtained were stored at −20°C in an inert atmosphere.

The fatty acid compositions of TGL and TGSO were as follows: TGL–myristate 2%; palmitate 24%; palmitoleate 2%; stearate 15%; oleate 49%; linoleate 8%; TGSO–palmitate 6%; stearate 5%; oleate 25%; linoleate 64%.

The phenolics thymol and carvacrol were from E. Merck, Germany.

2.2. Methods

Inhibition of the lipid substrates was achieved by adding aliquots of a solution of the antioxidant in purified acetone to a weighed lipid sample followed by the removal of the solvent under nitrogen. Samples containing 0.02, 0.05, 0.10 and 0.20 wt.% thymol and carvacrol were prepared.

Oxidation at 22°C (±2°C) was performed in the dark using a 1-mm layer in a Petri dish having a diameter of 5 cm. Under these conditions, the process took place in a kinetic regime, i.e. at a sufficiently high oxygen concentration at which the difflision rate does not influence the oxidation rate (Emanuel et al., 1965). Three replicate samples were stored. The oxidation was followed by withdrawing samples (*ca* 0.1 g) at measured time intervals and subjecting them to iodometric determination of the peroxide concentration, i.e. the peroxide

value PV (Yanishlieva et al., 1978). The efflectiveness of the antioxidants was estimated on the basis of the induction period IP, which was detemiined by the method of the tangents to the two parts of the kinetic curve (Yanishlieva and Popov, 1971a; Le Tutour and Guedon, 1992). The rate of non-inhibited W_O and inhibited W_{inh} oxidation was found from the tangents to the initial phase of the kinetic curves of peroxide accumulation and expressed as M s^{-1} (Marinova and Yanishlieva, 1992a).

3. Results and discussion

The results obtained are interpreted on the basis of the main regularities of inhibited lipid oxidation. The introduction of an antioxidant (inhibitor, InH) into the oxidising lipid system leads to a change in mechanism of the process and, as a result, in process kinetics (Scheme 1 and 2) (Denisov and Khudyakov, 1987). The effect of the inhibitor InH depends on the participation of its molecules and the radicals formed from the latter in a series of reactions (Scheme 2) (Denisov and Khudyakov, 1987):

In the schemes below, LH is the oxidizing lipid substrate, $LO_2^\bullet$ is the peroxide radical, and InH is the inhibitor.

The peculiarities of the inhibitor action are described by two kinetic characteristics (Yanishlieva and Marinova, 1992): (i) effectiveness, representing the possibility of blocking the radical chain process by interaction with peroxide radicals (reaction (7)), which is responsible for the duration of the induction period IP, and (ii) strength, expressing the possibility for the inhibitor moieties to participate in other reactions, e.g. (−7),(10),(11),(12),(14), which lead to a change in oxidation rate during the IP. A measure of the effectiveness is the stabilisation factor F:

$$F = IP_{inh}/IP_o$$

where IP_{inh} is the induction period in the presence of an inhibitor, and IP_o is the induction period of the non-inhibited system.

The oxidation rate ratio ORR is a measure of the strength

$$ORR = W_{inh}/W_o$$

where W_{inh} is the oxidation rate in the presence of an inhibitor, and W_o is the initial oxidation rate of the non-inhibited system. ORR is an inverse measure of the strength.

When ORR is larger than one, then the oxidation proceeds faster in the presence of an inhibitor than in the case of its absence, which, for example, is observed at high tocopherol concentrations (Marinova and Yanishlieva, 1992a). The lower the ORR, the stronger the inhibitor.

The general parameter antioxidant activity A (Yanishlieva and Marinova, 1992) unifies tie effectiveness of an inhibitor in termination of the autoxidation chain, on the one hand, and its ability to decrease the oxidation rate during the IP, on the other. A is equal to F/ORR:

$$A = F/ORR$$

Figs. 2 and 3 illustrate, by way of example, the kinetic curves of peroxide accumulation during oxidation of TGL and TGSO in the presence of 0.02 and 0.05% of thymol and carvacrol (Fig. 1). The kinetic curves of oxidation of non-inhibited lipid substrates are also presented. All kinetic curves are the mean result of three independent experiments. The values obtained varied by no more than 5%.

The kinetic parameters, obtained after processing the kinetic curves for all investigated concentrations are given in Tables 1 and 2. The mean rate of inhibitor consumption W_{InH} also is presented as determined according to the formula:

$$W_{InH} = [InH]_o/IP_{inh}\ (M\,s^{-1}) \quad (1)$$

where $[InH]_o$ is the initial concentration of the antioxidant (M), and IP_{inh} is the duration of the IP(s).

(0) $2LH + O_2 \rightarrow 2L^\bullet + H_2O_2$
(1) $L^\bullet + O_2 \rightarrow LO_2^\bullet$
(2) $LO_2^\bullet + LH \rightarrow LOOH + L^\bullet$
(3) $LOOH \rightarrow LO^\bullet + {}^\bullet OH$
(3′) $2LOOH \rightarrow LO_2^\bullet + LO^\bullet + H_2O$
(3″) $LOOH + LH \rightarrow LO^\bullet + L^\bullet + H_2O$
(4) $L^\bullet + L^\bullet \rightarrow L\text{-}L$
(5) $L^\bullet + LO_2^\bullet \rightarrow + LOOL$
(6) $LO_2^\bullet + LO_2^\bullet \rightarrow$ products

Scheme 1. Non-inhibited oxidation.

(7) $LO_2^\bullet + InH \rightarrow LOOH + In^\bullet$
(−7) $In^\bullet + LOOH \rightarrow InH + LO_2^\bullet$
(8) $In^\bullet + LO_2^\bullet \rightarrow In\text{-}OOL$
(9) $In^\bullet + In^\bullet \rightarrow$ products
(10) $In^\bullet + LH \rightarrow InH + L^\bullet$
(11) $InH + LOOH \rightarrow$ products
(12) $InH + O_2 \rightarrow In^\bullet + HO_2^\bullet$
(13) $InOOL \rightarrow InO^\bullet + LO^\bullet$
(14) $In^\bullet + O_2 \rightarrow InOO^\bullet$

Scheme 2. Inhibited oxidation.

Fig. 4 illustrates the dependence of the stabilisation factor F on the concentration of thymol and carvacrol during oxidation of TGL and TGSO at 22°C. It is clear (curve 3 in Fig. 4) that during the oxidation of TGSO, thymol shows the highest antioxidative effectiveness in a wide concentration range (below 1×10^{-2} M). At concentrations not higher than 3.3×10^{-3} M F, for the inhibited systems carvacrol-TGL, thymol-TGL and carvacrol-TGSO, have practically the same values.

Fig. 5 illustrates the changes of antioxidant activity A with increasing antioxidant concentration. Here again thymol demonstrates the highest activity during oxidation of TGSO (curve 3, Fig. 5).

The dependence of F on antioxidant concentration is linear only in the case of carvacrol-inhibited TGSO oxidation (line 4 in Fig. 4). The absence of linearity of the dependencies 1, 2 and 3 in Fig. 4 is due to the participation of the inhibitor molecules in reactions other than the main reaction of chain termination (7), namely reaction (11) or/and (12). In this case, there is a relationship between the mean rate of inhibitor consumption W_{InH} and the inhibitor concentration [InH] (Emanuel et al., 1965):

$$W_{InH} = W_i/f + K_{eff}[InH]^n \quad (2)$$

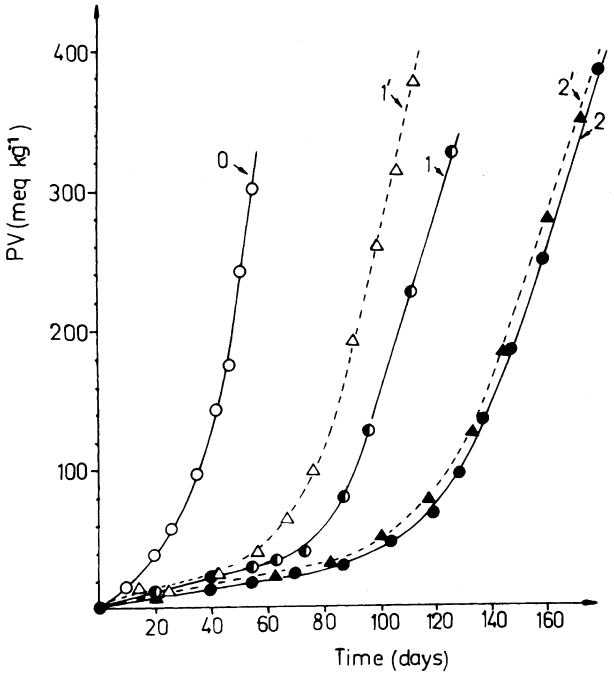

Fig. 2. Kinetic curves of peroxide accumulation (PV) during oxidation of TGL at 22°C in the absence (0) and in the presence of 0.02% (1,1′) and 0.05% (2,2′) thymol (1,2) and carvacrol (1′,2′).

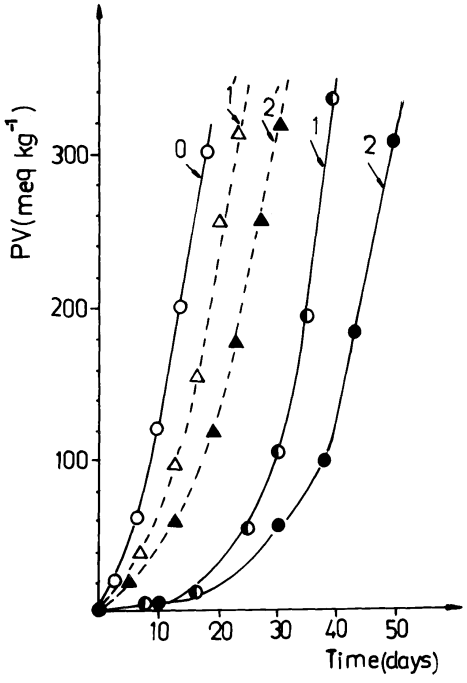

Fig. 3. Kinetic curves of peroxide accumulation (PV) during oxidation of TGSO at 22°C in the absence (0) and in the presence of 0.02% (1,1′) and 0.05% (2,2′) thymol (1,2) and carvacrol (1′,2′).

Table 1
Kinetic parameters characterizing inhibited oxidation of triacylglycerols of lard (TGL) at 22°C, $PV_o = 0$ meq kg^{-1}, $IP_o = 37$ days, $W_o = 8.9\times10^{-9}$ M s^{-1}

No.	Antioxidant	Inhibitor concentration		F	ORR	A	$W_{inh}(\times10^9)$ (M s^{-1})	$W_{InH}(\times10^{10})$ (M s^{-1})
		[InH] (%)	[InH]($\times10^3$) (M)					
1	Thymol	0.02	1.33	2.2	0.31	7.1	2.76	3.30
		0.05	3.33	3.5	0.19	18.4	1.65	4.24
		0.10	6.67	4.8	0.14	34.3	1.22	5.51
		0.20	13.33	6.4	0.11	52.8	0.96	7.68
2	Carvacrol	0.02	1.33	2.1	0.38	5.5	3.40	3.85
		0.05	3.33	3.4	0.25	13.6	2.22	4.28
		0.10	6.67	4.7	0.20	23.5	1.78	5.67
		0.20	13.33	6.0	0.14	42.9	1.29	8.34

Table 2
Kinetic parameters characterizing inhibited oxidation of triacylglycerols of sunflower oil (TGSO) at 22°C, $PV_o = 0$ meq kg^{-1}, $IP_o = 5$ days, $W_o = 4.63 \times 10^{-8}$ M s^{-1}

No.	Antioxidant	Inhibitor concentration		F	ORR	A	$W_{inh}(\times 10^9)$ (M s^{-1})	$W_{InH}(\times 10^{10})$ (M s^{-1})
		[InH] (%)	[InH]($\times 10^3$) (M)					
1	Thymol	0.02	1.33	6.0	0.08	75.0	3.86	6.15
		0.05	3.33	7.9	0.08	98.8	3.86	11.2
		0.10	6.67	9.2	0.08	115.0	3.86	18.8
		0.20	13.33	7.4	0.08	92.2	3.86	48.2
2	Carvacrol	0.02	1.33	2.0	0.71	2.8	33.1	30.8
		0.05	3.33	3.4	0.42	8.1	19.2	32.1
		0.10	6.67	5.9	0.29	20.3	13.6	31.5
		0.20	13.33	10.5	0.17	61.8	7.7	32.5

where W_i is the mean rate of initiation during the induction period of the inhibited oxidation (M s^{-1}), and f is the stoichiometric coefficient of inhibition determining how many radicals perish in an inhibitor molecule.

During TGSO oxidation, the carvacrol molecule did not participate in side reactions because the mean rate of its consumption W_{InH} did not depend on its concentration (Table 2).

The presentation of the results for the inhibited systems thymol-TGL and carvacrol-TGL, as a plot of the mean rate of their consumption, W_{InH}, against concentration [InH] at different n, showed that, for both antioxidants in TGL, this plot was linear at $n = 1$ (Fig. 6), which indicated their participation in one side reaction. From the slopes of the lines, the rate constants K_{eff} of this reaction was determined, having the same value for both inhibitors in TGL oxidation, e.g. $(3.7 \pm 0.1) \times 10^{-8}$ s^{-1}. K_{eff} for α-tocopherol in TGL oxidation at room temperature was 5.8×10^{-8} s^{-1} (Marinova and Yanishlieva, 1992a).

Two side reactions, (11) and (12), must be considered to identify the side reaction in which thymol and carvacrol participate during TGL oxidation.

Previous investigations have shown that K_{eff} depends on the character of the lipid system and, in particular, on the different decomposition rate of linoleic and oleic acid hydroperoxides (Yanishlieva and Popov, 1971a; Yanishlieva, 1973; Marinova and Yanishlieva, 1994, 1996; Yanishlieva and Marinova, 1995b). As seen from Scheme 2, reaction (11) is dependent on the hydroperoxide reactivity. That is why the main side reaction in which the antioxidants in inhibited lipid oxidation should participate is reaction (11). The same should be

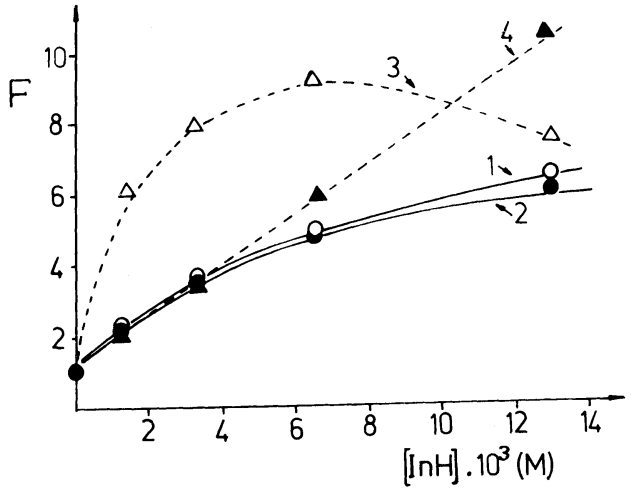

Fig. 4. Dependence of the stabilization factor F of thymol (1,3) and carvacrol (2,4) on their concentration [InH] during oxidation of TGL (1,2) and TGSO (3,4) at 22°C.

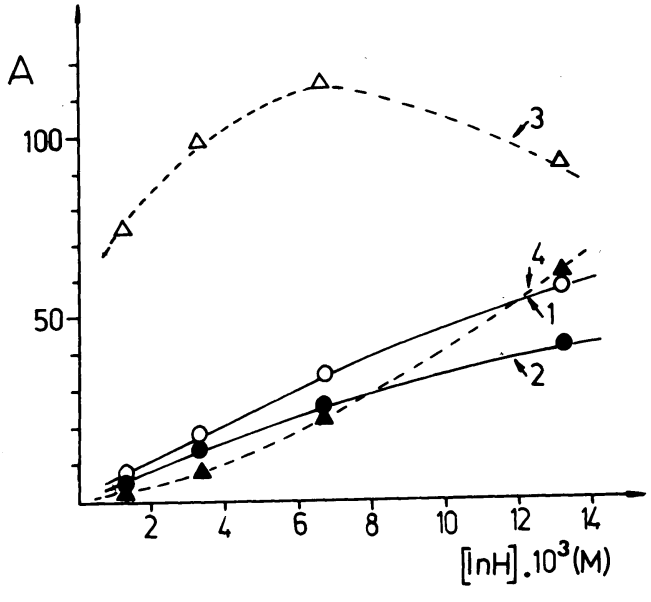

Fig. 5. Dependence of the antioxidative activity A of thymol (1,3) and carvacrol (2,4) on their concentration [InH] during oxidation of TGL (1,2) and TGSO (3,4) at 22°C.

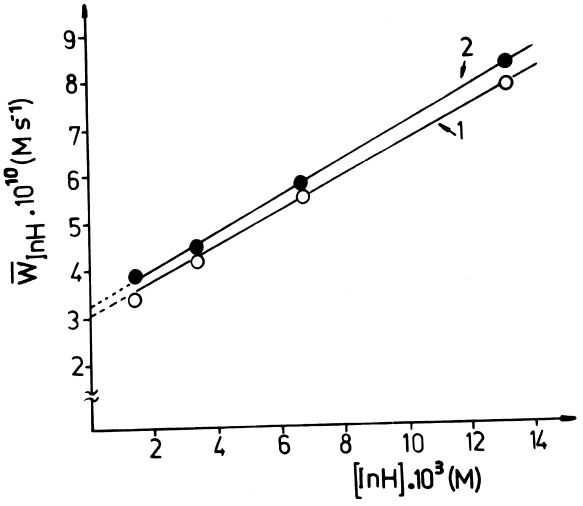

Fig. 6. Dependence of the mean rate of consumption W_{InH} of thymol (1) and carvacrol (2) on their concentration [InH] during oxidation of TGL at 22°C.

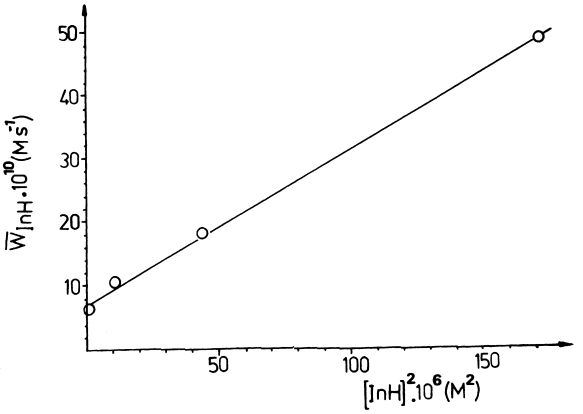

Fig. 7. Dependence of the mean rate of consumption W_{InH} of thymol on its concentration $[InH]^2$ during oxidation of TGSO at 22°C.

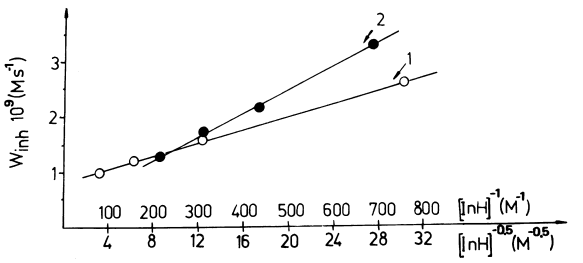

Fig. 8. Dependence of the rate of inhibited oxidation W_{inh} on the concentration of thymol, $[InH]^{-1}$, and of carvacrol $[InH]^{-0.5}$, during oxidation of TGL at 22°C.

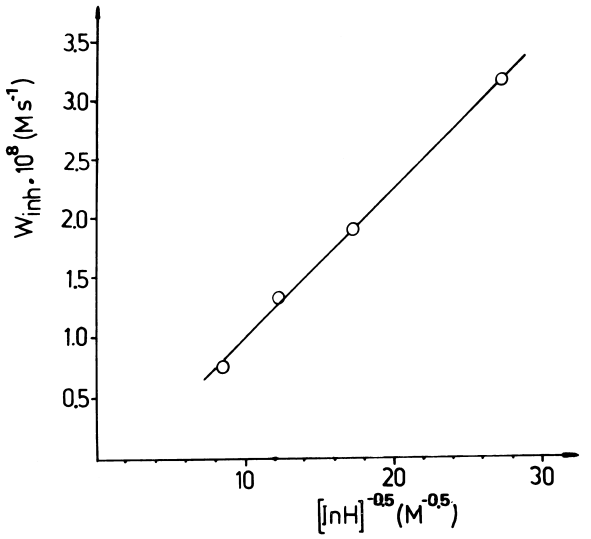

Fig. 9. Dependence of the rate of inhibited oxidation W_{inh} on the concentration of carvacrol, $[InH]^{-0.5}$, during oxidation of TGSO at 22°C.

true for thymol and carvacrol in TGL oxidation at room temperature.

Fig. 7 shows that the plot (2) for thymol-inhibited oxidation of TGSO at 22°C is linear at $n=2$. In this case, the antioxidant participates in both side reactions of inhibited oxidation, e.g. (11) and (12), and $K_{eff}=2.2\times10^{-5}\,M^{-1}\,s^{-1}$.

How do thymol and carvacrol participate in chain initiation and propagation?

The parameter W_i/f takes into account the participation of the antioxidant in the initiation reactions. W_i/f was determined from Figs. 6 and 7 by extrapolation to zero concentration of the antioxidants. The values for W_i/f were $3.0\times10^{-10}\,M\,s^{-1}$ (thymol in TGL), $3.3\times10^{-10}\,M\,s^{-1}$ (carvacrol in TGL), $7.0\times10^{-10}\,M\,s^{-1}$ (thymol in TGSO), and $32\times10^{-10}\,M\,s^{-1}$ (carvacrol in TGSO). W_i/f for α-tocopherol and ferulic acid in TGL oxidation at room temperature were $0.1\times10^{-10}\,M\,s^{-1}$ and $1\times10^{-10}\,M\,s^{-1}$, respectively (Marinova and Yanishlieva, 1992a). Comparison of the data obtained showed that α-tocopherol takes part in chain initiation with an order of magnitude lower rate than do ferulic acid, thymol and carvacrol. Also, thymol and carvacrol contribute in a higher degree to chain initiation during TGSO oxidation than during TGL oxidation.

Previous research (Denisov and Khudyakov, 1987) has proved that, if the inhibitor radical $In^{\bullet}$ participates in one reaction of chain propagation, reaction (−7), or (10), or (14), the relationship Eq. (3) is valid:

$$W_{inh} \sim [InH]^{-0.5} \quad (3)$$

If $In^{\bullet}$ does not participate in chain propagation, relationship Eq. (4) is valid:

$$W_{inh} \sim [InH]^{-1} \quad (4)$$

Processing of the results obtained (Tables 1 and 2) on the basis of the relationships Eqs. (3) and (4) showed

that, for carvacrol in TGL and TGSO oxidation, relationship Eq. (3) is valid (curve 2 in Figs. 8 and 9). This means that the radicals of carvacrol participate in one reaction of chain propagation. As discussed previously (Yanishlieva and Marinova, 1995b), this reaction should be reaction (10): $In^{\bullet} + 1{,}11 \rightarrow InH + L^{\bullet}$.

During TGL oxidation, the thymol radical did not participate in chain propagation because it obeyed relationship (Eq. (4)) (Fig. 8, line 1).

In TGSO oxidation at room temperature the rate of inhibited oxidation in the presence of thymol, W_{inh}, did not depend on its concentration (Table 2). This means that under these circumstances, thymol is so active that $LO_2^{\bullet}$ reacts faster with InH than with LH (Denisov and Khudyakov, 1987).

4. Conclusion

Thymol is a better antioxidant in lipids than carvacrol. This fact is due to the greater steric hindrance of the phenolic group in thymol than in carvacrol.

Acknowledgements

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