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Effects of antioxidants on the stability of triacylglycerols and methyl esters of fatty acids of sunflower oil

Nedjalka Vl. Yanishlieva & Emma M. Marinova

Institute of Organic Chemistry with Center of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

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The autoxidation of kinetically pure triacylglycerols and methyl esters of sunflower oil (TGSO and MESO) in the presence of four different concentrations of 3,4-dihydroxybenzoic, ferulic, sinapic and caffeic acids, and in the absence of metal ions, at 100°C was studied. It was established that the effectiveness and strength of the phenolic acids was greater in TGSO than in MESO. The interpretation of the results by kinetic and computational methods allowed the mechanism of action of the antioxidants to be elucidated. In both lipid substrates the molecules of the phenolic acids participate in one side reaction. The rate constants of this reaction in TGSO and MESO are practically the same, which shows that the binding of the fatty acids to the triacylglycerol structure of the sunflower oil does not change the mechanism of phenolic acid consumption in side reactions. The phenolic acids participate in reactions of chain initiation, which have a three to six times higher rate in MESO than in TGSO. The radicals of 3,4-dihydroxybenzoic, sinapic and caffeic acids in MESO, and of 3,4-dihydroxybenzoic and caffeic acids in TGSO, take part in one reaction of chain propagation (with the lipid substrate). The radicals of ferulic acid in MESO, and of ferulic and sinapic acids in TGSO, participate in more than one reaction of chain propagation. The effectiveness and strength of the phenolic acids are higher in TGSO than in MESO because, during the oxidation of TGSO, the contribution of the inhibitor radicals and molecules to chain initiation and propagation is smaller than in the case of MESO oxidation.

INTRODUCTION

Recently we performed some studies aimed at elucidating the activity and mechanism of action of natural antioxidants such as α-tocopherol and ascorbylpalmitate (Marinova & Yanishlieva, 1992*a*), phenolic acids (Marinova & Yanishlieva, 1994), and some hydroxycoumarins (Marinova *et al.*, 1994), depending on the degree of unsaturation of the lipid medium (Roginskii, 1990).

The results of some investigations have shown that the binding of fatty acids to triacylglycerols affects both the trend to oxidation of the lipid substrate containing no antioxidants (Yanishlieva & Popov, 1973; Katsuki *et al.*, 1987; Yanishlieva & Kortenska, 1989) and the inhibiting effect of the antioxidants (Yanishlieva & Popov, 1973; Katsuki *et al.*, 1987).

The purpose of the present study is therefore to discover how the binding of the fatty acids to the natural triacylglycerol system influences the effectiveness, strength and mechanism of action of some phenolic acids. The investigations were performed by using kinetically pure triacylglycerols of sunflower oil (TGSO) and the results obtained were compared with the published data on the inhibited oxidation of kinetically pure methyl esters of sunflower oil (MESO) (Marinova & Yanishlieva, 1994). The lipid substrates were inhibited with different concentrations (0.02–0.20 wt%) 3,4-dihydroxybenzoic, ferulic, sinapic and caffeic acids (Fig. 1).

MATERIALS AND METHODS

Materials

The triacylglycerols (TGSO) of commercially available sunflower oil were cleansed from pro- and antioxidants and trace metals by adsorption chromatography (Popov *et al.*, 1968): 100 g lipid substrate in 1000 ml distilled hexane were passed through a column (i.d. 2 cm) filled with 70 g alumina (type 507C, neutral, activity stage II, Fluka AG, Buchs, Switzerland) activated for 4 h at 180°C, and collected in nitrogen in the dark. The TGSO

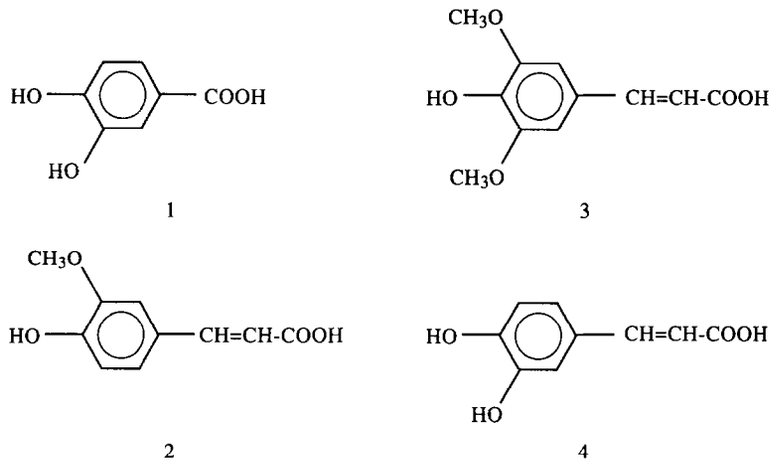

Fig. 1. Structure of the investigated phenolic acids: 1-3,4-dihydroxybenzoic, 2-ferulic, 3-sinapic, 4-caffeic.

were stored in an inert atmosphere at −20°C for no more than 10 days. Control oxidation experiments at 80°C in the presence of 0.01 and 0.02 wt% citric acid demonstrated that the chelating agent had no effect on oxidation kinetics. The initial peroxide value (PV_0) of the purified substrate at the start of each experiment was zero.

The phenolics 3,4-dihydroxybenzoic, 3-methoxy-4-hydroxycinnamic (ferulic) and 3,5-dimethoxy-4-hydroxycinnamic (sinapic) acids, were from Fluka, Switzerland, and 3,4-dihydroxycinnamic (caffeic) acid was from Merck, Germany.

Methods

Inhibition of the lipid substrate was achieved by adding aliquots of a solution of the antioxidant in purified acetone to a weighed lipid sample. Samples containing 0.02, 0.05, 0.10 and 0.20 wt% phenolic acids were prepared.

Oxidation was carried out at 100°C(±0.2°C) by blowing air through the samples (5 g) in the dark at a rate of 100 ml min^{-1}. The process 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 effectiveness of the antioxidants was estimated on the basis of the induction period IP, which was determined by the method of tangents to the two parts of the kinetic curve (Yanishlieva & Popov, 1971; Le Tutour & Guedon, 1992). The rate of non-inhibited W_0 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}. Recalculation of the rate from meq kg^{-1} h^{-1} into M s^{-1} was performed according to the following formula (Marinova & Yanishlieva, 1992*b*):

$$1 \text{ meq kg}^{-1}\text{h}^{-1} = 1.4 \times 10^{-7}\text{M s}^{-1}$$

Table 1. Kinetic parameters characterizing inhibited oxidation of triacylglycerols of sunflower oil (TGSO) at 100°C, PV_0 = 0 meq kg^{-1}, IP_0 = 0.5 h, $W_0 = 9.26 \times 10^{-6}$ M s^{-1} (acids as labelled in Fig. 1)

N	Antioxidant (Phenolic acid)	Inhibitor concentration		F	ORR	$W_{inh} \times 10^6$ (M s^{-1})	$W_{inh} \times 10^7$ (M s^{-1})
		[*InH*] (wt%)	[*InH*]x10^3 (M)				
1	3,4-Dihydroxybenzoic	0.02	1.30	3.6	0.60	5.5	2.78
		0.05	3.25	5.8	0.50	4.6	3.76
		0.10	6.49	7.4	0.42	4.0	5.63
		0.20	13.0	7.4	0.42	4.0	11.3
2	Ferulic	0.02	1.03	2.6	0.60	5.5	3.85
		0.05	2.53	3.2	0.60	5.5	5.97
		0.10	5.15	4.7	0.60	5.5	8.41
		0.20	10.3	5.4	0.60	5.5	13.6
3	Sinapic	0.02	0.89	7.6	0.27	2.5	0.75
		0.05	2.23	9.4	0.27	2.5	1.44
		0.10	4.46	13.0	0.27	2.5	2.06
		0.20	8.93	15.2	0.27	2.5	3.43
4	Caffeic	0.02	1.11	33.6	0.075	0.69	0.19
		0.05	2.78	48.6	0.054	0.50	0.32
		0.10	5.56	60.0	0.044	0.41	0.52
		0.20	11.1	60.0	0.041	0.37	1.05

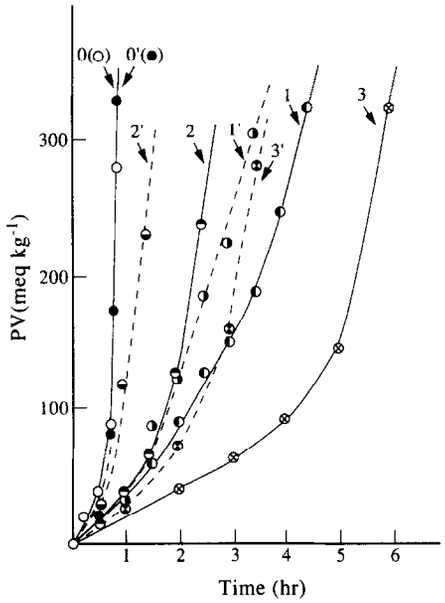

Fig. 2. Kinetic curves of peroxide accumulation (PV) during the oxidation of triacylglycerols TGSO (0,1,2,3) and methyl esters MESO (0′,1′,2′,3′) of sunflower oil at 100°C in the presence of 0.05% phenolic acids: 0 and 0′ —without additive, 1 and 1′ —3,4-dihydroxybenzoic acid, 2 and 2′ —ferulic acid, 3 and 3′ —sinapic acid. The data for MESO are taken from Marinova & Yanishlieva (1994).

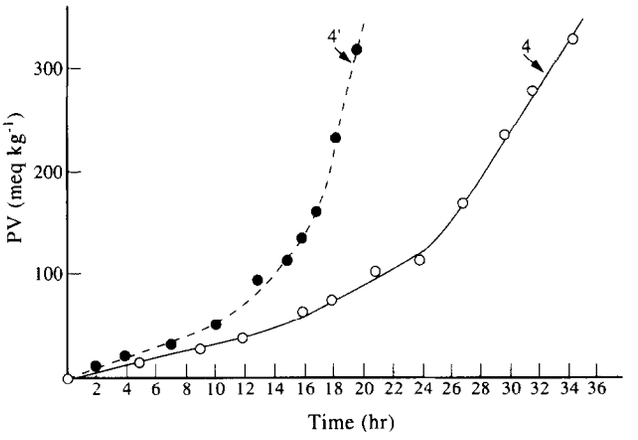

Fig. 3. Kinetic curves of peroxide accumulation during the oxidation of TGSO (4) and MESO (4′) at 100°C in the presence of 0.05% caffeic acid (4,4′). The data for MESO are taken from Marinova & Yanishlieva (1994).

RESULTS AND DICUSSION

The introduction of an inhibitor (antioxidant) into the oxidizing lipid system leads to a change in mechanism of the process and, as a result, a change in process kinetics. The effect of the inhibitor InH depends on the participation of its molecule and the radicals formed from the latter in a series of reactions (Denisov & Khudyakov, 1987):

$$(7)\quad LO_2^{\cdot} + InH \longrightarrow LOOH + In^{\cdot}$$

$$(-7)\quad In^{\cdot} + LOOH \longrightarrow InH + LO_2^{\cdot}$$

$$(8)\quad In^{\cdot} + LO_2^{\cdot} \longrightarrow In\text{–}OOL$$

$$(9)\quad In^{\cdot} + In^{\cdot} \longrightarrow products$$

$$(10)\quad In^{\cdot} + LH \longrightarrow InH + L^{\cdot}$$

$$(11)\quad InH + LOOH \longrightarrow products$$

$$(12)\quad InH + O_2 \longrightarrow In^{\cdot} + HO_2^{\cdot}$$

$$(13)\quad InOOL \longrightarrow InO^{\cdot} + LO^{\cdot}$$

$$(14)\quad In^{\cdot} + O_2 \longrightarrow InOO^{\cdot}$$

The peculiarities of the inhibitor action are described by two kinetic characteristics (Yanishlieva & 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 of the inhibitor moieties participating in other reactions, e.g. (−7), (10), (11), 12) and (14), which lead to a change in oxidation rate during the *IP*. A measure of the effectiveness is the stabilization factor **F**:

$$\mathbf{F} = IP_{inh}/IP_0$$

where IP_{inh} is the oxidation rate in the presence of an inhibitor, and IP_0 is the induction period of the non-inhibited system.

The oxidation rate ratio **ORR** is a measure of the strength

$$\mathbf{ORR} = W_{inh}/W_0$$

where W_{inh} is the oxidation rate in the presence of an inhibitor, and W_0 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 its absence, which, e.g. is observed at high tocopherol concentrations (Marinova & Yanishlieva, 1992*b*). The lower the **ORR**, the stronger the inhibitor.

Figures 2 and 3 illustrate, by way of example, the kinetic curves of peroxide accumulation during oxidation of TGSO and MESO in the presence of 0.05 wt% of phenolic acids (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. It can be seen that the effectiveness and strength of the inhibitors are lower in MESO than in TGSO. The kinetic parameters for all investigated concentrations of the phenolic acids in TGSO are given in Table 1. The mean rate of inhibitor consumption $\bar{W}_{InH}$ also is presented as determined according to the formula:

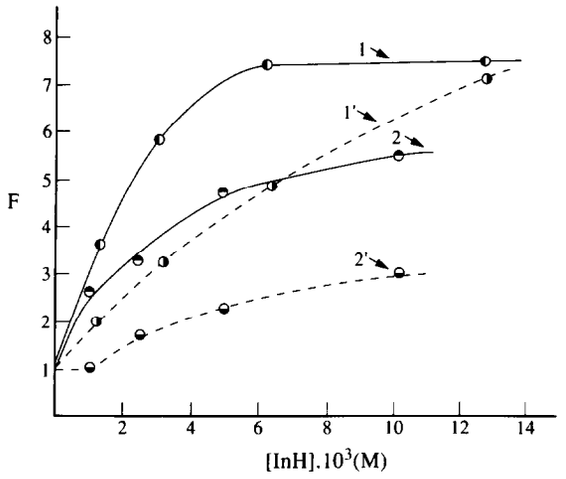

Fig. 4. Dependence of the stabilization factor **F** on the concentration of the phenolic acids [*InH*] during the oxidation of TGSO (1,2) and MESO (1′,2′) at 100°C: 1 and 1′ — 3,4-dihydroxybenzoic acid, 2 and 2′ — ferulic acid. The data for MESO are taken from Marinova & Yanishlieva (1994).

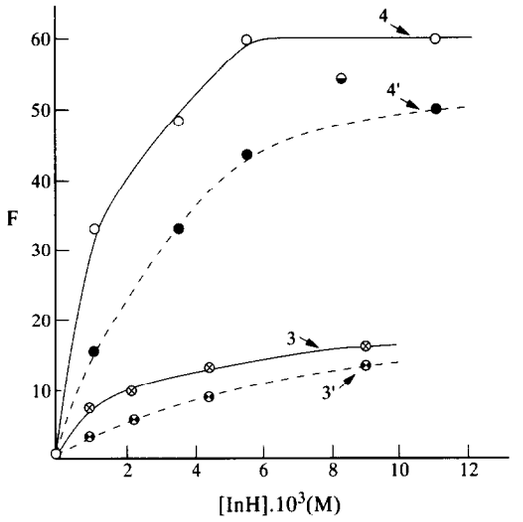

Fig. 5. The same as in Fig. 4, but for sinapic (3,3′) and caffeic (4,4′) acids.

$$\bar{W}_{InH} = [InH]_0/IP_{inh}(\mathrm{M\,s^{-1}}) \qquad (1)$$

where $[InH]_0$ is the initial concentration of the antioxidant (M), and *IP* is the duration of the *IP*(s).

Figures 4 and 5 illustrate the dependence of the stabilization factor **F** on the concentration of the phenolic acids. Obviously, for all acids in TGSO and MESO, respectively, these dependences are not linear. In addition, the effectiveness of the antioxidants in both systems increases in the sequence ferulic < 3,4-dihydroxybenzoic < sinapic < caffeic acid.

The absence of linearity of the dependences 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 $\bar{W}_{InH}$ and the inhibitor concentration [*InH*] (Emanuel *et al.*, 1965):

$$\bar{W}_{InH} = \bar{W}_i/f + K_{eff}[InH]^n \qquad (2)$$

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.

The presentation of the results as a dependence of the mean rate of consumption of phenolic acids $\bar{W}_{InH}$ on their concentration [*InH*] at different n showed that, for all phenolic acids in both lipid systems, this dependence was linear at $n = 1$ (Figures 6 and 7), which indicated their participation in one side reaction.

The values for K_{eff} in TGSO are presented in Table 2. Comparison of these values with those in MESO (Marinova & Yanishlieva, 1994) (3,4-dihydroxybenzoic acid 4.2×10^{-5} s^{-1}, ferulic acid 10.0×10^{-5} s^{-1}, sinapic acid 2.4×10^{-5} s^{-1}, caffeic acid 0.88×10^{-5} s^{-1}) reveals that, in the two systems, K_{eff} values have close (sinapic acid) or equal values (ferulic and caffeic acids). In the case of 3,4-dihydroxybenzoic acid alone, the K_{eff} value in TGSO is 1.6 times higher than that in MESO. That is why one may conclude that the binding of the fatty acids to the triacylglycerol structure of the sunflower oil does not change the mechanism of phenolic acid consumption in side reactions.

Figure 8 presents the dependence of the **ORR** on the concentration of the phenolic acids in TGSO and MESO. Comparison of these dependences with those of the stabilization factor **F** (Figures 4 and 5) shows that the sequence of change in strength of the phenolic acids is the same as the sequence of their effectiveness. Besides, the phenolic acids act as stronger inhibitors in TGSO than in MESO.

The oxidation rate during the IP is in direct proportion to the rate of chain initiation and propagation. The lower the rate constants of reactions (−7), (10), (11), (12), (13) and (14), e.g. of the reactions of participation of the inhibitor moieties in chain initiation

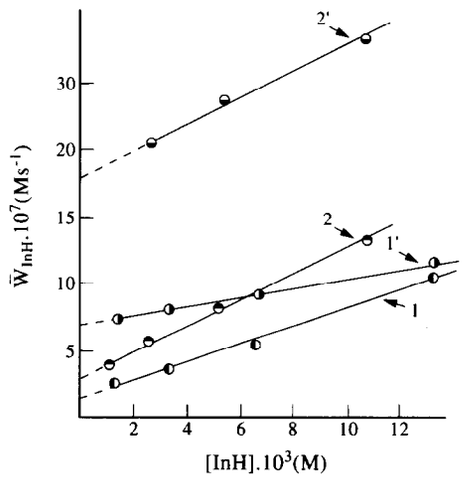

Fig. 6. Dependence of the mean rate of consumption of phenolic acids $\bar{W}_{InH}$ on their concentration [*InH*] during oxidation of TGSO (1,2) and MESO (1′,2′) at 100°C: 1 and 1′ — 3,4-dihydroxybenzoic acid, 2 and 2′ — ferulic acid. The data for MESO are taken from Marinova & Yanishlieva (1994).

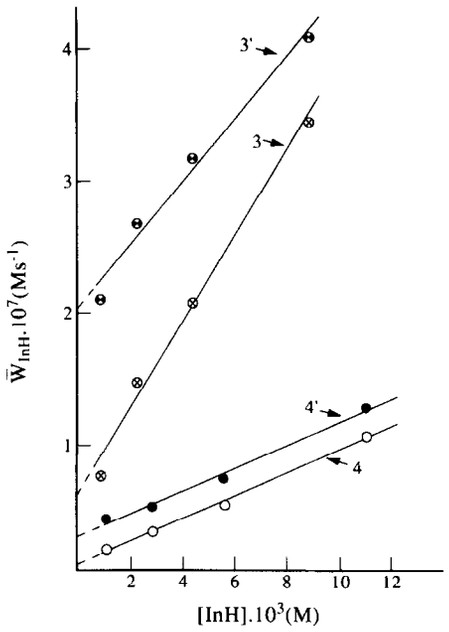

Fig. 7. The same as in Fig. 6, but for sinapic (3,3') and caffeic (4,4') acids.

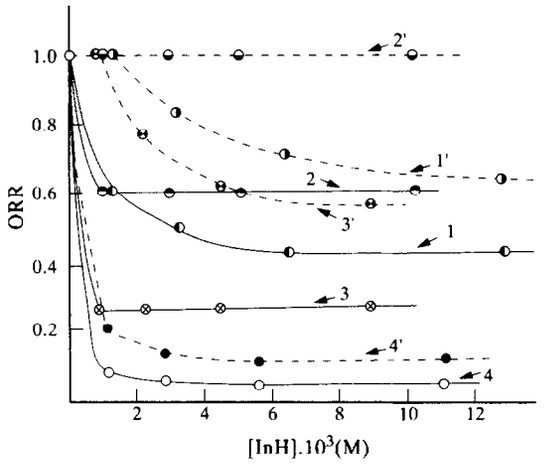

Fig. 8. Dependence of the oxidation rate ratio **ORR** on the concentration of phenolic acids during oxidation of TGSO and MESO at 100°C. Symbols as in Figs 4 and 5. The data for MESO are taken from Marinova & Yanishlieva (1994).

and propagation, the lower the oxidation rate during the IP and the stronger the inhibitor.

The parameter W_i/f takes into account the participation of the antioxidant in the initiation reactions. W_i/f was determined from Figures 6 and 7 by extrapolation to zero concentration of the phenolic acids and is presented in Table 2. Comparison of these data for W_i/f in TGSO with those obtained in MESO (Marinova & Yanishlieva, 1994) (3,4-dihydroxybenzoic acid 7.0×10^{-7} M s^{-1}, ferulic acid 18.0×10^{-7} M s^{-1}, sinapic acid 2.0×10^{-7} M s^{-1} and caffeic acid 0.3×10^{-7} M s^{-1}) shows that, during the oxidation of MESO, the phenolic acids cause a three to six times higher initiation rate than during the oxidation of TGSO.

Previous research (Denisov & Khudyakov, 1987) showed that, if the inhibitor radical *In*', participates in one reaction of chain propagation (reaction −7, or 10, or 14), the dependence (3) is valid:

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

When *In* does not participate in chain propagation, dependence (4) is valid

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

Processing of the results obtained (Table 1) on the basis of the dependences (3) and (4) showed that, for 3,4-dihydroxybenzoic and caffeic acid during TGSO oxidation, dependence (3) is valid (Fig. 9), and W_{inh} does not depend on the concentration of ferulic and sinapic acids.

As far as MESO oxidation is concerned, a linear dependence of W_{inh} on $[InH]^{-0.5}$ for 3,4-dihydroxybenzoic, sinapic and caffeic acids is observed (Marinova & Yanishlieva, 1994).

Which reaction of chain propagation, in which the phenolic acid radicals participate, is the most probable one: (−7), or (10), or (14)?

During the initial stage of the process, when the hydroperoxide concentration is low, the rate of reaction (−7) is negligibly low in comparison with the rate of reaction (10) (Mukai & Okauchi, 1989). The higher values of **ORR** during inhibited oxidation of MESO

Table 2. Kinetic parameters characterizing the mean rate of initiation, W_i/f, and the effective rate constant of inhibitor consumption, K_{eff}, during oxidation of triacylglycerols of sunflower oil (TGSO) at 100°C in the presence of different phenolic acids

Antioxidant (Phenolic acid)	$W_i/f \times 10^7$ (M s^{-1})	$K_{eff} \times 10^5$ (s^{-1})
3,4-Dihydroxybenzoic	1.5	7.30
Ferulic	3.0	10.6
Sinapic	0.6	3.20
Caffeic	0.1	0.82

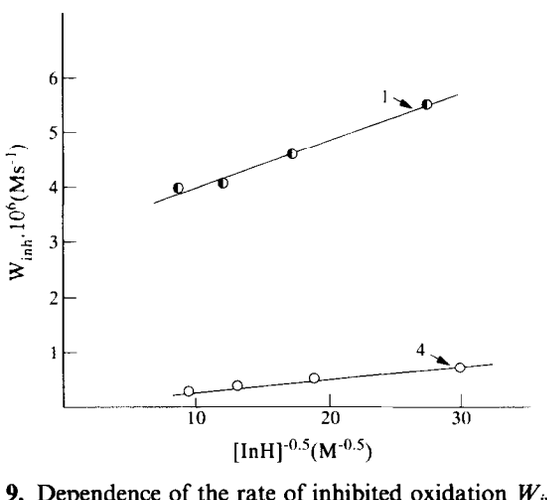

Fig. 9. Dependence of the rate of inhibited oxidation W_{inh} on the concentration of phenolic acids $[InH]^{-0.5}$ during the oxidation of TGSO at 100°C: 1 — 3,4-dihydroxybenzoic acid, 4 — caffeic acid.

than those during inhibited oxidation of TGSO (compare curves 1 and 1′, 2 and 2′, 3 and 3′, 4 and 4′ in Fig. 8) demonstrate the greater likelihood that reaction (10) and not reaction (14) will take place, because reaction (14) does not depend on the character of the substrate being oxidized.

The results show why the effectiveness and strength of the phenolic acids are higher in TGSO than in MESO: during the oxidation of TGSO the contribution of the inhibitor radicals $In^{\cdot}$ and molecules InH to chain propagation and initiation is smaller than in the case of MESO oxidation.

The W_{inh} values for ferulic and sinapic acids determined during the oxidation of TGSO and the W_{inh} values for ferulic acid determined during the oxidation of MESO (Marinova & Yanishlieva, 1994) show no linear dependence on either $[InH]^{-0.5}$ or $[InH]^{-1}$. This fact indicates that the radicals of phenolic acids, mentioned above, are involved in more than one reaction of chain propagation during the oxidation of both lipid systems.

ACKNOWLEDGEMENTS

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