The inhibitory activity of natural phenolic antioxidants in the oxidation of lipid substrates

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The suppression of the oxidation of triglyceride and methyl esters of lard and olive and sunflower oils by additives of natural phenolic acids (hydroxy and methoxy derivatives of *para*-hydroxybenzoic and cinnamic acids) at 100 °C was studied. The rate constants of the interaction of these acids with peroxyl radicals in the oxidation of cumene at 60 °C were determined by the chemiluminescence method. Caffeic acid is the most efficient lipid antioxidant, exceeding ionol and α-tocopherol.

Key words: natural phenolic acids, antioxidants; chemiluminescence.

Phenolic acids are contained in plant extracts[1—3] and can be used as nontoxic ecologically safe stabilizers of oil, food, and medicines.[4,5] In the present work, the antiradical activity of several natural phenolic acids was estimated by the chemiluminescence method, and the suppression effects of additives of these acids in lipid substrates with different degrees of unsaturation were studied. The inhibitory effects of antioxidants of plant origin were compared with the suppression effects of the known lipid antioxidants, α-tocopherol and ionol.

Experimental

Phenolic acids **1**—**6**, **8** (Fluka) (Table 1), and **7** (Merck) and α-tocopherol (Sigma) were used without additional purification. Cumene and the other organic solvents were purified by standard procedures. Lard, triglycerides, and methyl esters of olive and sunflower oils were purified by passing them through activated alumina.[6] Azo-bis-isobutyronitrile (AIBN), twice recrystallized from ethanol followed by drying *in vacuo* to constant weight, was used as the initiator of free radicals.

Lipid substrates were oxidized at 100 °C in a bubbling-type cell in a flow of air (100 cm^3 min^{-1}). The concentration of peroxides was determined by a modified iodometric method.[7]

The intensity of chemiluminescence (CL) during the AIBN-initiated oxidation of cumene (cumene—chlorobenzene 1 : 1 mixture, 60 °C) was measured on an SNK-7 chemiluminescence setup in the photocurrent amplification mode.[8] The intensity of CL was amplified by additives of the activator Eu^{3+} 1,10-phenanthroline-tris(thenoyltrifluoroacetonate) synthesized by a known procedure[9] and recrystallized from ethanol. This activator was used in combination with naphthalene.[10] The effective rate constants (k_i) of the reactions of the inhibitors (InH) with peroxyl radicals were calculated from the kinetic curves of CL of the inhibited oxidation[11,12] or from the value of the minimum intensity of CL ($I_{\min}$) in the presence of the inhibitor,

$$(k_i)_{\min} = (1 - I_{\min})\sqrt{2w_i k_t}/\left(f[\mathrm{InH}]\sqrt{I_{\min}}\right), \quad (1)$$

or from the slope of the CL curve at the inflection point (tanφ)

$$(k_i)_{\varphi} = \sqrt{2k_t}\tan\varphi/\left(0.237\sqrt{w_i}\right), \quad (2)$$

here w_i is the rate of the initiation of a radical. The values of the rate constants calculated by these two equations differed by not more than 10%.

Results and Discussion

Table 1 and Figs. 1 and 2 present the experimental data on the periods (τ) of the suppression of the accumulation of hydroperoxides (ROOH) during the oxidation of lard (see Table 1), triglycerides, and methyl esters of olive (see Fig. 1) and sunflower oils (see Fig. 2) in the presence of the phenolic antioxidants. The values of the rate constant k_i presented in Table 1 characterize the activity of the compounds in the reactions with peroxyl radicals ($RO_2^{\cdot}$):

$$\mathrm{PhOH} + \mathrm{RO_2^{\cdot}} \longrightarrow \mathrm{ROOH} + \mathrm{PhO^{\cdot}}.$$

In the present work, k_i was measured in oxidized cumene at 60 °C. The effects of the nature of the oxidized substrate and of the medium on the value of k_i for ionol **9** and α-tocopherol **10** have been considered in detail in previously published reviews.[13,15] The range of the k_i values is significant: $2.2 \cdot 10^4$—$1.0 \cdot 10^4$ mol^{-1} L s^{-1} for ionol[13] and $9.2 \cdot 10^7$—$1.7 \cdot 10^4$ mol^{-1} L s^{-1} for

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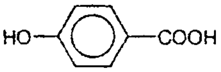
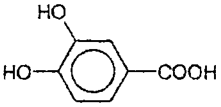
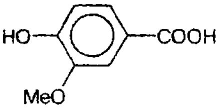
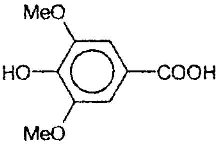
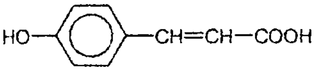
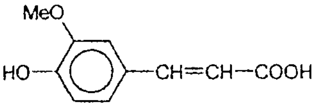
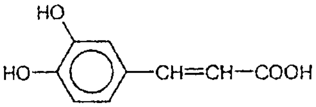
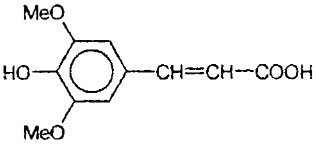
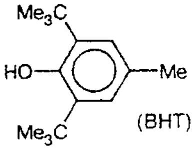
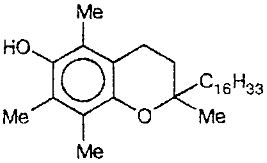
Table 1. Rate constants (k_i) of reactions with peroxyl radicals (cumene, 60 °C) and suppression periods (τ) in the oxidation of lard

Compound	Antioxidant	Formula	$k_i \cdot 10^{-4}$ /mol L^{-1} s^{-1}	τ/h
1	4-Hydroxybenzoic acid		0.27	1.0
2	3,4-Dihydroxybenzoic acid		4.6	9.0
3	Vanillic acid		1.05	1.1
4	Syringic acid		4.6	3.0
5	*p*-Coumaric acid		0.8	1.5
6	Ferulic acid		0.9	3.1
7	Caffeic acid		9.7	54.0
8	Sinapic acid		7.4	15.0
9	Ionol		1.65	5.0
10	α-Tocopherol		450	7.0

Note. Reaction conditions: 100 °C, [Inhibitor] = 0.001 mol L^{-1}, τ_0 = 1.0 h.

α-tocopherol.[15] The k_i values presented in Table 1 were obtained in the same measurement series under the same conditions. Thus the systematic errors are reduced to a minimum, and the comparison of compounds **1**—**10** by their antiradical activity is more correct.

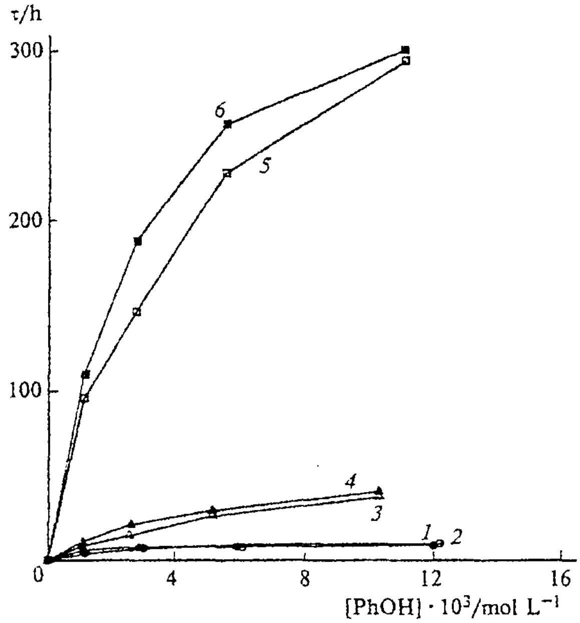


Fig. 1. Effect of phenolic acids on the oxidation of triglycerides (*1, 3, 5*) and methyl esters (*2, 4, 6*) of olive oil at 100 °C: *1* and *2*, compound **5**; *3* and *4*, **6**; *5* and *6*, **7**.

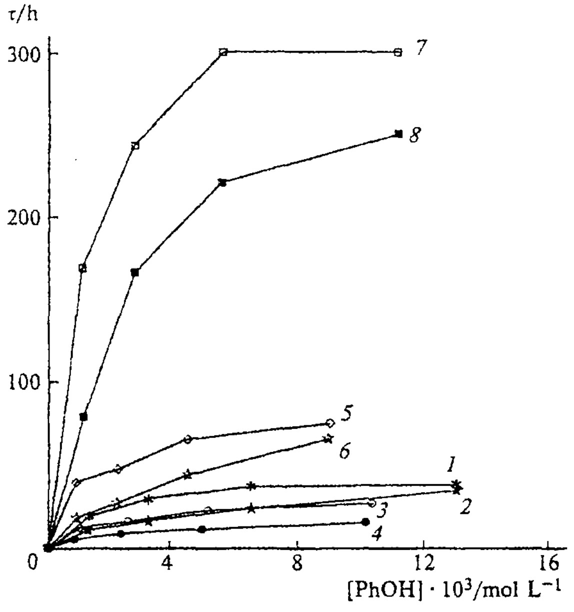


Fig. 2. Dependences of the suppression periods on the concentration of phenolic acids in the oxidation of triglycerides (*1, 3, 5, 7*) and methyl esters (*2, 4, 6, 8*) of sunflower oil at 100 °C: *1* and *2*, compound **1**; *3* and *4*, **5**; *5* and *6*, **8**; *7* and *8*, **7**.

It can be seen from the data in Table 1 that compounds **5**—**8** (cinnamic acid derivatives, in which the electron-withdrawing group is separated by a vinyl bridge from the aromatic ring) are characterized by higher values of k_i and longer suppression than the corresponding benzoic acid derivatives (compounds **1**—**4**). It follows from the comparison of the data for compounds **1**, **3**, and **4**, as well as for **5**, **6**, and **8**, that the donating methoxy groups in the aromatic ring increase the k_i values.

In both series, the *ortho*-substituted in the aromatic ring compounds **2** and **7** are the strongest inhibitors. When the k_i values are equal, the suppression period for compound **2** is three times longer than that for syringic acid **4**. A similar situation is observed in the case of caffeic and sinapic acids. It is noteworthy that phenolic acids **2**, **7**, and **8** suppress the oxidation of lard more efficiently than the known lipid antioxidants α-tocopherol and ionol. α-Tocopherol is the most abundant natural phenolic antioxidant and is characterized by very high activity in reactions with peroxyl radicals (see Table 1 and Refs. 13—15). However, in oxidized oils at high temperatures, the inhibitory efficiency of α-tocopherol decreases due to side reactions involving phenol or the corresponding phenoxyl radical.[13,15] It follows from the data in Table 1 that at 100 °C the suppression of the oxidation of lard by α-tocopherol is almost the same as that by comparatively poorly reactive ionol (τ is equal to 7 and 5 h, respectively), while in the presence of caffeic acid in the same concentration, the suppression period is equal to 54 h.

The changes in the suppression periods in the oxidation of triglycerides and methyl esters of acids of olive oil and sunflower oil as the concentration of added phenolic acids increases are presented in Figs. 1 and 2. It can be seen that the dependences are nonlinear, and the relative efficiency of the inhibitors decreases as the concentration increases, which is inherent in the autooxidation processes, in which the rate of radical initiation increases as hydroperoxides accumulate.[11]

It is reasonable that the longer the suppression period, the slower the formation of peroxides (low rate constant of chain propagation) and the higher the antiradical activity of the inhibitor (see Figs. 1 and 2). The suppression periods in sunflower oil, which has a high content of readily oxidizable linoleic acid (>60%), are an order of magnitude shorter than those in olive oil and lard (5—10% of linoleates).

In all the systems studied, the observed increase in the suppression period due to the addition of caffeic acid is likely related to the participation of the transformation products in the termination of oxidation chains. In the reactions with peroxyl radicals in oxidized hydrocarbons, the compounds dihydroxy-substituted in the aromatic ring, compounds **2** and **7** (QH_2), form semiquinone radicals ($QH^{\cdot}$), which are characterized by homo- and cross-disproportionation reactions:

$$QH^{\cdot} + QH^{\cdot} \rightleftharpoons Q + QH_2, \qquad (3)$$

$$QH^{\cdot} + R_j^{\cdot} \longrightarrow Q + R_jH. \qquad (4)$$

The formation of semiquinone radicals due to reversible reaction (3)[16,17] testifies that a strong inhibitor that terminates the chains by reaction (4) with the rate constant $>10^8$ mol^{-1} L s^{-1}, appears in the system. Under the autooxidation conditions, this should result in an increase in the suppression period. In addition, quinones can be reduced by alcohols and radicals (potential donors of hydrogen) to $QH^{\cdot}$ and QH_2 providing, in several cases, even negative catalysis of chain oxidation.[18]

It is also noteworthy that reaction (3), in essence, is a reaction of disproportionation—recombination of the radicals of the inhibitor. The initial inhibitor (QH_2), into which the inhibitor radicals formed from ionol and α-tocopherol disproportionate, is regenerated.[13—15] However, the other phenolic methoxy-substituted acids considered in this work form radicals that cannot disproportionate and, hence, cannot regenerate the inhibitor in a recombination reaction. Apparently this is the reason why acids **3—6**, despite their relatively high activity, provide considerably shorter peroids of suppression of the oxidation of lipid substrates than **2** and **7**.

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